Expression of p53-Regulated Proteins in Human Cultured
Lymphoblastoid TSCE5 and WTK1
Cell Lines during Spaceflight

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The aim of this study was to determine the biological effects of space radiations, microgravity, and
the interaction of them on the expression of p53-regulated proteins. Space experiments were performed
with two human cultured lymphoblastoid cell lines: one line (TSCE5) bears a wild-type p53 gene status,
and another line (WTK1) bears a mutated p53 gene status. Under 1 gravity or microgravity conditions, the
cells were grown in the cell biology experimental facility (CBEF) of the International Space Station for 8
days without experiencing the stress during launching and landing because the cells were frozen during
these periods. Ground control samples were simultaneously cultured for 8 days in the CBEF on the ground
for 8 days. After spaceflight, protein expression was analyzed using a Panorama™ Ab MicroArray protein
chips. It was found that p53-dependent up-regulated proteins in response to space radiations and space
environment were MeCP2 (methyl CpG binding protein 2), and Notch1 (Notch homolog 1), respectively.
On the other hand, p53-dependent down-regulated proteins were TGF-β, TWEAKR (tumor necrosis fac-
tor-like weak inducer of apoptosis receptor), phospho-Pyk2 (Proline-rich tyrosine kinase 2), and 14-3-3/τ
which were affected by microgravity, and DR4 (death receptor 4), PRMT1 (protein arginine methyltrans-
ferase 1) and ROCK-2 (Rho-associated, coiled-coil containing protein kinase 2) in response to space radi-
ations. ROCK-2 was also suppressed in response to the space environment. The data provides the p53-
dependent regulated proteins by exposure to space radiations and/or microgravity during spaceflight. Our
expression data revealed proteins that might help to advance the basic space radiation biology.

INTRODUCTION

Long-duration spaceflights are now being planned to the
Moon and Mars as a part of the “Vision for Space Exploration” program initiated by National Aeronautics and Space
Administration (NASA) in 2004. This report describes the
efforts to help design future missions for the International
Space Station (ISS), Lunar Base, and eventually a Mars
Expedition.1) Space radiations have been understood to be
one of the major hazards for personnel in space and have
emerged as the most critical issue to be resolved for future
long-term missions, both orbital and interplanetary.
Astronauts are exposed to severe high linear energy transfer
(LET) radiations in a complex radiation field consisting of
protons, heavy ions and secondary particles including neutrons with a broad range of energy. Space radiations pene-
trates the interior of the ISS, and physical dosimetry data has
been obtained with thermo-luminescent dosimeters (TLD),
plastic nuclear track detectors (CR39), phantoms and real
time dosimetry. Although a real time physical monitoring
facility for space radiations detected about 1 mSv per day 15
years ago,2,3) a recent estimate by NASA and the Japan
Aerospace Exploration Agency (JAXA) for the daily expo-
sure dose for a human body in the ISS was about 0.5 mSv.4,5)
Some effects of space radiations on the human body have
been reported to be chromosomal aberrations in lymphocytes, light flashes in the eye, central nervous system effects, and cataracts in the eye. On the other hand, it is not possible to answer questions concerning microgravity effects on biological reactions to radiation in space because contradictory data have been reported such as enhanced abnormal differentiation and decreased radiosensitivity, and no effects on mutation frequency and DNA repair activity.

An accumulation of 53 kDa tumor suppressor protein (p53) was reported in the skin and muscle of rats after spaceflight. p53 is generally thought to contribute to the genetic stability of cells in the presence of DNA damage by operating through p53-centered signal transduction pathways.

A proposal for the “RadGene” project was accepted by JAXA in 2000. This project was designed to investigate the expression of p53-regulated genes and proteins in cultured human lymphoblastoid cells using large-scale expression profiling analysis. Cells with different p53 gene status were chosen, because the cellular fate after exposure to radiation is mostly determined by p53 gene status. It was reported that wild-type p53 (wt p53) cells showed more apoptosis, slightly stronger arrest in G1, but less lethal aberrations and a lower viability when compared to mutated p53 (mp53) cells, whereas cells absent in p53 are characterized by an intermediate response. Since the difference of radiation-induced gene expression in mp53 cells versus wt p53 cells was more than that of p53 null versus wt p53 cells, to clarify the dependency of protein expression on cellular p53 gene status, two cell lines were used; one cell line bears a wt p53 gene, and another cell line bears a mp53 gene. These experiments were scheduled as the first life science experiment to be conducted on the Japanese Experimental Module of the ISS. This module is known as “Kibo” which means hope in Japanese, and is Japan’s first human-rated space experiment facility.

