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Gene expression profile of *Xenopus* A6 cells cultured under random positioning machine shows downregulation of ion transporter genes and inhibition of dome formation

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Abstract

Random positioning machine (RPM) devices that generate a simulated microgravity environment of approximately 0*g* prevent the formation of dome structures in *Xenopus* kidney-derived A6 cells. In the present study, the gene expression profile of A6 cells cultured under RPM was determined using the *Xenopus* 22K scale microarray, and those genes up- or downregulated twofold or more were investigated. We identified 29 genes (up, 25 genes; down, 4 genes) on day 5, 68 genes (up, 25 genes; down, 43 genes) on day 8, 111 genes (up, 69 genes; down, 42 genes) on day 10, and 283 genes (up, 153 genes; down, 130 genes) on day 15 of culture under RPM. These genes were classified according to categories described in the KOG database, such as "extracellular structure", "cytoskeleton", and "transcription". Almost all the genes involved in "inorganic ion transport and metabolism" were downregulated under RPM. Our study further investigated some of these including the epithelial Na^+ channel (ENaC) and Na^+/K^+-ATPase transporter genes. A specific inhibitor of Na^+/K^+-ATPases, ouabain, inhibited dome formation in the A6 cells, even under control culturing conditions of 1*g* (the static condition). Together these data suggested that downregulation of sodium ion transporter gene expression plays a significant role in the RPM-dependent prevention of the dome formation in kidney epithelial cells.

Keywords: A6 cell; *Xenopus laevis*; Dome formation; Random positioning machine; Simulated microgravity

1. Introduction

The A6 cell line was established by Rafferty in 1969 from adult kidney epithelial cells of *Xenopus laevis*. A6 cells spontaneously form 'domes', which are fluid-filled, blister-like structures characteristic of morphological and functional changes taking place in the A6 cells (Moberly and Fanestil, 1988). These dome structures are probably formed from fluid accumulation between the cell monolayer and the culture-dish surface as the cells reach confluency.

A6 cells exhibit apical–basolateral polarity, with the apical membrane exposed to the culture medium and the basolateral membrane attached to the culture dish. This polarized cell axis is maintained during cell culturing (Ichigi and Asashima, 2001).

The apical membrane of the renal epithelial cell contains the epithelial Na^+ channel (ENaC), while Na^+/K^+-ATPase in the basolateral membrane (Feraille and Doucet, 2001; Kellenberger and Schild, 2002). ENaC is composed of α, β, and γ subunits, and its functional channel consists of a tetrameric assembly of α–β–α–γ (Kellenberger and Schild,

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2002). The Na^+/K^+-ATPase is composed of two main subunits, α and β, that associate with each other in a 1:1 molar ratio (Feraille and Doucet, 2001). ENaC and Na^+/K^+-ATPase are also found in the apical and basolateral membranes, respectively, of distal nephron epithelia in vivo (Verrey et al., 2003), with the same localization as in A6 cells (Blazer-Yost et al., 2001; Verrey et al., 1989). Based on these findings it was proposed that sodium ions in the A6 cell culture medium are incorporated into the cells by ENaCs in the apical membrane, and pumped out into the extracellular space by Na^+/K^+-ATPases in the basolateral membrane. The ions are thereby accumulated in the space between the A6 cells and the plastic surface, resulting in the osmotic flow of water into this space and causing the typical dome structures of A6 cells.

A6 cells can be easily cultured at room temperature without additional CO_2 using Leibovitz's L-15 medium, with a doubling time at 25 °C of approximately 36 h. These cells are therefore suitable models for microgravitational experiments in a simulated microgravity environment or on a spaceflight.

The three-dimensional (3D) clinostat is a valuable device for simulating a microgravity *g*-force close to zero. We previously demonstrated a long-term culturing system using the 3D clinostat to compare the growth of A6 cells cultured under RPM with cells grown under static conditions at 1*g*. RPM inhibited dome formation (Ichigi and Asashima, 2001), and increased the expression of N-myc downstream-regulated gene 1 (NDRG1) in A6 cells (Kyuno et al., 2003a). In addition, we analyzed changes in the expression of 52 genes responding to the RPM-simulated microgravity using a *Xenopus* 8K microarray, and monitored morphological changes including the prevention of dome formation (Kitamoto et al., 2005).

