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Simulated Microgravity-Induced Epigenetic Changes in Human Lymphocytes

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ABSTRACT

Real space flight and modeled microgravity conditions result in changes in the expression of genes that control important cellular functions. However, the mechanisms for microgravity-induced gene expression changes are not clear. The epigenetic changes of DNA methylation and chromatin histones modifications are known to regulate gene expression. The objectives of this study were to investigate whether simulated microgravity alters (a) the DNA methylation and histone acetylation, and (b) the expression of *DNMT1*, *DNMT3a*, *DNMT3b*, and *HDAC1* genes that regulate epigenetic events. To achieve these objectives, human T-lymphocyte cells were grown in a rotary cell culture system (RCCS) that simulates microgravity, and in parallel under normal gravitational conditions as control. The microgravity-induced DNA methylation changes were detected by methylation sensitive-random amplified polymorphic DNA (MS-RAPD) analysis of genomic DNA. The gene expression was measured by Quantitative Real-time PCR. The expression of *DNMT1*, *DNMT3a*, and *DNMT3b* was found to be increased at 72 h, and decreased at 7 days in microgravity exposed cells. The MS-RAPD analysis revealed that simulated microgravity exposure results in DNA hypomethylation and mutational changes. Gene expression analysis revealed microgravity exposure time-dependent decreased expression of *HDAC1*. Decreased expression of *HDAC1* should result in increased level of acetylated histone H3, however a decreased level of acetylated H3 was observed in microgravity condition, indicating thereby that other HDACs may be involved in regulation of H3 deacetylation. The findings of this study suggest that epigenetic events could be one of the mechanistic bases for microgravity-induced gene expression changes and associated adverse health effects. J. Cell. Biochem. 111: 123–129, 2010.

KEY WORDS: DNA METHYLATION; SIMULATED MICROGRAVITY; EPIGENETICS; RAPD; DNMT1; HDAC1; HISTONE ACETYLATION

Several studies suggest that space flight conditions result in profound changes in various physiological systems causing adverse health effects including cardiovascular changes [Baevsky et al., 2007], bone loss [Vico et al., 2000], muscular atrophy [Hayes et al., 1992], and decline in cellular immune function [Leach et al., 1990]. The mechanism of these microgravity and space flight-associated physiological changes are not well understood.

Recent studies have shown that microgravity conditions affect the expression of genes including some of the genes associated with cellular functions. For example, altered expression of cell proliferation and growth factor cascades-associated genes was found in male rats exposed to spaceflight condition (NASA-STS-90 neurolab) for 17 days [Taylor et al., 2002]. A gene chip microarray analysis revealed microgravity-induced changes in the expression of genes belonging to various functional categories including those directly related to immune response, cell proliferation and differentiation, protein folding, transport and degradation, as well as apoptosis [Ward et al., 2006]. Our recent study revealed that simulated microgravity results in decreased expression of genes for DNA repair and cell cycles [Kumari et al., 2009]. While the role of genetic events of DNA mutations in gene expression changes is well established, accumulating evidences suggest that epigenetic changes of DNA methylation and histone modifications play important role in regulation of gene expression [Kang et al., 2001; Roh et al., 2006].

In normal cells, DNA methylation occurs predominantly in repetitive genomic regions, and CpG islands of the promoters generally remain unmethylated [Robertson, 2005]. The maintenance of the DNA methylation patterns is essential for mammalian development and for the normal functioning of the adult organism.