During this flight of 133 days, we separately measured two kinds of energy ranges according to measurements with physical dosimetry using a “Bio Passive dosimeter for life science experiments in space” (PADLES) containing four plates of CR-39 plastic nuclear track detectors and seven TLD dosimeter elements. The absorbed dose of ≥ 10 keV/μm and > 10 keV/μm which were 40.9 ± 3.2 mGy and 2.7 ± 0.5 mGy, respectively. The total equivalent dose of space radiation was 71.2 mSv. When calculating the results, the space samples exposures before cell culture began may have added up to about 52 mSv in the frozen state for 97 days. Thus, cells accumulated damage as a consequence of this space radiations exposure before culture. During culture conditions for 8 days, the space samples may have been exposed to about 4 mSv. Although the cells still accumulated approximately another 15 mSv during the rest of the spaceflight, they were in a frozen state during this period. Consequently, this 15 mSv exposure can be neglected in considering protein expression during spaceflight, since the cells were never cultured at 37°C during this period for an analysis of protein expression. The cells were thawed after arriving in this laboratory of Nara Medical University (NMU) and used for analysis of protein expression during spaceflight.

MATERIALS AND METHODS

Cells

Experiments were performed with two human lymphoblastoid cell lines: TSCE5 and WTK1. The cell lines were kindly provided by Dr. Fumio Yatagai (RIKEN, Saitama, Japan). The TSCE5 cell line was established from TK6 cells having a wt p53 gene status. TK6 and WTK1 cells were derived from the same donor source and thus are closely related, but differ in radiosensitivity and kinetics of apoptosis, mutability, chromosomal instability and p53 gene status. WTK1 cells overexpress an mp53 gene with a single base-pair substitution in exon 237 of exon 7 resulting in a change from Met (TAC) to Ile (TAT), and are dysfunction of p53. Although there are currently no reports concerning the full genetic make-up of these cell lines, there may possibly be other genetic differences in addition to the difference in their p53 gene status. Thus, these cells have been used as a useful tool to clarify the biological effects of p53 gene status. The cells are grown at 37°C in suspension cultures in a humidified 95% air/5% CO2 atmosphere in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% heat-inactivated horse serum (JRH Biosciences, Lenexa, KS, USA), 100 U/ml penicillin (Meiji Seika Kaisha Ltd., Tokyo, Japan), 100 μg/ml streptomycin (Meiji Seika Kaisha Ltd.) and 200 μg/ml sodium pyruvate (Nacalai Tesque, Kyoto, Japan). The cells were in an exponential growth phase, and thus were not synchronized with respect to the cell cycle before the experiment. Exponentially growing cells were washed and resuspended at a concentration of 5 × 10⁵ cells/ml in medium containing 10% dimethyl sulfoxide (DMSO) (Sigma-Aldrich Co., St Louis, MO, USA) at 4°C. 0.25 ml of a cell suspension was placed into a polypropylene syringe (SS-01T, Terumo Co., Tokyo, Japan), which was then placed into a cell culture bag (Fig. 1). The samples were then frozen at −80°C.

Cell culture bags

The cell culture bags were made of polypropylene (Hybrid MekkinBag HM-1304, HOGY, Osaka, Japan) with heat shielding (Fig. 1). A vacuum heat sealer (FCB-200, Fujimipulse Co. Ltd., Osaka, Japan) was used to prevent air bubbles from forming in sample bags. The two compartments were separated by a double temporary partition. The left compartment contained 10 ml of cell culture medium and frozen cells, and the right compartment contained 10
ml of a cryo-protectant solution such as medium containing 20% DMSO to stop cell growth (Fig. 1a). Before activation, the bags were frozen at –80°C. To start culture growth, the bags were moved into the CBEF at 37°C in a humidified 95% air/5% CO₂ atmosphere (Fig. 1b). To stop culture growth, the partition was broken, and this was confirmed when the two beads were visible together in the left compartment (Fig. 1c). Immediately after mixing the contents of the two compartments, the cells were frozen again at –80°C. Cell culture details have been reported previously.27)

