Characterization of Mouse 3T3-Swiss Albino Cells Available in Japan:
Necessity of Quality Control When Used as Feeders

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SUMMARY: Mouse 3T3-Swiss albino cells are widely used as feeder cells to culture the human epidermis for the treatment of burns. To minimize the risk of xenoinfection, quality control of the feeder cells is required in the Japanese guidelines on regenerative medicine using feeder cells. We characterized three lots of 3T3-Swiss albino cells that are publicly or commercially available in Japan. One lot, which propagated more rapidly than the other two without showing typical contact inhibition, was found to release endogenous murine leukemia virus upon iododeoxyuridine treatment. Southern blotting of restriction fragments showed that the rapidly growing lot consisted of genetically altered cells that had probably emerged during the passages. The data support the guidelines that require quality control of the feeder cells.

INTRODUCTION

Mouse 3T3-Swiss albino cells, particularly the J2 clone, are widely used as feeder cells for culturing the human epidermis, which is clinically used for the treatment of burns (1-3). Since the epidermis is produced in direct contact with the feeder layer, it usually contains a small number of the feeder cells. Thus, it is important to perform quality control of the feeder cells to minimize the risk of xenoinfection.

The proper method of ensuring quality control in Japan is described in the “Public Health Guidelines on Infectious Disease Issues in Skin-graft by Co-cultivation of Human Keratinocytes with Mouse Feeder Cells” (http://www.mhlw.go.jp/general/seido/kousei/i-kenkyu/isyoku2/sisin.html [in Japanese]). The feeder cells should be negative for abnormal growth and for the induction of endogenous murine leukemia virus (MLV), which is known to be present in the cellular genome (4). An efficient test for the MLV induction is to treat cells with iododeoxyuridine (IdU) (5,6).

3T3 cells were originally established from Swiss albino mouse embryos by Todaro and Green in the 1960s, and now they are being circulated throughout the world through several channels (7). Each lot of the cells has been repeatedly passed and could be genetically altered. Furthermore, the situation is complicated by the existence of other cells with similar names containing 3T3, which is an abbreviation for the passage scheme of seeding \(3 \times 10^5\) cells on a 50-mm dish every 3 days. Other mouse fibroblast cell lines have been established through the same “3T3” passage scheme, namely NIH/3T3 cells (8) and BALB/3T3 cells (9). These cells must be recognized accurately.

It is important to determine the quality of the 3T3-Swiss albino cells available in Japan at present, because the cells are candidates to be used as feeders for culturing the clinical human epidermis. In this study we obtained 3T3-Swiss albino cells from one commercial and two public sources and examined them for their growth characteristics and their capacity to produce endogenous MLV. In addition, we confirmed that 3T3-Swiss albino cells were distinguishable from NIH/3T3 cells or BALB/3T3 cells by Southern blotting of restriction fragments.

MATERIALS AND METHODS

Cells: The frozen stocks of 3T3-Swiss albino (Cat.CCL-92), NIH/3T3 (Cat.CRL-1658), and BALB/3T3 clone A31 (Cat.CCL-163) were obtained from the American Type Culture Collection (ATCC) (Manassas, Va., USA). The frozen stocks of 3T3-Swiss albino (Cat.CJRB9019), NIH/3T3 clone 5611 (Cat.CJRB0615), and BALB/3T3 clone A31 (Cat.CJRB9005) were obtained from the Japanese Cell Research Bank (JCRB) through the Health Science Research Resources Bank (Osaka, Japan). The live cultures of 3T3-Swiss albino (Cat.08-092) and NIH/3T3 (Cat.09-1658) were purchased from the Dainippon Sumitomo Pharma Co., Ltd. (Osaka, Japan). The frozen stocks of NIH3T3-3-4 (Cat.RCB1862) and BALB/3T3 clone A31 (Cat.RCB0005) were obtained from the RIKEN Bioresource Center (RIKEN BRC) (Ibaraki, Japan). All of the cells were cultured in Dulbecco’s Modified Eagle Medium containing antibiotics and 10% fetal bovine serum (growth medium) at 37°C under 5% CO\(_2\) in a humidified incubator. For all the experiments, cells were used within 5 passages after reception, and, when necessary, one cycle of cryopreservation was included.