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Change in DNA methylation pattern has also been reported to increase mutation rate and genomic instability [Robertson and Jones, 2000]. Three important mechanisms have been proposed for increased mutations and genomic instability as a result of DNA methylation changes. First of all, 5-methylcytosine (5meCs) serves as sites of transition mutations by hydrolytic deamination of 5meC to thymine, and therefore is considered as endogenous mutagen. For example, CpG sites in the p53 coding region are methylated in all human tissues studied [Denissenko et al., 1997] and contribute to approximately 50% of inactivating mutations of p53 in colon cancers [Greenblatt et al., 1994]. Similar characteristic point mutations were found in several other important genes including *Rb*, and *c-H-ras-1* [Ghazi et al., 1990]. Secondly, epigenetic inactivation of DNA repair genes by promoter hypermethylation may predispose to genetic instability. For instance, hypermethylation of mismatch repair (MMR) gene *hMLH1* precedes the microsatellite instability plus phenotype in sporadic colon, gastric, and endometrial cancers [Esteller et al., 1999; Fleisher et al., 2001]. 5-aza-2'-deoxycytidine treatment to colon cell lines containing a hypermethylated *hMLH1* gene resulted in re-expression of *hMLH1* and restoration of MMR ability indicating that hypermethylation of the *hMLH1* CpG island was the inactivating event [Herman et al., 1998]. Thirdly, genome-wide global hypomethylation of repeat sequence could result in illegitimate recombination events, transposition and can cause transcriptional deregulation of nearby genes and genomic instability [Schmutte and Fishel, 1999; Robertson, 2005]. These reports suggest that epigenetic events play important role in induction of genomic instability and gene expression changes. Therefore, the purpose of this study was to determine whether simulated microgravity condition leads to (a) epigenetic changes of DNA methylation and histone acetylation, and (b) changes in the expression of genes that controls epigenetic events of DNA methylation and histone modification.

MATERIALS AND METHODS

CHEMICALS AND REAGENTS

The Taq DNA polymerase (stoffel fragment) was purchased from Applied Biosystems (Foster City, CA). Oligonucleotide random 10-mer primers (OPA and OPC series) were purchased from Operon Technologies (Huntsville, AL). Restriction enzymes were purchased from Roche Applied Sciences, Indianapolis, IN. Trizol® reagent for total RNA isolation was purchased from Invitrogen, Inc. (Carlsbad, CA). One-step RT-PCR kit for real-time PCR was obtained from BioRad, Inc. (Hercules, CA). Antibodies for Western blot analysis were purchased either from Santa Cruz Biotechnology, Inc. (CA). or from Upstate Inc. (CA).

CELL CULTURE AND SIMULATION OF MICROGRAVITY

Human T-lymphocytes (Coriell Laboratories) cell cultures were maintained in DMEM F-12 medium supplemented with 10% fetal bovine serum. For ground-based simulation of microgravity, a Rotary Cell Culture System (RCCS) developed by NASA was used. Rotation in the RCCS mimics the microgravity environment by nullifying the gravitational vector. RCCS is a suspension culture vessel and is optimized to minimize mechanical stress on cells in culture [Villa et al., 2005]. The system is also optimized to provide oxygenation to cells by diffusion and excluding undissolved gases from the vessel [Hammond and Hammond, 2001]. To investigate the effect of simulated microgravity, actively growing lymphocyte cells were maintained in the RCCS for 72 h and 7 days. After seeding the cells in culture media, the RCCS vessel was completely filled with additional culture fluid, and then the air bubbles were removed from the culture vessel. The RCCS contains no impellers, airlifts, bubbles, or agitators, the cell damage or stress from impact and turbulence is essentially insignificant [Villa et al., 2005]. To maintain the cells in healthy and actively growing state during 7 days exposure of simulated microgravity, cells were collected at day 3, cell viability was confirmed by trypan blue staining method, and then reseeded in RCCS using fresh culture media at the same cell density that was used in the beginning. The cell viability was also confirmed after collecting the cells at day 7. The cell viability data confirmed no significant difference in the number of dead cells when a comparison was made between the bioreactor and ground condition [Kumari et al., 2009]. In parallel, cells at similar cell densities were maintained in culture flasks as controls. Both groups of cells were grown under sterile conditions in a cell culture incubator maintained at 5% CO_2 and 37°C.