**Spaceflight**

The space shuttle (Endeavor, STS-126) was launched at 9:55 am Nov. 15th, 2008 (Japanese time) from the Kennedy Space Center (KSC). Cell culture experiments were performed from February 20th to 28th, 2009 in space. The cell culture work was handled by a member of the space crew, Sandra H. Magnus. Experiments were designed to obtain information concerning microgravity effects on biological processes in the presence of space radiations by comparing results between cells grown in microgravity (10⁻⁴–10⁻⁶ gravity) (Fig. 2a) and cells grown under 1 gravity. The 1 gravity conditions were obtained by using a centrifuge (approximately 1.0–1.2 gravity) (Fig. 2b) in the CBEF aboard the ISS. Data were obtained daily from the ISS which indicated that the culture conditions (temperature and CO₂ levels) were maintained at normal conditions. The cells were then re-frozen in MELFI (Minus Eighty degree Celsius Laboratory Freezer for ISS) at –80°C 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. The cells were then thawed after arriving in this laboratory of NMU in Japan and used for analysis of protein expression during spaceflight.

**Ground controls**

Ground control samples were kept in a freezer at the JAXA during spaceflight (Fig. 2c). Cell culture was performed 3 days later at JAXA Tsukuba by obtaining information from the ground control facility (Fig. 2c).

**Protein expression analysis using protein chips**

After being returned to this laboratory in NMU, the frozen cells were thawed, washed with phosphate buffered saline

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**Fig. 1.** Cell culture bags. **a,** before activation (maintained at –80°C); **b,** after activation (maintained on the CBEF at 37°C in a humidified 95% air/5% CO₂ atmosphere); **c,** after deactivation (maintained at –80°C).

**Fig. 2.** Experimental schedules. **a,** spaceflight for microgravity (μg) experiments; **b,** spaceflight for 1 gravity (1 g) experiments; **c,** ground control experiments. JAXA, Japan Aerospace Exploration Agency; KSC, Kennedy Space Center; NMU, Nara Medical University.
(PBS) at 4°C, immediately frozen in liquid N₂ and stored at –80°C. 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 cyanine dye 3 (Cy3) and cyanine dye 5 (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.) 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 an Array-Pro Analyzer® Ver.4.5 (Media Cybernetics Inc., Bethesda, MD, USA).

Protein levels in TSCE5 and WTK1 cells
In analysis of individual induced or suppressed protein levels, the expression level value was obtained by a comparison of samples grown in different environments (space 1 g gravity, space microgravity, and the ground environment). The operational definition for proteins with an increased level of expression is proteins with an expression level more than 1.5-fold (ratio ≥ 1.5). In addition, the operational definition for proteins with a suppressed level of expression is proteins with an expression level less than 0.66-fold (ratio ≤ 0.66). From these results, the cause of the altered expression in TSCE5 and WTK1 cells is indicated in Tables 1 and 2, respectively.

p53-Dependent up-regulated proteins
The p53-dependent up-regulated genes were defined as formula; W/M ≥ 1.5

(W ≥ 1.5 and M ≤ 1.5)

W was the ratio of gene expression (spaceflight/ground) in wtp53 cells. M was the ratio of that in mp53 cells.

Table 1. Protein levels in TSCE5 (wtp53) cells.

