Effects of microgravity on DNA damage response in  
*Caenorhabditis elegans* during Shenzhou-8 spacecraft

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**Abstract**

**Purpose:** Space radiations and microgravity both could cause DNA damage in cells, but the effects of microgravity on DNA damage response to space radiations are still controversial.

**Materials and methods:** A mRNA microarray and microRNA microarray in dauer larvae of *Caenorhabditis elegans* (*C. elegans*) that endured spaceflight environment and space radiations environment during 16.5-day Shenzhou-8 space mission was performed.

**Results:** Twice as many transcripts significantly altered in the spaceflight environment than space radiations alone. The majority of alterations were related to protein amino acid dephosphorylation and histidine metabolic and catabolic processes. From about 900 genes related to DNA damage response, 38 differentially expressed genes were extracted; most of them differentially expressed under spaceflight environment but not space radiations, although the identical directions of alteration were observed in both cases. cel-miR-81, cel-miR-82, cel-miR-124 and cel-miR-795 were predicted to regulate DNA damage response through four different anti-correlated genes.

**Conclusions:** Evidence was provided that, in the presence of space radiations, microgravity probably enhanced the DNA damage response in *C. elegans* by integrating the transcriptome and microRNA expression.

**Keywords:** Space radiations, microgravity, DNA damage response, Gene expression, microRNA

**Introduction**

During spaceflight missions, crews endure both radiations and gravity regimens that profoundly differ from those experienced on Earth (Horneck 1999, Manti 2006). Space radiations have been characterized as high linear energy transfer (LET) and low dose rate, which may induce serious DNA damage over a wide spectrum when compared with radiation exposure on the ground (Ohnishi et al. 2002, Cucinotta and Durante 2006, Dziegielewski et al. 2006). Cells have evolved highly conserved DNA repair machinery to process the damage by an efficient DNA damage response (DDR), involving the activation of cell cycle checkpoints to induce a cell cycle arrest, DNA repair, apoptosis, or a combination thereof (Harper and Elledge 2007). The transcriptional profile also changes in response to DNA damage, probably in order to promote cell survival (Abbots et al. 2014). Disrupting DDR may elicit genome instability and increasing risks of carcinogenesis (Cucinotta et al. 2001, Cucinotta and Durante 2006, Hei et al. 2011).

Microgravity is another inevitably risk factor to health during spaceflight, which could impair signal transduction and immune response (Morrison 1994, Aubert et al. 2005). Simulated microgravity is reported to affect the expression of genes involved in DNA repair, resulting in accumulation of DNA damage (Takahashi et al. 2012). Studies on the combined effects of microgravity and radiation have had conflicting results, with some showing that microgravity or simulated microgravity increased the DNA damage induced by ionizing radiation (IR) (Degan et al. 2005, Kumari et al. 2009, Wang et al. 2011, Yatagai et al. 2011), others showing they decreased the damage (Kobayashi et al. 2000, 2004), and some showing they had no effects (Pross et al. 2000, Mognato and Celotti 2005). Therefore, whether real microgravity can affect the DDR to space radiations is still unclear.

MicroRNA (miRNA), a class of small non-coding RNA, mediates post-transcriptional regulation of specific target mRNA in various cellular processes (Bartel 2009). Recently, miRNA has been shown in regulating a variety of physiological activities such as DDR process under microgravity and/or radiation environments (Kato et al. 2009, Mangala et al. 2011, Wouters et al. 2011). Girardi reported that simulated microgravity altered the miRNA expression signature of irradiated cells by decreasing the quantity of radio-responsive miRNA. Genes in the Gene Ontology (GO) category ‘Response to DNA damage stimulus’ were enriched under 1 g but not microgravity conditions, indicating that simulated microgravity could affect the DDR process to IR in human lymphocytes (Girardi et al. 2012). In our previous study, 23 altered miRNA of *Caenorhabditis elegans* showed different expression patterns under spaceflight environment and space radiations environment, and seven miRNA were predicted to regulate 12 genes by integration analysis of the miRNA and mRNA expression profiles (Xu et al. 2014). Therefore, miRNA and...
their target genes could be involved in the DDR process of living organisms when exposed to space environment.