DNA AND RNA EXTRACTION

For genomic DNA isolation, lymphocytes were collected by centrifugation and subjected to digestion with 1% SDS and 0.5 mg/ml proteinase K (Roche Applied Science, Indianapolis, IN) at 50°C for 3 h. DNA was subsequently isolated from the digested tissues by phenol-chloroform extraction and ethanol precipitation as described previously by us [Singh et al., 2004]. Similarly total RNA was isolated using TRIzol (Invitrogen, Inc.) following manufacturer's protocol. The concentrations of DNA and RNA were determined by spectrophotometer and quality was checked by ethidium-bromide staining after resolution on 1% agarose gels.

RAPD-PCR AMPLIFICATION PROTOCOL

RAPD-PCR was performed with genomic DNA by following our earlier published method [Singh and Roy, 2008]. For methylation analysis, an aliquot of 2 μg DNA was digested with methylation sensitive restriction enzyme *Msp*I, and methylation insensitive restriction enzyme *Hpa*II. After overnight incubation at 37°C for complete digestion, the restriction enzymes were heat-denatured by incubation of DNA digest at 70°C. Restriction enzyme digested as well as undigested genomic DNA was diluted to 20 ng/μl for RAPD analysis. The RAPD-PCR amplifications were performed in 25 μl of reaction mixture containing 2.5 μl of 10× enzyme assay buffer, 100 μM each of dATP, dCTP, dGTP, dTTP (Applied Biosystems), 100 nM of random primer (10-bp), 2.5 mM $MgCl_2$, 0.5 unit of AmpliTaq DNA polymerase (Applied Biosystems) and 75 ng of genomic DNA as template. The amplifications were performed in a DNA thermal cycler (Stratagene) programmed for 45 cycles as follows: 1st cycle (3.5 min at 92°C, 1 min at 34°C, 2 min at 72°C), next 44 cycles (1 min at 92°C, 1 min at 34°C, 2 min at 72°C) followed by a final extension cycle of 15 min at 72°C. The PCR products were resolved on 1.5% agarose gel and detected by ethidium bromide

TABLE I. A List of Primers and Their Sequence Used for RAPD and Gene Expression Analyses

Primer	Sequence (5′–3′)
For methylation analysis by RAPD-PCR	
OPC03	GGGGGTCTTT
OPC06	GAACGGACTC
OPC07	GTCCCGACGA
OPC11	AAAGCTGCGG
OPC15	GACGGATCAG
OPC16	CACACTCCAG
OPC17	TTCCCCCCAG
OPC19	GTTGCCAGCC
OPC20	ACTTCGCCAC
For gene expression analysis by real time-PCR	
GAPDH-F	GGTGGTCTCCTCTGACTTCAACA
GAPDH-R	GTTGCTGTAGCCAAATTCGTTGT
DNMT1-F	CCCATGCATAGGTTCACTTCCTTC
DNMT1-R	TGGCTTCGTCGTAACTCTACCT
DNMT3a-F	CCTGAAGCCTCAAGAGCAGT
DNMT3a-R	AGCCAAGTCCCTGACTCTCA
DNMT3b-F	CCCATTCGAGTCCTGTCATT
DNMT3b-R	GGTTCCAACAGCAATGGACT
HDAC1-F	TGGAAATCTATCGCCCTCAC
HDAC1-R	TCTCTGCATCTGCTTGCTGT

staining. The primers sequences used for RAPD-PCR are given in Table I.

QUANTITATIVE REAL-TIME PCR

Gene expression was measured by quantitative real-time PCR (qRT-PCR) method and by using the One-Step RT-PCR kit with SYBR green (BioRad). Single-step RT-PCR amplifications starting with total RNA (200 ng) were performed in 96-well optical reaction plates using an iCycler (Bio-Rad Laboratories, Hercules, CA) programmed for reverse transcription at 50°C for 15 min, denaturation and reverse transcriptase enzyme inactivation at 95°C for 5 min, followed by 40 cycles of 10 s denaturation at 95°C and 30 s annealing and extension at 60°C. The specificity of PCR products was verified by melting curve analysis between 55 and 95°C at 0.5°C temperature increments. Threshold cycle number (Ct value) was analyzed using iCycler IQ optical system software (Bio-Rad, version 3.0a). Quantitative RT-PCR results were normalized to the Ct value of *GAPDH* from the same sample and the fold- change in the expression of each gene was calculated by using the delta-delta Ct method [Livak and Schmittgen, 2001]. Amplification reactions for each sample were performed in triplicate and each experiment was repeated twice. A non-template control was included in each experiment. Primer sequences used in the quantitative PCR experiments to amplify each gene are given in Table I.

