GRAVITY-INDUCED CHANGES OF GENE EXPRESSION IN PC12 CELLS

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ABSTRACT
Microgravity in space may affect many physiological changes (e.g., reduction of bone mass, blood ejection rate, and muscle strength) in astronauts but the mechanisms are not yet understood. The objective of this study is to analyze the genes and gene ontology categories significantly changed in rat pheochromocytoma (PC12) cells cultured in the NASA-developed Rotating Wall Vessel (RWV) bioreactors using the cDNA microarray technique. PC12 cell line is one of the readily available cell lines with phenotypic plasticity, abundance, and ease of maintenance. This study observes that 173 genes in PC12 cells cultured for four days under the simulated microgravity condition show significant changes in gene expression compared with the unit-gravity control. Genes involved in the oxidoreductase activity category are most significantly expressed under microgravity conditions. This result may provide a resource for further research about mass transport under variable gravity conditions.

INTRODUCTION
Optimized suspension cell culture conditions for simulating microgravity of outer space on earth can be obtained from using RWV bioreactors [1]. The unique culture system has been used to understand and investigate basic cellular responses to microgravity [2,3]. Since changes of gene expression cause a different pattern of the physiology of cells and tissues, the understanding of the functions of genes is essential for exploring the biological roles of the organisms. Also, changes in the multi-gene patterns of expression can provide clues about regulatory mechanisms [4]. One of the most commonly used approaches is the cDNA microarray technique, which can measure expressions of level of thousands of genes. The microarray technique is a powerful method for comparing gene expressions in the same cell or tissue under different culture conditions [5].

MATERIALS AND METHODS
Cell culture
The PC12 cells were cultured in RPMI 1640 medium supplemented with 10%(v/v) fetal bovine serum, 10%(v/v) horse serum, and 1%(v/v) penicillin-streptomycin-glutamine (all reagents; Gibco). Cells were maintained in a humidified incubator with 5% CO2 and 95% air mixture at 37°C. For this experiment, Cytodex-3 microcarrier beads (Amersham Biosciences) were pretreated according to the manufacture's instructions to promote cell adhesion. Cell suspension (4×10^5 cells/mL) was first placed into 100mm Petri dishes by adding beads (5mg/mL). Figure 1 shows how the RWV bioreactors are oriented to grow PC12 cells under conditions of simulated microgravity (micro-g) and normal gravity by simply changing the position of the bioreactor (unit-g condition). The RWVs were rotated at an initial speed of 15 rpm.

Figure 1. Experimental orientations of the RWV: (A) modeled micro-g and (B) unit-g condition.
Microarray

Total RNA was isolated from PC12 cells released from microcarrier beads using RNeasy kit (Qiagen) according to the manufacturer's instructions. Then it was labeled as follows: MI and M4 for the samples cultured under the simulated micro-g condition on day 1 and day 4, and C1 and C4 for unit-g on day 1 and day 4, respectively (Fig. 1). The rat 70-mer oligonucleotide library version 3.0 (27,342 optimized oligos) was printed using a high-speed robotic Omniprint machine (GeneMachines). The cDNA was labeled with monofunctional reactive Cyanine-3 and Cyanine-5 dyes (Cy3 and Cy5; Amersham Biosciences). Imaging and data generation were carried out using a GenePix 4000A and GenePix 4000B (Axon Instruments). Data normalization and statistical analyses were performed using SAS statistical software package (SAS Institute Inc). Hierarchical clustering was performed using the average linkage clustering method.

RESULTS

Of ~10,000 genes analyzed, 173 genes in PC12 cells cultured for four days under the simulated microgravity condition showed significant changes in gene expressions compared with the unit-gravity control. Of these 173 genes, 104 genes revealed at least two-fold-change. 65 genes were up-regulated and 39 genes down-regulated. Hierarchical clustering is a method used to group genes according to their expression profiles. In Fig. 2, red indicates gene up-regulation between the 1st and the 2nd culture conditions of each column, while green indicates down-regulation, and black means no change.

![Figure 2. Parts of Cluster diagram with gene Ensembl ID and names (column 1: M4/C4, 2: M1/C1, 3: C4/C1, 4: M4/M1).](image)

Then genes were compared with functional assignment lists to determine which gene categories were significantly enriched with differentially expressed genes. This analysis was performed using Expression Analysis Systematic Explorer (EASE, http://apps1.niaid.nih.gov/david) in order to explore the biology of any given group of genes [6]. EASE score was calculated for each gene category in the significant list, and the gene categories were ranked by significance. In the functional analysis result, genes, involved in oxidoreductase activity category, which functions as catalysis of an oxidation-reduction reaction, showed significant difference in gene expression (Table 1).

DISCUSSION

These results have demonstrated that simulated microgravity conditions altered the expressions of several genes. The contribution of microarray technique to understanding and investigating the gene expression under different culture conditions was also demonstrated. It was found that oxidoreductase activity category was most significantly changed under the microgravity condition. We will conduct additional experiments and analyses with long-term series data and confirmation of the cDNA microarray results by Northern blot and/or RT-PCR. The present report will provide the foundation for the further research on understanding the cellular response and the mass transport under different gravity conditions and culture environments.

<table>
<thead>
<tr>
<th>Gene Category</th>
<th>List Hits</th>
<th>List Total</th>
<th>Genbank Accession</th>
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<tr>
<td>Oxidoreductase activity</td>
<td>11</td>
<td>54</td>
<td>AF106860; D37920; J03481; J05031; L35317; M11670; M21048; M36410; M86870; X01964; X07467</td>
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<td>Glucose metabolism</td>
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<td>50</td>
<td>AF106860; L22294; L36250; X01964; X07467</td>
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<td>Response to stress</td>
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<td>50</td>
<td>AF290895; A1122691; J02962; L35317; M11670; M60921; U73030; Y00047</td>
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<td>Isomerase activity</td>
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<td>54</td>
<td>DI4046; L36250; M21018; M86870</td>
</tr>
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</table>

Table 1. Functional analysis of most significantly changed gene categories [EASE results]

ACKNOWLEDGEMENTS

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REFERENCES