ANTIGENIC PROTEIN IN MICROGRAVITY-GROWN HUMAN MIXED MÜLLERIAN OVARIAN TUMOR (LN1) CELLS PRESERVED IN RNA STABILIZING AGENT


1Enterprise Advisory Services, Inc., Houston, TX; 2National Space Biomedical Research Institute; 3Baylor College of Medicine, Houston, TX; 4Wyle Life Sciences, Houston, TX; 5Biological Systems Office, NASA, Johnson Space Center, Houston, TX.

Cellular biotechnology experimentation on the International Space Station (ISS) sometimes requires that the collection of cells be preserved for long periods of time under adverse conditions. During Expedition 3, four different cell lines were grown using the Cellular Biotechnology Operations Support System (CBOSS), and were then preserved in either formalin or RNAlater™ (Ambion) at refrigerator temperatures. RNAlater™ is marketed to preserve RNA in the refrigerator for 30 days. Cells treated with RNAlater™ have previously been shown to contain antigenic proteins that can be visualized using Western blot analysis. These proteins appear to be stable for several months when stored in this RNA stabilizer at 4ºC. Antigenic protein can also be recovered from cells that have been processed using an RNAqueous® kit (Ambion) to remove RNA (1). Prior work demonstrated that mixed Müllerian ovarian tumor cells (LN1) (2, 3) were capable of being grown in rotating cell cultures that are analogs for microgravity (4). These cells grew on the ISS in the static bioreactor, Biological Specimen Temperature Controller (BSTC), and produced cytokines, although in reduced amounts compared with the ground controls (5).

In this set of experiments, LN1 cells grown on the ISS during Expedition 3 were examined for antigenic stability after removal of RNA. The cells were grown in 15 mL of media on Cytodex™ 3 beads (Amersham) in Teflon bags, stored for three months in 9mL of RNAlater™ in the refrigerator, and RNA was extracted using an RNAqueous® kit. The RNA filtrate containing the protein was precipitated with a final volume of 5% trichloroacetic acid (TCA), washed in TCA, and suspended in buffer containing sodium dodecyl sulfate (SDS). Samples containing equal concentrations of protein as determined by the Bicinchoninic acid (BCA) method (6) were loaded onto SDS-polyacrylamide gels (7). After electrophoresis, gels stained with SYPRO Orange (Molecular Probes) were scanned for equal loading of protein. Further, gels were transferred by Western blot procedures (8, 9) to polyvinylidene fluoride membranes. Primary antibodies were as noted below. A horse-radish peroxidase secondary antibody was used and the Western blots were stained with an enhanced chemiluminescent ECL® Plus detection kit (Amersham) and scanned using a Storm™ 840 gel image analyzer (Amersham). ImageQuant™ TL software (Amersham) was used to quantify the densities of the protein bands.

The ground cell samples from day 3 (G3), day 9 (G9) and day 14 (G14) and flight cell samples from the same days (F3, F9 and F14) showed similar staining patterns over time with mouse antibodies to vimentin and epithelial membrane antigens, and rabbit antibody to glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Triplicate blots were made for each antibody. The graphs (Figures 1-3) show the average of the scanned pixel volumes for the stained protein band in each lane. A control using untreated cells was run on each blot and the pixel volumes normalized to the control value. Due to the normal variability of blots, the numbers were calculated as a percent of the control. Blots are shown below the corresponding bar on each graph.

Figure 1. Average of three blots using mouse anti-vimentin antibody (Oncogene) with 5µg protein/lane compared to control (C) untreated cells (100%). Samples corrected for protein loading using SYPRO Orange stained gel.

Figure 2. Average of three blots using mouse antibody to epithelial membrane antigen, EMA (DAKO) with 5µg protein/lane. The highest molecular weight bands are the EMA according to the manufacturer’s instructions.
These data demonstrate the presence of antigenic protein in the RNA-stabilized LN1 cells, even after long periods of time in refrigerator storage. The antigenic protein is recoverable even after the RNA has been removed from the cells by the filtration method. All three proteins examined here had similar profiles at different times in the flight and ground samples. Previously, proteins from human renal cortical epithelial (HRCE) cells had been shown to exhibit similar characteristics (1). This work further demonstrates that the technique can be generalized to other cell lines and might be a good way to preserve proteins for long term storage. Since this preservative is even more effective in protecting RNA when stored frozen, it seems likely that protein protection would be similarly increased in freezing conditions. Further work is needed to determine if freezing also protects the proteins for longer periods of time. In addition, a more comprehensive study should be undertaken to determine if this holds true over a large range of protein varieties and sizes.

(Grant support to J. Becker: NAG9-1341)

REFERENCES


Bicinchoninic acid (BC) protein assay kit procedure #TPRO-562, Sigma7. Laemmlii, UK