WESTERN BLOT ANALYSIS

Control and simulated microgravity grown cells were harvested and total cellular protein was extracted by lysis in extraction buffer {50 mM Tris–HCl (pH 8), 150 mM NaCl, 1% NP-40, 0.1% sodium dodecyl sulfate (SDS), 1 mM phenylmethylsulfonyl fluoride}. The samples were then centrifuged and the protein content of the supernatant was determined by Bradford Assay [Bradford, 1976]. Thirty micrograms of total cell lysate from each sample was electrophoretically separated on a 10% SDS–PAGE gel, followed by protein transfer onto nitrocellulose. The membranes were blocked with 5% non-fat dried milk in 1× PBS containing 0.05% Tween 20, overnight at 4°C. The blots were then incubated with a 1:100 dilution of primary antibody against HDAC1, acetylated histone H3 or GAPDH (Santa Cruz Biotechnology) for 1 h at room temperature. After three washes of 5 minutes each with PBST, the membranes were incubated in the appropriate horseradish peroxidase-conjugated secondary antibody (Sigma, St. Louis, MO) at a dilution of 1:1,000 for 1 h at room temperature. Membranes were again given 3 washes of 5 min each with PBST. The specific protein bands were then visualized by using an enhanced chemiluminescence system according to the manufacturer's instructions (Amersham, Piscataway, NJ).

RESULTS

DETECTION OF SIMULATED MICROGRAVITY-INDUCED GENOMIC INSTABILITY BY RAPD

In order to detect the genomic instability as a result of mutational changes in the genome, RAPD fingerprinting analysis was performed with genomic DNA isolated from lymphocytes grown in simulated microgravity condition and also from cells grown in normal gravitational condition in parallel as control. Interestingly, none of the 20 primers used in this study revealed any change in DNA fingerprints when RAPD analysis was performed with undigested genomic DNA. Representative RAPD fingerprints showing no change in DNA are given in Figure 1. However, some of these primers revealed changes in the DNA fingerprints of 7 days simulated microgravity grown lymphocytes, when RAPD-PCR was performed with restriction enzymes *Hpa*II digested DNA. For example, the comparison of DNA fingerprints of control to that of microgravity grown cells revealed loss of 1,300 bp fragment with primer OPC07 and 1,200 and 560 bp fragment with primer OPC20, indicating mutations in these genomic regions (Fig. 2). Similarly, the gains of 200 bp with primer OPC07; 1,150, 800, and 120 bp with primer OPC11; and 1,130, 680, 620, and 500 bp with primer OPC20 revealed presence of mutations in these genomic regions (Fig. 2).

DETECTION OF SIMULATED MICROGRAVITY-INDUCED METHYLATION CHANGES BY MS-RAPD

In contrast to the RAPD-PCR with undigested DNA, the methylation sensitive (MS)-RAPD-PCR with restriction enzyme digested DNA revealed several changes in the RAPD fingerprint pattern. Comparison of the DNA fingerprint of 7 days simulated microgravity grown cells with that of the cells grown in normal gravity (ground condition) for 7 days revealed the losses of 750 bp band with primer OPC15; 590 bp band with primer OPC16; 670, 560, and 450 bp bands with primer OPC17 (Fig. 3); and 640, 510 bp bands with primer OPC11 (Fig. 2) in *Msp*I digested DNA of control but not in simulated microgravity grown cells. However, these same DNA bands were present in control but absent in simulated microgravity grown cells DNA digested with *Hpa*II (Figs. 2 and 3). This indicates that the primer binding sites for these three primers in the genomic DNA was methylated and therefore got cut by *Msp*I, resulting in the loss of priming site and therefore the loss of PCR amplification of that genomic region. In *Hpa*II digested samples, the presence of this