Growth assay: Cells (1.5 to 2 \(10^5\)) were seeded on a 35-mm dish (BD, Franklin Lakes, N.J., USA), and 48 h later they were trypsinized and counted with a Z2 Coulter counter (Beckman Coulter, Inc., Fullerton, Calif., USA). Similarly, cells (1 \(10^5\)) were seeded on a 60-mm dish (BD) and counted 72 h later.

Treatment of cells with IdU: Cells (1.5 \(10^5\)) in 1.5 ml
growth medium were seeded on a 35-mm dish. The next day, IdU (30 μg/ml) was added to the medium. Twenty-four h later the medium containing IdU was removed and the cells were washed twice with the growth medium. The cells were cultured in the growth medium, which was harvested daily and replaced with fresh medium. The harvested medium was filtered through a 0.45-μm PVDF filter (Milex HV, Millipore, Billerica, Mass., USA) and kept frozen at –80°C until a reverse transcriptase (RT) assay was performed.

RT assay: RT activity was measured by using the HS-Mn2+-RT kit (Cavidi Tech AS, Uppsala, Sweden) according to the manufacturer’s protocol, which contained the following steps: elongation from the immobilized primer and incorporation of bromodeoxyuridine (BrdU) by RT, immunodetection of BrdU, and colorimetric assay. The RT standard provided by the kit was used to quantitate the RT activity of the samples.

Southern blotting: Cellular DNA was purified by using QIAGEN genomic tips (QIAGEN GmbH, Hilden, Germany). After digestion with an appropriate restriction enzyme, DNA was purified by phenol/chloroform extraction, chloroform extraction, and ethanol precipitation. Ten microgram of each sample was electrophoresed on a 1% agarose gel and transferred to a nylon membrane (Hybond XL; GE Healthcare UK Ltd., Little Chalfont, UK). Radiolabeled probes were prepared by using Rediprime II (GE Healthcare UK). The full-length cDNA of MLV (a kind gift from Dr. Yoshihiro Kitamura) and mouse minisatellite Pc-1 DNA (a kind gift from Dr. Ryo Kominami) (10) were excised from plasmid Kitamura) and mouse minisatellite Pc-1 DNA (a kind gift from Dr. Yoshihiro Kitamura) and mouse minisatellite Pc-1 DNA (a kind gift from Dr. Ryo Kominami) and mouse minisatellite Pc-1 DNA (a kind gift from Dr. Yoshihiro Kitamura). Hybridization was carried out at 74°C for 1 h in ExpressHyb (Clontech Laboratories, Inc., Mountain View, Calif., USA). After hybridization, membranes were washed in 2× SSC (1× SSC contains 15 mM sodium citrate and 150 mM sodium chloride) containing 0.1% sodium dodecyl sulfate (SDS) at 70°C for 1 h with three changes of the buffer and then in 0.1× SSC containing 0.1% SDS at 70°C for 1 h with one change of the buffer. Phosphoimages were obtained using Storm860 (GE Healthcare UK).

RESULTS

Morphology and growth of 3T3-Swiss albino cells: During the initial propagation of the cells, we noticed that the purchased 3T3-Swiss albino cells (designated 3T3-P) were morphologically different from the other two (designated 3T3-A and 3T3-J) (Fig. 1); the 3T3-P cells were round with irregular projections. The 3T3-P cells grew much faster than the 3T3-A or 3T3-J cells (Table 1). 3T3-P, unlike 3T3-A and 3T3-J, had lost its contact inhibition and grew to a higher density (data not shown).

Induction of MLV from 3T3-Swiss albino cells: Endogenous MLV was induced from 3T3-P by stimulation with IdU. The 3T3-Swiss albino cells were cultured in the growth medium containing IdU for 24 h. The culture medium was harvested and replaced with fresh medium every 24 h, and the RT activity of the harvested medium was measured by a colorimetric assay (Fig. 2). RT activity was detected only in the IdU-treated 3T3-P culture medium. The peak of RT activity appeared at 1 to 2 days after the IdU-treatment and reached 3 to 4 mU/ml.

Southern blotting of DNA extracted from 3T3-Swiss albino cells, NIH/3T3 cells, and BALB/3T3 cells: 3T3-Swiss albino cells, NIH/3T3 cells, and BALB/3T3 cells showed their distinctive Southern blotting patterns (Fig. 3). The patterns obtained from EcoRI-digested DNA samples probed with MLV DNA (Fig. 3A) or Pc-1 DNA (Fig. 3B) were similar in NIH/3T3 cells and BALB/3T3 cells, but not in 3T3-P cells. However, those obtained from HaeIII-digested (Fig. 3C), Hinfi-digested (Fig. 3D), and MboI-digested (Fig. 3E) DNA samples probed with Pc-1 DNA were unique to each cell line.