\emph{C. elegans} is simpler than the mammalian system while still sharing high genomic homology and thus employed for space biological studies as an excellent model (Szewczyk et al. 2008). Under spaceflight environment, the sensitivity of physiological processes in \emph{C. elegans} varied from endpoints to endpoints, such as developmental timing (Zhao et al. 2005, Szewczyk et al. 2008), locomotion (Oczykop et al. 2012), apoptosis (Higashitani et al. 2005), mutant rate (Zhao et al. 2006), muscle development (Higashibata et al. 2006, Adachi et al. 2008), and aging (Honda et al. 2012). However, it was not clear whether these changes were generated by space environment or by metagenesis during the space missions, because larvae used in most studies could keep breeding during spaceflight. To avoid the influence from metagenesis (Morey-Holton et al. 2007), an effective solution would be chosen a diapause stage to maintain \emph{C. elegans} worms in a synchronized stage. The dauer diapause stage of \emph{C. elegans} is a stress-resistant stage characterized by no feeding, lifespan extending and developmental arrest (Wang and Kim 2003, Hu 2007, Jeong et al. 2009). Hence, dauer larvae were employed in our study.

To elucidate the effects of microgravity on the DDR process in the presence of space radiations, transcriptome and microRNA of space-flown \emph{C. elegans} were integrated to analyze gene expression and the corresponding miRNA expression. The genes involved in the processes of DNA repair, apoptosis, cell cycle arrest, and others related to DDR process, were focused on to search for a possible miRNA regulating gene expression in the dauer larvae of \emph{C. elegans} flown on Shenzhou-8 spaceflight.

\section*{Materials and methods}

\subsection*{Sample preparation of \emph{C. elegans}}

The wild-type strain \emph{(Bristol N2)} of \emph{C. elegans} was acquired from the Caenorhabditis Genetics Center (Minneapolis, MN, USA). Worms were cultured on Nematode Growth Medium (NGM) at 23°C and synchronized to dauer larvae by 1% sodium dodecyl sulfonate (SDS) (Sangon Biotec, Shanghai, China) according to the protocols in Wormbook (Stiernagle 2006). \emph{C. elegans} were divided into three groups as shown in Table I: spaceflight group, spaceflight control group, and ground control group. As described in previous studies (Horneck 1999, Takahashi et al. 2012), experiments were designed to obtain biological effects concerning space radiations (SR) by comparing results between spaceflight control group and ground control group (group 2 vs. 3), and to obtain biological effects concerning spaceflight environment (SF) by comparing results between spaceflight group and ground control group (group 1 vs. 3).

\subsection*{Spaceflight experiments}

Shenzhou-8 was launched in the Jiuquan Satellite Launch Center on 1 November 2011. About 10h before the launch, fresh solid NGM and approximately 10⁵ dauer worms were successively loaded in the Experiment Unique Equipment (Supplementary Figure 1A, available online at http://informahealthcare.com/abs/doi/10.3109/09553002.2015.1043754 available online), which was housed in the Experiment Containers (Supplementary Figure 1B available online at http://informahealthcare.com/abs/doi/10.3109/09553002.2015.1043754). Assembled containers were settled into the centrifugal and static slots of BIOBOX (Supplementary Figure 1C and D available online at http://informahealthcare.com/abs/doi/10.3109/09553002.2015.1043754). The Shenzhou-8 mission was flown from 1–17 November 2011, with a total mission time of 16.5 days. Throughout the duration of the experiment, the Experiment Containers maintained a temperature of 23 ± 0.5°C. G-force peaks did not exceed levels of ± 0.01 g, while most of the time values oscillated between ± 0.005 g. Space radiations dose was measured by the thermoluminescent detector (TLD) in the static slot (1.92 mGy) and centrifuge slot (2.27 mGy), respectively. Seven hours after landing, worms were collected and kept in liquid nitrogen. The corresponding ground controls of the experiment were performed in parallel at the Payload Integration Test Center in Beijing 2 days later. Here, the samples were kept in static slot in BIOBOX and temperature was maintained at 23 ± 0.5°C. The ground group was stopped after 16.5 days and the samples were collected after 7 h later. Finally, worms were sent back to Dalian Maritime University for further analysis.