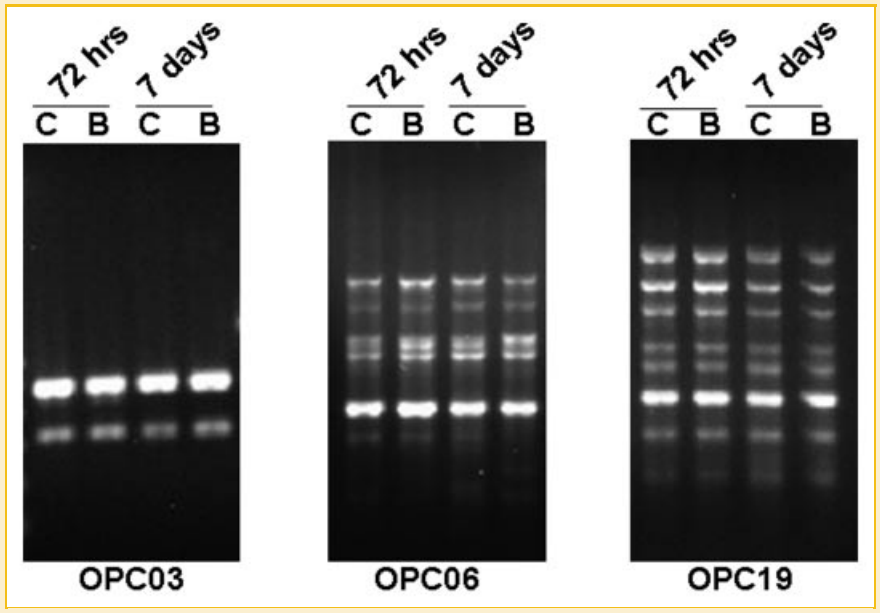

Fig. 1. Representative RAPD profiles showing similar DNA fingerprints between lymphocytes grown under ground condition (C) and simulated microgravity conditions (B). Lymphocytes were grown in rotary cell culture system to simulate microgravity conditions (B) as well as in ground condition in parallel as control (C) for 72 h and 7 days and the genomic DNA isolated from these cells was used to perform RAPD fingerprints as described in Materials and Methods Section.

band in control and its absence in simulated microgravity grown cells suggest that simulated microgravity exposure resulted in hypomethylation in the primer binding sequence, and therefore got cut by *Hpa*II causing the loss of priming site and hence no PCR amplification. The results of this methylation sensitive-RAPD-PCR analysis revealed the genomic targets for methylation changes, however, this method do not identify the specific methylated CpG sites. Nevertheless, this data provides the basis for further analysis by additional methods designed to detect site-specific methylation changes.

DETECTION OF GENE EXPRESSION CHANGES IN RESPONSE TO SIMULATED MICROGRAVITY BY REAL-TIME PCR AND WESTERN BLOT ANALYSIS

The expression of *DNMT1* was increased by 3.2 folds in the lymphocytes grown for 72 h in simulated microgravity condition,