| Protein expression | Gene symbol | Genbank accession | Ratio | Sample comparison | Cause |
|--------------------|-------------|-------------------|-------|-------------------|-------|
| Induced proteins   | Phospho-FAK | NP_032008.1,NP_722560.1,NP_037213.1 | 2.26  | Space 1 g/Ground  | Space radiations |
|                    | MeCP2       | NP_004983.1,NP_073164.2,NP_034918.1 | 1.60  |                   |       |
|                    | NF-κB       | NP_032715.2,NP_003989.2 | 1.50  |                   |       |
|                    | KPN2        | NP_002257.1,NP_034785.1 | 1.83  | Space μg/Ground   | Space environment |
|                    | Notch1      | NP_060087.3,NP_032740.2 | 1.81  |                   |       |
|                    | PDGFRβ      | NP_002600.1 | 1.71  | Space μg/Ground   | Space environment |
|                    | p300/CBP    | NP_064389.2,NP_003875.3,NP_001019423.1 | 1.60  |                   |       |
|                    | LAP2        | NP_003267.1 | 1.52  |                   |       |
|                    | 14-3-3 θ/t  | NC_000002.11 | 1.51  |                   |       |
|                    | Bcl-xL      | NP_612815.1,NP_113723.2,NP_038737.3 | 1.92  | Space μg/Space 1 g | Microgravity |
| Suppressed proteins| Phospho-c-Jun| NP_002219.1,NP_034721.1 | 0.66  |                   |       |
|                    | ROCK-2      | NP_033098.2,NP_037154.1,NP_004841.2 | 0.64  | Space 1 g/Ground  | Space radiations |
|                    | Bmf         | NP_612186.2,NP_277038.1 | 0.63  |                   |       |
|                    | DR4         | NP_003835.2 | 0.62  |                   |       |
|                    | PRMT        | NP_062804.1,NP_938075.2 | 0.60  |                   |       |
|                    | Bcl-xL      | NP_612815.1,NP_113723.2,NP_038737.3 | 0.61  | Space μg/Ground   | Space environment |
|                    | ROCK-2      | NP_033098.2,NP_037154.1,NP_004841.2 | 0.62  |                   |       |
|                    | Bcl-xL      | NP_612815.1,NP_113723.2,NP_038737.3 | 0.61  | Space μg/Ground   | Space environment |
|                    | DR4         | NP_003835.2 | 0.60  |                   |       |
|                    | NF-κB       | NP_032715.2,NP_003989.2 | 0.64  |                   |       |
|                    | Phospho-Pyk2 | NP_059014.2,NP_775268.1 | 0.55  |                   |       |
|                    | TGF-β       | NP_000651.3,NP_003229.1,NP_003230.1 | 0.54  |                   |       |
|                    | p19Ink4d    | NP_524145.1 | 0.48  | Space μg/Space 1 g | Microgravity |
|                    | TWEAKR      | NP_057723.1 | 0.44  |                   |       |
|                    | 14-3-3 θ/t  | NC_000002.11 | 0.41  |                   |       |
|                    | KPN2        | NP_002257.1,NP_034785.1 | 0.38  |                   |       |
### Table 2. Protein levels in WTK1 (mp53) cells.

| Protein expression | Gene symbol | Genbank accession | Ratio | Sample comparison | Cause               |
|--------------------|-------------|--------------------|-------|-------------------|---------------------|
| Induced proteins   |             |                    |       |                   |                     |
| KPNA2              | NP_002257.1,NP_034785.1 | 3.02              |       |                   |                     |
| TWEAKR             | NP_057723.1 | 2.39              |       |                   |                     |
| p19ink4d           | NP_524145.1 | 2.03              |       |                   |                     |
| TGF-β              | NP_000651.3,NP_003229.1,NP_003230.1 | 2.03 |       |                   |                     |
| Importin α3        | NP_002259.1,NP_00101479.3,NP_032493.1 | 1.89 |       |                   |                     |
| Phospho-FAK        | NP_032008.1,NP_722560.1,NP_037213.1 | 1.84 |       |                   |                     |
| HDAC10             | XP_001073452.1,NP_114408.3,NP_954668.1 | 1.70 |       |                   |                     |
| E2F3               | NP_001940.1 | 1.57              |       |                   |                     |
| Suppressed proteins|             |                    |       |                   |                     |
| APRIL              | NP_742085.1 | 0.65              |       |                   |                     |
| MKK3               | NP_032954.1,NP_659731.1,XP_239239.2 | 0.63 |       |                   |                     |
| Phospho-c-Jun      | NP_002219.1,NP_034721.1 | 0.61 |       |                   |                     |
| Bmf                | NP_612186.2,NP_277038.1 | 0.66 |       |                   |                     |
| Apaf1              | NP_863659.1,NP_033814.2 | 0.66 |       |                   |                     |
| APRIL              | NP_742085.1 | 0.65              |       |                   |                     |
| DR4                | NP_003835.2 | 0.64              |       |                   |                     |
| Bmf                | NP_612186.2,NP_277038.1 | 0.66 |       |                   |                     |
| Bcl-xL             | NP_612185.1,NP_113723.2,NP_033873.3 | 0.66 |       |                   |                     |
| HDAC10             | XP_001073452.1,NP_114408.3,NP_954668.1 | 0.66 |       |                   |                     |
| KPN2               | NP_002257.1,NP_034785.1 | 0.66 |       |                   |                     |
| HSPA9              | NP_004125.3,NP_034611.2 | 0.66 |       |                   |                     |
| Apaf1              | NP_863659.1,NP_033814.2 | 0.66 |       |                   |                     |
| Bmf                | NP_612186.2,NP_277038.1 | 0.66 |       |                   |                     |
| Bcl-xL             | NP_612185.1,NP_113723.2,NP_033873.3 | 0.66 |       |                   |                     |
| ROCK-2             | NP_033098.2,NP_037154.1,NP_004841.2 | 0.64 |       |                   |                     |
| TGFB               | NP_000651.3,NP_003229.1,NP_003230.1 | 0.64 |       |                   |                     |
| TWEAKR             | NP_057723.1 | 0.55              |       |                   |                     |
| Phospho-Pyk2       | NP_059014.2,NP_775268.1 | 0.49 |       |                   |                     |