Among 3T3-Swiss albino cells, 3T3-A and 3T3-J showed a similar pattern, but there were extra hybridization signals specific to 3T3-P alone (indicated with arrowheads in Fig. 3, B to E). 3T3-P is not a cloned cell line, but the majority of the cells are likely to have the same genotype because the 3T3-P-specific signal was as intense as other signals common to all 3T3-Swiss albino cells. It is very likely that a single cell with the genetic alteration had emerged and become dominant over the others during the passages. The genetic alteration is probably associated with the capacity to produce endogenous MLV.

Table 1. Growth of 3T3-Swiss albino cells

<table>
<thead>
<tr>
<th>Lot of cells</th>
<th>Fold-increase of cell number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5 x 10⁵ cells</td>
<td>Cell growth at 48 h in a 35-mm dish seeded at 1.5 x 10⁵ cells</td>
</tr>
<tr>
<td>3T3-P</td>
<td>6.7 ± 0.2*</td>
</tr>
<tr>
<td>3T3-A</td>
<td>2.4 ± 0.1</td>
</tr>
<tr>
<td>3T3-J</td>
<td>3.7 ± 0.2</td>
</tr>
<tr>
<td>1 x 10⁶ cells</td>
<td>Cell growth at 72 h in a 60-mm dish seeded at 1 x 10⁶ cells</td>
</tr>
<tr>
<td>3T3-P</td>
<td>7.2 ± 0.6*</td>
</tr>
<tr>
<td>3T3-A</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>3T3-J</td>
<td>3.0 ± 0.6</td>
</tr>
</tbody>
</table>

*: P < 0.01. Average of four measurements with standard deviation is shown.

Fig. 1. Phase-contrast micrographs of 3T3-Swiss albino cells used in this study. (A) 3T3-Swiss albino cells obtained from ATCC (designated 3T3-A). (B) 3T3-Swiss albino cells obtained from JCRB (3T3-J). (C) 3T3-Swiss albino cells purchased from Dainippon Sumitomo Pharma Co., Ltd. (3T3-P). The micrographs were taken with a BZ-8000 microscope (Keyence, Osaka, Japan). Scale bar represents 50 μm.
MLV after the IdU-treatment (Fig. 2).

All NIH/3T3 cells showed the same pattern, and one lot of BALB/3T3 cells differed from the others as in the case of 3T3-Swiss albino cells.

**DISCUSSION**

In this study, we demonstrated that among three publicly or commercially available lots of 3T3-Swiss albino cells, the 3T3-P cells were phenotypically and genetically different from the other two. The data indicate that the 3T3-Swiss albino cells have the potential to be transformed by unintentional mutations that occur during the passages and that some mutations are associated with the easy induction of endogenous MLV. Therefore, the quality control of each lot of the feeder 3T3-Swiss albino cells should be conducted according to the guidelines.

It is likely that performing standardized passages of the 3T3-Swiss albino cells is important for maintaining the original cellular characteristics. Todaro and Green cultured several sets of cells that originated from a set of mouse embryos using different schemes: $3 \times 10^5$ cells on a 50-mm dish every 3 days (3T3), $3 \times 10^5$ cells on a 50-mm dish every 6 days (3T6), and $3 \times 10^5$ cells on a 50-mm dish every 12 days (3T12) (7). The resulting three cell lines behaved differently. For example, MLV was released only from 3T12 cells after a long-term culture (11). Also, MLV was induced by the BrdU-treatment from a clonal cell line derived from 3T6 (12).

We cannot trace the passage history of 3T3-P, but the following sequence of events is plausible. During the passages, a faster-growing mutant cell emerged, gradually became a major species, and finally dominated. Changes in the growth property might be difficult to recognize without setting appropriate controls, because the mutant cells probably outgrow the other cells gradually, and the loss of contact inhibition is not detected during routine passages. Therefore, the bank system, which is useful for minimizing repeated passages of qualified cells using the master cell-bank and the working cell-bank, is strongly recommended.

Southern blotting is primarily useful for the identification of cell strains and for the detection of some, but by no means all, genetic alterations, as was done in this study. In such cases, adequate selection of the restriction enzymes and careful comparison with the reference patterns are important for detecting the genetic alterations.
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REFERENCES