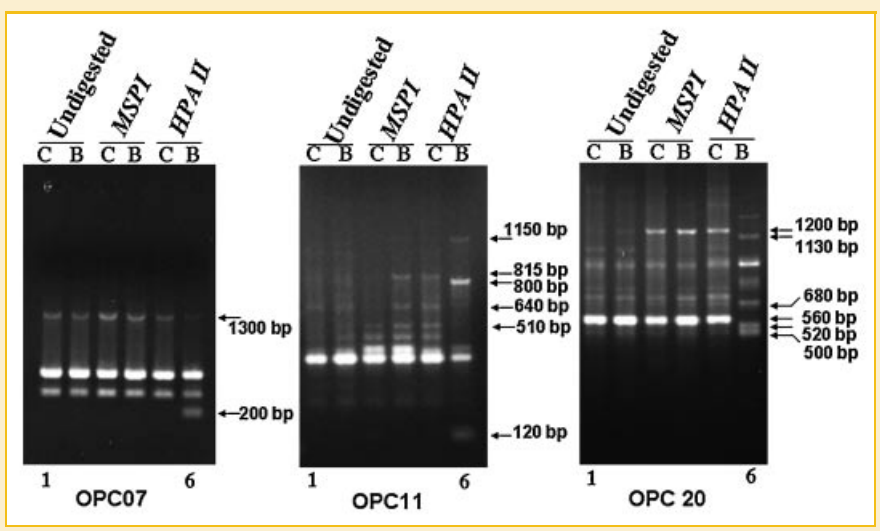

Fig. 2. Representative RAPD fingerprints showing simulated microgravity-induced genomic instability as revealed by loss/gain of amplification products. Genomic DNA isolated from 7 days simulated microgravity exposed cells was digested with restriction enzymes (*Msp*I and *Hpa*II) and then used for RAPD amplifications as described in Materials and Methods Section. The loss/gain of RAPD amplification products in the fingerprints from lymphocytes grown in simulated microgravity and ground conditions are indicated by arrows. The primers used are indicated at the bottom of each fingerprint.

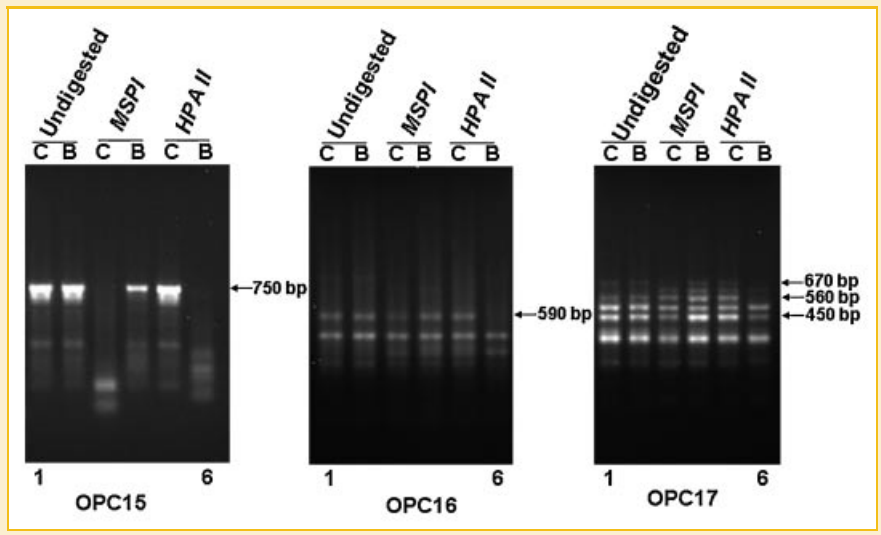

Fig. 3. Representative MS-RAPD fingerprints showing simulated microgravity-induced methylation changes as revealed by loss/gain of amplification products. Genomic DNA isolated from 7 days simulated microgravity exposed cells was digested with methylation sensitive restriction enzymes (*MspI* and *HpaII*) and then used for RAPD amplifications as described in Materials and Methods Section. The loss/gain of RAPD amplification products in the fingerprints from lymphocytes grown in simulated microgravity and ground conditions are indicated by arrows. The primers used are indicated at the bottom of each fingerprint.

whereas 6.4 folds decreased expression of *DNMT1* was observed when lymphocytes were exposed to simulated microgravity for 7 days (Fig. 4). The expression of *DNMT3a* and *DNMT3b* was also increased by 2.7 and 2.6 folds, respectively, in microgravity grown cells for 72 h. The simulated microgravity exposure of 7 days however resulted in decreased expression of *DNMT3a* and *DNMT3b* by 2.7 and 22.5 folds, respectively. Simulated microgravity exposure time-dependent decrease by 1.7-fold at 72 h and 2.6-fold at 7 days was observed in the expression of *HDAC1*. Western blot analysis further confirmed a time-dependent decrease in the expression of *HDAC1* and in level of acetylated histone H3 (Fig. 5). The decreased expression of *DNMT1* at 7 days correlates with the DNA hypomethylation as observed by methylation sensitive-RAPD analysis. The decreased level of acetylated histone H3 however does not correlate with the observed decreased expression of *HDAC1*.