\subsection*{Total RNA isolation}

About 2000 worms from each group were collected and total RNA was isolated using Invitrogen™ TRizol (Invitrogen, Carlsbad, CA, USA) according to manufacturers’ instructions. Quality and purity of the RNA preparations were assessed by spectrophotometric determination (NanoDrop® 2000c UV-Vis Spectrophotometer) (Thermo Fisher Scientific Inc., Wilmington, DE, USA) of the ratio of absorbance at 260/280 nm (OD_{260}/OD_{280} > 1.9) and by quantification of the ratios of 28S:18S ribosomal RNA (GelDoc-ITM 310 Imaging System) (UVP, Cambridge, CA, USA).

\subsection*{mRNA microarray and miRNA microarray analysis}

The NimbleGen Gene Expression Profiling service and miRCURY™ LNA Array microRNA Expression Profiling service were performed by KangChen Bio-tech Inc. (Shanghai, China) as previously described (Xu et al. 2014). GO analysis was applied to determine the biological process of genes. Genes involving in DDR process belong to categories of DNA repair (GO: 0006281), apoptotic process (GO: 0006915), cell cycle arrest (GO: 0007050), response to DNA damage stimulus (GO: 0006974), telomere maintenance (GO:0000723) and other processes involved in DDR according to the database from AmiGO (version 1.8) (Ashburner et al. 2000), KEGG pathway, and Wormbook (O’Neil and Rose 2006). To predict the target miRNA of differentially expressed genes, computational analysis was performed with combination of miRanda (Betel et al. 2008), MicroCosm Targets (Version 5), and TargetScanWorm 6.2.

\subsection*{Quantitative real-time polymerase chain reaction analysis}

The data of mRNA microarray were validated by quantitative real-time polymerase chain reaction (qRT-PCR) using SuperScript® III Reverse Transcriptase (Invitrogen, Carlsbad, CA, USA) and SYBR® Green Master Mix (Applied Biosystems, Foster City, CA, USA). Total RNA was extracted from independent worm samples for qRT-PCR. The reactions were incubated in an ABI PRISM7900 HT system (Applied Biosystems, Foster City, CA, USA).
CA, USA) in PCR plates (Applied Biosystems, Foster City, CA, USA) for 10 min at 95°C, followed by 40 cycles of 10 s at 95°C and 60 s at 60°C. Results were normalized to the threshold cycle (Ct) value of house-keeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (gpd-2), and the relative expression levels of genes were calculated using the 2−ΔΔCt method. Primers were shown in Supplementary Table I available online at http://informahealthcare.com/abs/doi/10.3109/09553002.2015.1043754 (available online).

Statistical analysis
Raw data were log2-transformed to achieve the statistical analysis under MS Excel 2010 software. R version 2.15.2 software was used to acquire the features of the datasets. Differentially expressed mRNAs (log2-fold changes ≤ −1 or ≥1) and miRNA (log2-fold change ≤ −1 or ≥1) were identified through fold change filtering. Cluster 3.0 version 1.24 and Java TreeView version 1.0.4 were used to achieve hierarchical clusters of differentially expressed miRNA.