### Table 3. p53-Dependent protein expression. A classification of p53-dependency means there were changes in protein synthesis levels in wt53 cells when compared to levels in mp53 cells after cells were grown in space.

| p53-dependent | Gene symbol | Genbank accession | Value | Sample comparison | Cause               |
|---------------|-------------|--------------------|-------|-------------------|---------------------|
| Up-regulated  | MeCP2       | NP_004983.1,NP_073164.2,NP_034918.1 | 1.85  | Space 1 g/Ground  | Space radiations    |
| Notch1        | NP_060087.3,NP_032740.2 | 1.63 |       |                   |                     |
| Down-regulated|              |                    |       |                   |                     |
| NICD          | NC_00002.11 | 0.42              |       |                   |                     |
**Classification of results**

Results were interpreted to be responses to space radiations by using comparisons between the 1 gravity space samples and the ground samples. Results were interpreted to be responses to the space environment by using comparisons between the space microgravity samples and ground samples. Results were interpreted to be responses to microgravity by using comparisons between the space microgravity samples and the space 1 gravity samples.

**RESULTS**

The aim of this study was to compare protein expression profiles in wt-p53 and mp53 cells during spaceflight. Protein expression profiles were measured using Sigma-Aldrich protein chip technology including 642 human protein-recognizing antibodies and about 80 p53-related proteins. In wt-p53 cells, there were 10 proteins with induced expression. These were Phospho-FAK (phosphorylation of focal adhesion kinase), MeCP2 (methyl CpG binding protein 2), NF-κB, (nuclear factor of κ light polypeptide gene enhancer in B-cells), KPNA2 (karyopherin alpha 2), Notch1 (Notch homolog 1, translocation-associated, Drosophila), PDGFRβ (platelet-derived growth factor receptor β), p300/CREB (p300 and the cAMP response element-binding protein (CREB)- binding protein), LAP2 (lamin-associated polypeptide 2), 14-3-3 θ/τ (tyrosine 3-monooxygenase/tryptophan 5- monooxygenase activation protein, theta polypeptide) and Bcl-xL (BCL2-like 1). There were 13 proteins with suppressed expression. These were Phospho-c-Jun (Jun oncogene), ROCK-2 (Rho-associated, coiled-coil containing protein kinase 2), Bmf (Bcl2 modifying factor), DR4 (death receptor 4), PRMT (protein arginine methyltransferase 2), Bcl-xL, NF-κB, Phospho-Pyk2 (Proline-rich tyrosine kinase 2), TGF-β (Transforming growth factor-β), p19Ink4d (p19, inhibits cyclin-dependent kinase 4), TWEAKR (tumor necrosis factor-like weak inducer of apoptosis receptor), 14- 3-3 θ/τ and KPNA2 (Table 1). In mp53 cells, there were 15 proteins with induced expression. These were KPNA2, TWEAK R, p19, TGF-β, KPNA4, Phospho-FAK, HDAC10 (histone deacetylase 10), E2F3 (E2F transcription factor 3), PDGFRβ, Rab7 (member RAS oncogene family), NF-κB, Bmf, Apaf1 (apoptosis protease activator protein-1), APRIL (a proliferation-inducing ligand) and Phospho-c-Jun. There were 16 proteins with suppressed expression. These were APRIL, MKK3 (mitogen-activated protein kinase kinase-3), Phospho-c-Jun, Bmf, Apaf1, Bcl-xL, DR4, Bmf, HDAC10, KPNA2, HSPA9 (heat shock 70kDa protein 9), p19Ink4d, 53BP1 (p53-binding protein 1), NF-κB, LIMK1 (LIM domain kinase 1), Phospho-FAK and MeCP2 (Table 2). Regardless of p53 gene status, the number of induced proteins was only 2 and these were Phospho-FAK in response to space radiations and PDGFRβ in response to the space environment (Tables 1 and 2). In addition, there were 7 proteins with p53-independent suppressed expression. These were Phospho-c-Jun and Bmf in response to space radiations, Bcl-xL and DR4 in response to the space environment, and NF-κB, p19Ink4d and KPNA2 in response to microgravity environments (Tables 1 and 2).