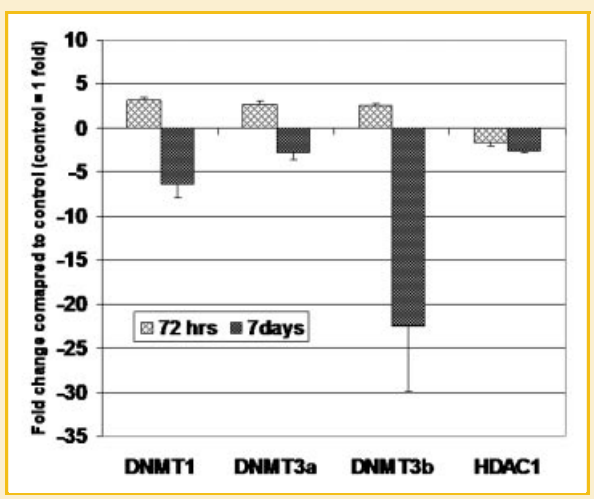

Fig. 4. Effect of simulated microgravity on the expression of *DNMT1*, *DNMT3a*, *DNMT3b*, and *HDAC1* as determined by quantitative real-time PCR (qRT-PCR). Lymphocytes were grown in a Rotary Cell Culture System for 72 h and 7 days, and the expression of *DNMT1*, *DNMT3a*, *DNMT3b*, and *HDAC1* at transcript level was measured by qRT-PCR as mentioned in Materials and Methods Section. Threshold cycle number (Ct value) for target genes obtained by qRT-PCR was normalized to the Ct value of *GAPDH* from same sample and the fold-change in expression for each gene was obtained by using the delta-delta Ct method. The graph shows the means of triplicate values.

DISCUSSION

The important finding of this study is that the ground-based simulated microgravity exposure can alter expression of genes that regulate the epigenetic events, and as a consequence it can induce changes in the chromatin compactness as well as the DNA

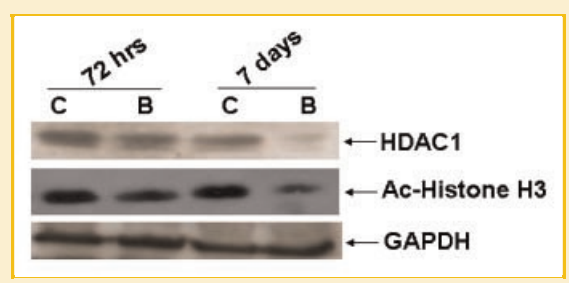

Fig. 5. Representative Western blots showing the effect of simulated microgravity on the expression of *HDAC1* and the level of acetylated form of histone H3 (Ac-histone H3). Lymphocytes were grown in a Rotary Cell Culture System for 72 h and 7 days, and the expression of *HDAC1* protein and the amount of acetylated histone H3 was measured in whole cell lysates by Western blot analysis (C: Control group, lymphocytes grown under normal gravity condition; B: Test group, lymphocytes grown under simulated microgravity condition).

methylation status in the genome. Increasing evidences suggest that in addition to the genetic changes of mutations, the epigenetic changes of DNA methylation and histone modifications play important role in regulation of gene expression [Kang et al., 2001; Roh et al., 2006]. While there are several reports on the microgravity condition-induced genetic changes at chromosome and DNA level, there are no reports on the epigenetic effects of microgravity. Therefore, to our knowledge this is the first report on epigenetic changes of histone modification and DNA methylation induced by simulated microgravity condition.