Results
Global gene expression profile under different space environments
mRNA microarray analysis showed that 2262 transcripts changed among the 18,186 detected transcripts under SF environment. By contrast, only half the number of altered transcripts (1078) were observed under SR environment (Figure 1A). The percentage of up-regulated transcripts and down-regulated transcripts appeared nearly to be equal in both the SF and SR environments (Figure 1B). The distribution characteristics of global gene expression profile were evaluated by the interquartile range (IQR). The value of IQR under SF environment (0.827) was higher than that under SR environment (0.621), indicating that more genes were significantly altered in response to SF environment (Figure 1C and 1D). Differentially expressed transcripts were subjected to GO analysis as shown in Table II. In the all of biological processes, ‘protein amino acid dephosphorylation’ represented the largest group of up-regulated genes, followed by ‘dephosphorylation,’ ‘post-translational protein modification,’ and so on under SF environment. ‘Histidine metabolic process,’ ‘histidine catabolic process,’ ‘histidine family amino acid metabolic process,’ and ‘histidine family amino acid catabolic process’ represent important groups of down-regulated genes under SF environment. Under SR environment, differentially expressed genes were the most enriched in ‘polyl metabolic process,’ ‘acyl-CoA metabolic process’ and ‘thioester metabolic process’.

Expression profile of genes involving in DDR process
About 900 genes involved in DDR process were screened out (Figure 2), and of those, 38 differentially expressed genes...
were extracted under SF and SR environments (Table III). Under SF environment, 31 genes were altered, including seven in DNA repair, 17 in the apoptosis process, and the rest were altered in various other processes involved in DDR process. In contrast, four differentially expressed genes were observed under SR environment, including two in ‘DNA repair’ and two in ‘apoptosis process’. Besides, *agt-1*, *him-6* in ‘DNA repair’ and T05C3.6 in ‘apoptosis’ were altered in both cases. Genes in cell cycle arrest (GO: 0007050) were found to have no change in both cases (data not shown).

**Anti-correlation analysis of miRNA expression**

The miRNA microarray utilized the same worm samples in mRNA microarray covered 157 miRNA, and hierarchical clustering analysis showed 23 altered miRNA under SF and SR environments (Supplementary Figure 2A, available online at http://informahealthcare.com/abs/doi/10.3109/09553002.2015.1043754 available online). According to the anti-correlation analysis on miRNA and mRNA profiling, the target genes of miR-81, 82, 124, and 795 were predicted to be *air-2*, *bath-41*, *pme-1* and *daf-16*, respectively (Table IV).

**Microarray data validation by qRT-PCR**

Microarray data from mRNA expression profiling were validated by qRT-PCR from independently-isolated RNA samples (Figure 3). Ten genes were selected from differentially expressed genes in microarray, including H21P03.2, **exo-3**, *him-6*, **air-2**, *cdh-3*, *dyf-2*, *sir-2.2*, *hda-4* and Y56A3A.33. The direction and amplitude of the fold changes determined by qRT-PCR closely matched microarray data for genes tested.
Discussion

Space radiations and microgravity could induce DDR in living organisms, while the interaction between them is not determined. The combined influence of space radiations and microgravity with which humans in space have to cope must be taken into consideration. In this study, an analysis of mRNA microarray and miRNA microarray was performed to elucidate the effects of microgravity on the DDR process of space-flown C. elegans in the presence of space radiations.

The cellular responses invoked by DNA damage consist of a broad network of transcriptionally regulated pathways covering most aspects of cellular physiology, such as cellular signaling, metabolic pathways and protein modification (Jen and Cheung 2003, Forrester et al. 2012). In this study, the global gene expression profiles under different experimental conditions were analyzed in order to study the effect of microgravity on DDR process in the presence of space radiations. Results showed the number of differentially expressed genes under SF environment was about double that under SR environment, and altered genes appeared to have much more obvious changes under SF environment than SR environment (Figure 1). GO analysis showed that altered genes under SR environment were enriched in six categories (Table II). In animals, acetyl-CoA is mainly produced from fatty acid metabolism and is essential for lipid metabolism. Polyol metabolic process is probably related to worm energy supply (Blaise et al. 2007). Hence, space radiations may lead to a shift in energy metabolic homeostasis during spaceflight (Mao et al. 2014), which probably is a relevant adjustment to space environmental stress in C. elegans at dauer or developmental stage (Selch et al. 2008). In contrast, a large number of altered genes function in protein metabolic processes and modification under SF environment. Genes in metabolic and catabolic processes of histidine and related amino acid may be involved in antioxidant effect (Farshid et al. 2013) and resistance to metal toxicity (Murphy et al. 2011). Protein amino acid dephosphorylation and phosphorylation changes have functional pluripotency in almost all physiological activities induced by radiation or reduced gravity, such as signal transduction (Taubber et al. 2013), transcription (Trivigno et al. 2013), endothelial dysfunction (Versari et al. 2013), muscle damage/recovery (Shifman et al. 2013). Higashibata also found that expression of phosphoprotein changed significantly in space-flown C. elegans (Higashibata et al. 2007). Therefore, the findings indicated that microgravity could affect transcriptional modulation involved in DDR under spaceflight environment.