On the other hand, the number of p53-dependent up-regulated proteins was only 2, and the number of down-regulated proteins was 7 (Table 3). Still, the number of p53-dependent profiled proteins (Table 3) includes about 1.4% of the protein chip. Up-regulated p53-dependent proteins consisted of MeCP2 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, TWEAKR, phosho-Pyk2 and 14-3-30/τ in response to microgravity environments, and DR4, PRMT1 and ROCK-2 in response to space radiations. ROCK-2 was also suppressed independently of p53 gene status in the space environment (Table 3).

**DISCUSSION**

The p53 tumor suppressor gene is frequently mutated in human cancer. The majority of tumor-derived p53 mutations is missense point mutation and clustered within the central DNA-binding domain. Mp53 is defective in sequence specific DNA binding, and have acquired additional functional independent of wtp53, called gain of function. On the other hand, wtp53-regulated proteins include apoptosis-related proteins [e.g. AIF (apoptosis inducing-factor), Apaf1,] Bax (Bcl-2 associated x protein), DR5 (death receptor 5), PERP (p53 apoptosis effector related to PMP-22), PUMA (p53-upregulated modulator of apoptosis), p53DINP1 (p53-dependent damage-inducible nuclear protein 1); cell cycle-regulated genes [e.g. Cdkn1a (cyclin-dependent kinase inhibitor 1A, formerly known as p21WAF1/CIP1), cyclin D, PCNA (proliferating cell nuclear antigen), PTEN (phosphatase and tensin homolog deleted from chromosome 10), and RB (retinoblastoma gene product)]; and DNA repair-regulated genes [e.g. p53R2 (p53-inducible ribonucleotide reductase small subunit)], and the p53-regulated gene [Hdm2 (human homolog of Mdm2)]. In this experiment, alterations in the expression of p53 or of these prominent p53-regulated proteins were not detected (Table 3). Although DR4, TGF-β and 14-3-3β were reported to be DNA damage-inducible p53-regulated genes, these proteins were suppressed in a p53-dependent manner in these space experiments. A direct accumulation of p53 proteins was observed in rat muscle and skin after spaceflight, but was not seen in cells grown in a space environment in this experiment. These results are supported by previous findings that the cellular amounts of p53 and WAF1 in cultured mammalian cells from flight samples were similar to those of the ground control samples. In addition, the expression of p53 gene which was analyzed using DNA
chip, was also not detected in a space environment. In common 642 kinds between protein chips and DNA chips, there were quite differences of p53-dependent profiled list in response to space radiations, microgravity and space environment. This discrepancy probably occurred because protein expression might be dependent on translation or stabilization levels not in transcription levels.

It is possible that the profiled proteins (Table 3) reported here may represent newly observed p53-regulated proteins which have not been documented until this report. In fact in support of this, it was recently demonstrated that the up-regulation of Notch1 gene expression by p53 occurs through a negative regulation of ROCK-2 as well as from these results (Table 3). After genotoxic stresses, Notch signaling determines cell fate and affects cell proliferation, differentiation, and apoptosis during cell development. Therefore, it is interesting to note that Notch1 appeared to accumulate in a p53-dependent manner after an exposure to space radiations and microgravity. Compared to radiation induced protein induction, the mechanisms through which microgravity induces gene activity are unknown.

Here, the emphasis was on examining the behavior of p53-dependent regulated proteins after exposure to space radiations, microgravity, and the space environment during spaceflight can be profiled. In future, these finding will contribute to advancing our knowledge of basic radiation biology.

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