In the present study, we used a 22K-scale microarray to establish a more detailed gene expression profile of A6 cells cultured under RPM, 5–15 days under the RPM. In addition, we investigated the effect of ouabain, a specific inhibitor of sodium ion transport, on dome formation in the cell monolayer. Our results attributed the observed inhibition of dome formation in the A6 cells to downregulation of ENaC and Na^+/K^+-ATPase gene expression under RPM.

2. Materials and methods

2.1. Cell culture and RPM by 3D-clinostat

As described in previous studies (Ichigi and Asashima, 2001; Kitamoto et al., 2005), A6 cells were cultured in 50% Leibovitz's L-15 medium containing 10% fetal bovine serum (FBS) and 0.2% antibiotic–antimyotic mixture without CO_2 supplement. The cells (0.6×10^6 per 25 cm^2) were seeded into a culture flask, incubated at 24 °C, and fed twice weekly with fresh culture medium.

For experiments, cells were seeded and cultured overnight, and then the flask filled with medium to remove all air bubbles, and set in a 3D-clinostat (MHI Co., Kobe, Japan). The 3D-clinostat is presumed to generate a microgravity environment of ca. 0*g* by choosing a random revolution velocity at a certain time interval with the inner frame rotated at 1.579–1.875 rpm and outer frame rotated at 1.5 rpm; this is called RPM. For controls, cells were cultured under static conditions at 1*g* using the same filled medium as in the simulated microgravity environment, and the flask was not placed in the 3D-clinostat. Cells were cultured for 5, 8, 10, and 15 days under the RPM (Fig. 1a). Experiments were performed in duplicate (5 and 8 days) or triplicate (10 and 15 days).

2.2. Analysis by 22K-scale microarray

The 22K-scale microarray constructed from *X. laevis* was customized by Agilent Technologies (Palo Alto, CA) to contain 21,495 genes. Sequence information for the probes used in the microarray analysis was obtained from *X. laevis* UniGene, June 10, 2004 (UniGene Build #55).

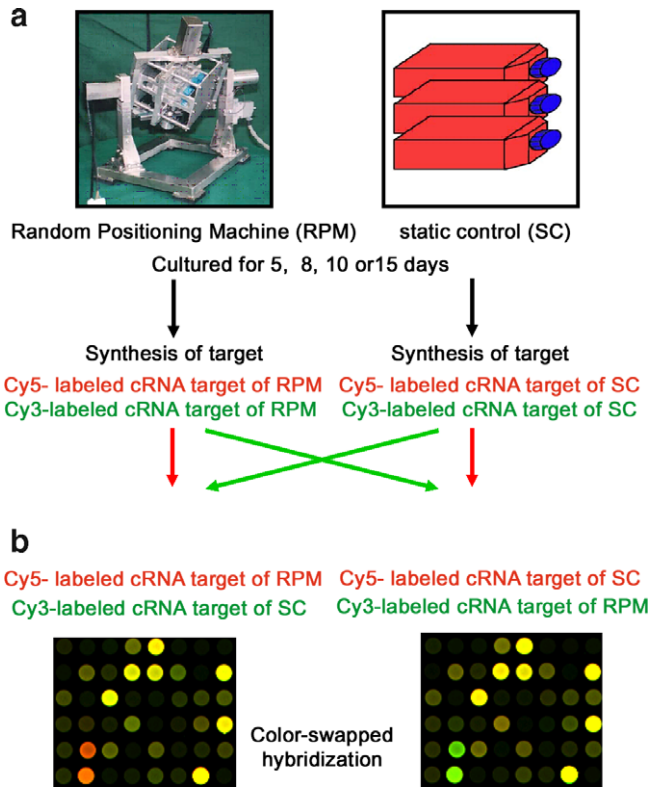

Fig. 1. Experimental procedures by microarray analysis. (a) Microarray target preparation: Cy5-labeled cRNA array target and Cy3-labeled cRNA array target was synthesized with total RNA extracted from A6 cells cultured under RPM or static conditions (SC), respectively. (b) Microarray experiment: Cy5-labeled cRNA targets for the RPM cells and Cy3-labeled targets for the SC cells were hybridized on the same microgravity slide. Therefore, the red fluorescent signals from the Cy5 represent RPM-induced gene expression, while the green signals from the Cy3 represent control gene expression. The yellow color represents an equal level of gene expression under both conditions. The color-swapped hybridization was performed using Cy3-labeled cRNA for RPM and Cy5-labeled cRNA for SC. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this paper.)