DDR is a complex pathway addressed to maintain genome integrity through the activation effector proteins of DNA repair, apoptosis and cell cycle arrest (Girardi et al. 2012). DDR to IR depends on the LET and the dose of radiation,
thus space radiations consisting of complex particles may induce a variety of DNA damage, such as single strand DNA lesions (SSL) or double-strands DNA breaks (DSB) (Hada and Georgakilas 2008, Lemmens and TijSterman 2011). Cells have developed a complex network of redundant DNA repair mechanisms, instead of a single DNA repair pathway, to avoid the risk of faulty DNA repair (Lemmens and TijSterman 2011) (Supplementary Table II, available online at http://informa-healthcare.com/abs/doi/10.3109/09553002.2015.1043754 available online).

Under SF environment, ung-1, exo-3 and pme-1 possessing nuclease activity in Base excision repair (BER) or Nucleotide excision repair (NER) pathways were altered, and polk-1 acting as DNA polymerase in Fanconi anemia (FA) process was altered as well. Under SR environment, whether combination with microgravity or not. The results indicated that cells probably activated more DNA repair mechanisms, instead of a single DNA repair pathway, to avoid the risk of faulty DNA repair (Lemmens and TijSterman 2011).

Table III. List of differentially expressed genes involved in DDR process.

| Gene symbol | Gene name | Brief description | SF | SR |
|-------------|-----------|------------------|----|----|
| DNA repair (GO:0006281) | | | |
| get-17 | GEX interacting protein 17 | E3 SUMO protein ligase | – 1.03 | – 0.31 |
| C11G6.2 | Unknown | Unknown | – 1.38 | 0.06 |
| pif-1 | PIF1p DNA helicase (yeast) homolog 1 | Negative regulates telomerase activity | 1.54 | 0.54 |
| unc-1 | Uracl DNA N-glycosylase 1 | A uracl-DNA glycosylase | 1.00 | 0.53 |
| exo-3 | Exonuclease 3 | AP (apurinic/apyrimidinic) endonucleases | 1.20 | 0.65 |
| pme-1 | Poly (ADP-ribose) metabolism enzyme 1 | Poly (ADP-ribose) polymerase | 1.21 | 0.59 |
| polk-1 | Polk (DNA polymerase kappa) homolog 1 | Y-family DNA polymerases | 1.23 | 0.53 |
| agt-1 | Alkylguanine DNA alkytransferase 1 | O6-Alkylguanine DNA-alkyltransferase | 1.16 | 1.23 |
| him-6 | High incidence of males 6 | DNA helicases, Bloom’s syndrome complex | 1.24 | 1.21 |
| lig-4 | Ligase 4 | DNA Ligase 4 complex | – 0.76 | – 1.48 |
| F35H10.5 | Unknown | Unknown | 0.90 | 1.14 |
| Apoptotic process (GO:0006915) | | | |
| ccc-1 | Conserved coiled-coil protein 1 | Regulator, conserved coiled-coil Protein | – 1.02 | – 0.47 |
| det-1 | daf-16/FoxO controlled, germline tumor affecting 1 | Positive regulator, pro-apoptotic activity | – 1.50 | – 0.26 |
| daf-17 | daf-16/FoxO controlled, germline tumor affecting 17 | Positive regulator, germline tumor affecting | 1.01 | – 0.05 |