The gene expression data revealed co-downregulation of *DNMT1* and *HDAC1* in lymphocytes grown in simulated microgravity condition for 7 days. There are several reports that suggest that *DNMT1* and *HDAC1* interacts [Geiman et al., 2004], and cooperatively initiate and contribute in epigenetic gene silencing [Bird, 2001; Fuks et al., 2000; Fuks, 2005]. For example, both *DNMT1* and *HDAC1* are upregulated in prostate cancer [Patra et al., 2001]. Histone deacetylase inhibitors downregulate *DNMT1* expression [You et al., 2008] and promote ubiquitin-dependent proteosomal degradation of *DNMT1* in human breast cancer cells [Zhou et al., 2008]. The finding of this study on co-downregulation of *DNMT1* and *HDAC1* in simulated microgravity condition is similar to these previous reports and further strengthens the view that *DNMT1* and *HDAC1* co-operates in epigenetic gene regulation.

In this study, a methylation sensitive-RAPD-PCR method was used to detect simulated microgravity-induced methylation changes at genome-wide level. Several previously reported studies [Machover et al., 2002; Estecio et al., 2006; Liu et al., 2009; Zama and Uzumcu, 2009] including ours [Singh and DuMond, 2007] have used this experimental method of methylation-sensitive RAPD-PCR/AP-PCR to detect global DNA methylation changes. Difference in DNA fingerprint between ground and simulated microgravity conditions-grown cells only after digesting the DNA with methylation sensitive restriction enzymes *Msp*I and *Hpa*II but not from undigested DNA indicate that the observed differences in DNA fingerprints are due to simulated microgravity-induced methylation changes. The DNA methylation changes observed in this study do not identify the target gene (s) that may be affected at expression level. However, this study provides a strong basis for future study with additional methods such as, bisulfite sequencing to identify methylations in specific gene promoter sequence, or methylated DNA immunoprecipitation (MeDIP) coupled with methylation microarray that will allow determining the promoter and genic DNA methylation at genome-wide level in microgravity exposed cells. Additionally, this will help to obtain information on DNA methylation changes-associated regulation of genes and its impact on cell physiology under microgravity conditions.

The finding of this study on simulated microgravity-induced methylation changes indicates that epigenetic regulation may be a potential mechanistic basis for the changes in the expression of some of the genes associated with phathopysiological changes as a result of exposure to microgravity conditions. For example, muscular atrophy [Hayes et al., 1992] and physiological decreases in muscle loading by hindlimb suspension (animal model for simulated microgravity) or spaceflight do decreases type I fiber percentage in rodents [Caiozzo et al., 1997] and humans [Edgerton et al., 1995; Trappe et al., 2009]. Myosin heavy chain (MHC) is the most abundant protein in skeletal muscle. Recent study provides direct evidence for the regulation of MHC gene by histone modifications [Pandorf et al., 2009]. Recently, we have reported decreased expression of DNA repair genes in simulated microgravity conditions [Kumari et al., 2009]. Expression of some of the DNA repair genes, such as *MLH1* [Kane et al., 1997] and *OGG1* [Guan et al., 2008] are known to be regulated by promoter hypermethylation. In this study, an increased expression of de novo DNA methylating enzymes DNMT3a and DNMT3b was observed in 72 h simulated microgravity grown cells. Whether the increased expression of de novo methylating enzymes DNMT3a and DNMT3b is involved in downregulating the gene expression by promoter hypermethylation in simulated microgravity condition remains to be identified. However, it is possible that decreased expression of some of the genes in microgravity condition as reported previously by us [Kumari et al., 2009] and others [Ward et al., 2006], especially those genes with CpG islands in their promoter, could be due to increased expression of *DNMT3a* and *DNMT3b*-mediated promoter hypermethylation. The observed decrease in the level of acetylated histone H3 resulting in compact chromatin structure could be another factor in regulating the genes in microgravity condition. The decreased level of acetylated histone H3 despite the decrease in histone deacetylase *HDAC1* suggest that other HDACs including the class II HDACs (4, 5, 6, 7, 9, and 10) might be responsible for deacetylating the H3 in microgravity condition.

In summary, this study provides the first evidence for the epigenetic changes induced by simulated microgravity condition. The finding of this study also provides the basis for further study on the role of microgravity-induced epigenetic changes in the regulation of gene expression and associated adverse health effects on astronauts as well as its potential countermeasure strategies.

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