Gene Expression Profiling of Human Epidermal Keratinocytes in Simulated Microgravity and Recovery Cultures

Jade Q. Clement*, Shareen M. Lacy, and Bobby L. Wilson

Department of Chemistry and NASA University Research Center for Biotechnology and Environmental Health, Texas Southern University, Houston, TX 77004, USA.

Simulated microgravity (SMG) bioreactors and DNA microarray technology are powerful tools to identify "space genes" that play key roles in cellular response to microgravity. We applied these biotechnology tools to investigate SMG and post-SMG recovery effects on human epidermal keratinocytes by exposing cells to SMG for 3, 4, 9, and 10 d using the high aspect ratio vessel bioreactor followed by recovery culturing for 15, 50, and 60 d in normal gravity. As a result, we identified 162 differentially expressed genes, 32 of which were "center genes" that were most consistently affected in the time course experiments. Eleven of the center genes were from the integrated stress response pathways and were coordinately down-regulated. Another seven of the center genes, which are all metallothionein MT-I and MT-II isoforms, were coordinately up-regulated. In addition, HLA-G, a key gene in cellular immune response suppression, was found to be significantly up-regulated during the recovery phase. Overall, more than 80% of the differentially expressed genes from the shorter exposures ($\leq$4 d) recovered in 15 d; for longer ($\geq$9 d) exposures, more than 50 d were needed to recover to the impact level of shorter exposures. The data indicated that shorter SMG exposure duration would lead to quicker and more complete recovery from the microgravity effect.

Key words: HEK001, HARV, DNA microarray, Northern blotting, expression profiling, microgravity

Introduction

Exposure to microgravity has been recognized as a major environmental factor of spaceflight. Some of the adverse effects resulting from spaceflight are a decline in cellular immune response (*1–3*), cardiovascular deconditioning (*4*), bone deterioration (*5*), and muscular atrophy (*6*). Elucidation of the molecular mechanisms underlying microgravity-induced health problems is critical for formulating effective countermeasures for spaceflight side-effects. Due to the cost effectiveness and the ability to separate microgravity effect from other complex factors of spaceflight, ground-based simulated microgravity (SMG) research has become popular and is widely practiced in space life sciences research.

Ground-based SMG conditions for mammalian cell and microorganism cultures are created through the use of high aspect ratio vessel (HARV) bioreactors (*7, 8*), which simulate microgravity by maintaining the cells in continuous free fall in liquid medium and are most commonly used in the United States (*9–12*). The HARV bioreactor does not allow the cells to receive a gravitational load in any fixed direction. Its constant rotation does not eliminate gravity, but it does allow the g-vector to be time-averaged to near zero (*13*). Ground-based SMG experiments using bioreactors such as HARV have become increasingly recognized as an effective approach in simulating certain aspects of microgravity, as it readily permits the more detailed experimentation towards the understanding of microgravity effects on genes and cellular activities during microgravity exposure.

Understanding gene and cellular activity changes in microgravity is essential for tackling the problems caused by microgravity exposure and for developing potential countermeasures. Studies at the cellular and molecular levels have been reported from both spaceflight and ground-based microgravity simulators such as HARV (*10, 14–17*). Microgravity has been found to influence major cellular events such as cell

*Corresponding author.
E-mail: clement_jq@tsu.edu

proliferation, cell cycle, cell differentiation, and programmed cell death (*18–20*). Many cell types, ranging from bacteria to mammalian cells, are sensitive to the microgravity environment, suggesting that microgravity affects fundamental cellular activities. The study of microgravity effects on cellular activities can benefit enormously from genome-wide gene expression technologies.

Gene expression profiling based on DNA microarrays is a genome-wide gene expression analysis method for assessing cellular and molecular activity changes in response to a change in the growth environment. Environmental change as drastic as sudden gravity change is likely to alter the functions and transcriptional activities of groups of genes. A genome-wide display and comparison of gene expression profiles in cells that are exposed to microgravity and different recovery stages could provide great insight into the array of genes that are directly or indirectly involved in gravity response. This is because any change in the physiological activity of a cell is most likely the result of changes in the expression of certain genes. Thus, the genome-wide expression analysis technology can be a powerful tool to identify the "space genes" that play key roles in cellular response to a microgravity environment. In recent years, microarray technology has become increasingly popular in space biosciences research and most of the microgravity-induced cellular and molecular effects have been reported on cells of the immune system (*21, 22*), muscle (*23–26*), and bone (*12, 27–30*). However, it is not clear if the affected cellular functions would recover once the cells are returned to 1 *g* conventional growth conditions.

To further understand the cellular and molecular mechanisms by which spaceflight alters cellular activities, the effects of microgravity on various human cell lines should be studied to identify the genes whose functions are most consistently affected by microgravity. Epidermal keratinocytes, the major cell type in the outermost layer of the skin, play an essential role in the first-line defense against invading microorganisms and in innate immunity (*31, 32*). To date, the molecular effect of microgravity on keratinocytes is not known. Thus, the aim of this study was to display time course gene expression profiles and identify key gravity sensitive genes for human kerotinocytes in response to microgravity. We studied the microgravity effect on a basal-like type of immortalized human epidermal keratinocytes, HEK001, using the HARV bioreactor SMG system. We applied DNA microar-

ray analysis for genome-wide expression profiling of the cells exposed to HARV bioreactor SMG conditions for 3, 4, 9, and 10 d, followed by recovery of 15, 50, and 60 d at 1 *g*. Our results indicated that cellular gene activity achieved >80% recovery from the shorter exposure time ($\leq$4 d) in a recovery period of 15 d, while a longer recovery period ($\geq$50 d) was needed for genes to recover to the shorter exposed impact levels after an exposure time as long as 9 and 10 d. In addition, we have identified 32 putative "major space genes" that were most consistently affected by SMG through the interlinked time course analysis. Interestingly, a cluster of eleven genes that are inducible through the integrated stress response pathways were all down-regulated in the presence of SMG. In contrast, a cluster of seven metallothionein genes were up-regulated through all the time points. Moreover, HLA-G, a key gene that mediates cellular immune suppression effect, was up-regulated during the recovery phase of SMG exposure. Our findings contribute significantly to the knowledge of epidermal response to SMG, which may suggest a mechanism in the whole body response to microgravity, particularly in microgravity-associated immune response suppression, integrated stress response, and tumor progression.

Results

Morphology of HEK001 cells cultured in HARV bioreactors and in conventional 2D cell culture flasks

Monolayer keratinocytes, HEK001 cells, formed small aggregates and spherical cellular morphology (Figure 1C and E) typical for cells cultured in the 3D environment of HARV bioreactors. When the HEK001 cells were removed from the HARV bioreactors after the shorter (3 and 4 d) SMG exposures and then cultured in conventional 2D cell culture flasks for 15 d, the keratinocytes spread out to form monolayer growth indistinguishable from the untreated controls (compare Figure 1A and B with D and F). Cells from the longer (9 and 10 d) SMG exposures were not recovered as complete as the shorter ones, and elongated cells were found in the recovery culture after 16 d (Figure 1G and H). Figure 1G shows cells after 7-day recovery from a 9-day SMG treatment; note that cell aggregates were visible at the early recovery stage. Although these cells regained their morphological