| pal-1 | Posterior alae in males 1 | Positive regulator, homeodomain protein of caudal ortholog | 1.05 | – 0.19 |
| eya-1 | EYA (drosophila eyes absent) homolog 1 | Negative regulator, protein tyrosine phosphatase | – 1.58 | – 0.74 |
| pdc-2 | PDCD (mammalian ProgrammeD Cell Death protein) homolog | Apoptosis protein | – 1.03 | – 0.51 |
| F43D9.3 | Unknown | Endocytosis | – 1.57 | – 0.86 |
| hsp-1 | Heat shock protein 1 | Hsp70A, member of the heat shock family of proteins, endocytosis | – 1.16 | 0.02 |
| hel-1 | Helicase 1 | Helicase | – 1.09 | – 0.20 |
| lgc-46 | Ligand-gated ion channel 46 | Ligand-gated ion channel | – 1.13 | 0.94 |
| ubq-1 | Ubiquitin 1 | Ubiquitin | – 1.73 | – 0.67 |
| mpz-4 | Multiple PDZ domain protein 4 | Inhibits CEP-1- and HUS-1-dependent germline apoptosis | 1.07 | 0.22 |
| bath-41 | BTB and MATH domain containing 41 | A protein with a mprin-associated Traf homology domain | 1.03 | 0.51 |
| knl-2 | Kinetochore null 2 | An ortholog of human MIS18 binding protein 1 | 1.27 | 0.48 |
| C35D10.13 | Unknown | Unknown | 1.05 | 0.36 |
| paf-11 | POF (pumilio/FBF) domain-containing 11 | RNA binding activity | 1.08 | 0.01 |
| rmd-1 | Regulator of microtubule dynamics 1 | Regulator of microtubule dynamics | 1.01 | 0.26 |
| T05C6.6 | Unknown | A protein with NACHT and WD domains | 1.76 | 1.37 |
| abl-1 | Related to oncogene ABL 1 | Checkpoint, inhibits germline apoptosis induced by radiation | 0.38 | 1.12 |
| Y32G9B.1 | Unknown | Unknown | 0.37 | – 1.12 |
| Other processes involved in DDR | | | |
| hda-4 | Histone deacetylase 4 | Histone deacetylase 4 | – 1.13 | – 0.86 |
| Y56A3A.33 | Unknown | Unknown | – 1.90 | – 0.56 |
| daf-16 | Abnormal dauer formation 16 | Solo fogo homologue, response to DNA damage stimulus | – 1.41 | – 0.50 |
| sir-2.2 | Yeast sir related 2.2 | NAD dependent protein deacetylase | – 1.31 | – 0.60 |
| air-2 | Aurora/Ipl1 related kinase 2 | Serine/threonine protein kinase, cytokinesis checkpoint | 1.19 | 0.28 |
| sep-1 | Separa homolog 1 | Separa, cytokinesis checkpoint | 1.13 | – 0.02 |
| trt-1 | Telomerase reverse transcriptase 1 | Telomerase reverse transcriptase | 1.18 | 0.77 |

GEX, gut on exterior; SUMO, small ubiquitin-like modifier; FoxO, the forkhead box O; PDZ, PSD-95/discs large/ZO-1; BTB, Broad complex, Tramtrack and Bric-a-Brac; MATH, meprin and Tram-C homology; Traf, Tumor necrosis factor receptor-associated factor; NAGT, NAFI (neuronal apoptosis inhibitor protein); C2TA (MHC class 2 transcription activator), HET-E (incompatibility locus protein from Podospora anserina) and TPI (telomerase-associated-associated factor); WD, tryptophan (W)-aspartic acid (D) acid; FBE, Fem-3 (Feminization of XX and XO animals) mRNA-Binding factor from C. elegans; NAD, Nicotinamide Adenine Dinucleotide; ABL, c-abl oncogene 1, receptor tyrosine kinase 1.
A draft of the text is as follows:

Pathways to repair DNA lesions under SF environment compared with SR environment. Microgravity also probably influenced the genomic stability by telomere maintenance because three genes were altered under SF environment.

telomere maintenance by GO identification. DNA (Boule et al. 2005, Eki et al. 2007). C11G6.2 is involved in the DNA damage-induced apoptosis (Lettre and Hengartner 2006). The cel-mir-81/82 and 124 and 795 were likely to regulate DDR process by targeting air-2, bath-41, pme-1 and daf-16, respectively (Table IV).

The cel-mir-81/82 family is partial homology to hsa-miR-143 (Lim et al. 2003). Hsa-miR-143, widely down regulating in many cancer cells, has been reported to protect cells from DNA damage-induced killing (Lin et al. 2011), and inhibit tumor cell growth of gastric cancer (Wu et al. 2013), breast carcinoma (Ng et al. 2014) and colorectal cancers (Pagliuca et al. 2013). Hence, down-regulated mir-81/82 in space-flown C. elegans implied the possible risk of tumors under space environment. mir-124 is a highly conserved miRNA proven to regulate DNA repair protein Ku70 in rats brain (Zhu et al. 2013), radiosensitize human glioma cells by targeting CDK4 (Deng et al. 2013), and inhibit Reactive oxygen species (ROS) formation and aging in C. elegans (Dallaire et al. 2012). Given that potential DDR from space radiations exposure results from direct DNA break or indirectly from the production of ROS (Cucinotta and Durante 2006), miR-124 was possible to regulate DDR by multiple pathways in response to spaceflight. mir-795 is speculated to promote apoptosis by decreasing daf-16 (Perrin et al. 2013), while little is known about mir-795. These results indicated that miRNA might be involved in the DDR process in space-flown C. elegans.

To disentangle the complex interplay of the parameters of spaceflight environment, a set of appropriate control experiments in space and on the ground are required. As reported widely in previous studies (Horneck et al. 2010, Takahashi et al. 2012), the use of an on-orbit 1 g centrifuge can provide an ideal method for ensuring that the experimental groups are exposed to the same overall space environmental factors with the exception of g-level. The approach permits a separate determination of the effects of radiation or microgravity, as well as interactions of both parameters of space (Horneck 1999). Given that the space radiations were always present during our practical spaceflight, we discussed the synergistic effects of microgravity by comparing the
difference between the responses to spaceflight with and without microgravity, and found that microgravity involved in several biological processes on gene expression and miRNA expression. Interestingly, most of genes involved in DDR processes significantly altered under SF environment but not SR environment, although the identical directions of alteration of genes were observed in both cases. These might result from a better resistance to ROS and genomic instability in dauer larvae (Burnell et al. 2005, Ruzanov et al. 2007). More possibly, the results indicated the limited impact of space radiations encountered during a 16.5-day spaceflight mission. Although several studies showed the effects of low-dose radiation (Hartman et al. 2001, Maalouf et al. 2011, Takahashi et al. 2012, Schenten et al. 2013), proper long duration space radiation (Hartman et al. 2001, Maalouf et al. 2011, Takahashi et al. 2012, Schenten et al. 2013), proper long duration space missions are still needed for studying the interaction of space radiation and microgravity.

In this study, gene expression profile and miRNA expression profile in dauer larvae of *Caenorhabditis elegans* during Shenzhou-8 spaceflight mission were analyzed. Results indicate that during the short-duration spaceflight, microgravity probably enhanced the biological response on transcription and post-transcriptional regulation, in particular on DDR process, and these findings suggest how safe or hazardous the radiation combined with microgravity exposure is for the astronauts, which may be helpful for space risk assessment.

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**Declaration of interest**

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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Supplementary material available online

Supplementary Figures 1, 2 and Tables I, II.