Feeder Independent Culture of Mouse Embryonic Stem Cells

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ABSTRACT

Embryonic stem cell classically cultured on feeder layer with FBS contained ES medium. Feeder-free mouse ES cell culture systems are essential to avoid the possible contamination of nonES cells. First we determined the difference between ES cell and MEF by Oct4 population. We demonstrate to culture and to induce differentiation on feeder free condition using a commercially available mouse ES cell lines.

(Key words : ES cells, Differentiation, Feeder free culture)

INTRODUCTION

Evans and Kaufman have demonstrated that it is possible to isolate pluripotential cells directly from early embryos and that they behave in a manner equivalent to EC cells isolated from teratocarcinomas (3). Embryonic stem cell will be grown in *in vitro* culture, and standard culture medium contains bovine serum. Common process involves the use of nonhuman serum, may be the source of possible contamination. Also, it will be culture on the nonhuman feeder layer. The development of techniques to grow *in vitro* human and murine embryonic pluripotent stem cells (hESC and mESC, respectively) and/or adult human and murine multipotent mesenchymal stromal stem cells (h and mMSC), and the deeper understanding of pathways of cell differentiation, have expanded the horizon of likely therapeutic uses. In general, the use of stem cells to create human tissues *ex vivo* for transplantation into patients with degenerated or injured tissues has attracted increased interest, inducing the Food and Drug Administration to set the rules of stem-cell-based therapies as health policies (4). Important issue for clinical application is the generation and expansion of stem cells without animal- derived components in the culture. Human ESC, which were cocultured with mouse embryonic fibroblast (MEF) as the feeder cells, had acquired a significant amount of nonhuman cell surface proteins creates a high risk for immune rejection after cell transplantation, most likely due to the presence of circulating antibodies within the human body. Furthermore, animal-derived products in the culture medium and extracellular matrix (ECM) can be a source for the transmission of nonhuman pathogens to human. If these potentially harmful situations could be avoided, it would facilitate the clinical use of human ES cells (6, 10). Moreover, to minimize the risk of transmitting infectious diseases from animals, hESC lines have been cultured in a conditioned medium obtained from human cells as feeders and human and recombinant serum components or serum-free conditions; hence, contrasting results have been widely obtained, and a heated debate has been opened on this topic(1, 2, 5, 9, 11).

Mouse ES cells have a similar risk to use for experiment and need feeder layer and FBS for culture medium. Feeder cells are not a xenogenic source. But feeder-free mouse ES cell culture systems are essential to avoid the possible contamination of nonES cells. Because feeder cell have a possibility to influence on ES cell character and a risk to mix with ES cells during doing treatment or experiment. Therefore we demonstrated difference between ES cell and MEF (mouse embryonic fibroblast) cell by Oct4 population.

Stem cells are defined by two fundamental functional properties, the ability to generate cells of identical properties (self-renew) and to give rise to differentiated cell types (8, 12). The differentiation potential of stem cells has been extensively demonstrated in the mouse (7, 8, 12). We demonstrate to culture and to induce differentiation on feeder free condition using a commercially available mouse ES cell lines.

MATERIALS AND METHODS

Cell Lines

E14TG2a, this is a derivative of one of several embryonal stem cell (ES) lines developed by M. Hooper

* This research was supported by Kyungpook National University Research Fund, 2012.

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in 1987. Origin strain is 129/Ola of mice and purchased from ATCC. The J1 ES line was derived from a male agouti 129S4/SvJae embryo and purchased from ATCC. R1 was Established in August 1991, from (129/Sv x 129/Sv-CP) F1 3.5-day blastocyst and purchased from life technologies. TT2 (13) mouse embryonic stem (ES) cells were from B6 X CBA embryo.

Feeder Preparation and Embryonic Stem (ES) Cell Culture

To culture ES cell, primary CF-1 mouse embryonic fibroblast (MEF) cells were treated with 100 ug/ml of mitomycin C for 2 h at 5% CO_2 incubator. The cells were trypsined and $7.5\times10^5\sim1\times10^6$ cells were seeded into T-25 cell culture flask, which pretreated with 0.1% gelatin for 2 hr at room temperature, and cultured at 5% CO_2 incubator for overnight. Mouse embryonic stem (ES) cells were thawed and seeded onto mitomycin C-treated feeder layer with ES medium (20% ES Cell qualified fetal bovine serum (Gibco, Grand Island, NY)-DMEM supplemented with 10^3 units/ml leukemia inhibitory factor (CHEMICON, ESGRO, ESG1107), 10^{-4} M β-mercaptoethanol, 1× MEM nonessential amino acid (Gibco, Grand Island, NY) and 100 mM of sodium pyruvate (Gibco, Grand Island, NY) for 5 h. The ES medium was changed with fresh ES medium and medium was changed every day. The ES cells were passaged before ES cell colonies are contacting each other.

Feeder Free Culture of Embryonic Stem (ES) Cell

To culture ES cell, cells were trypsined and $1\times10^5\sim1\times10^6$ cells were seeded into T-25 cell culture flask, which no treated for 30 min at 5% CO_2 incubator and transfer sup to another T-25 cell culture flask, which pretreated with 0.1% gelatin for 2 hr at room temperature, and cultured at 5% CO_2 incubator for overnight. Mouse embryonic stem (ES) cells were culture with ES medium (20% ES Cell qualified fetal bovine serum (Gibco, Grand Island, NY)-DMEM supplemented with 10^3 units/ml leukemia inhibitory factor (CHEMICON, ESGRO, ESG1107), 10^{-4} M β-mercaptoethanol, 1× MEM nonessential amino acid (Gibco, Grand Island, NY) and 100 mM of sodium pyruvate (Gibco, Grand Island, NY) for 5 h. The ES medium was changed with fresh ES medium and medium was changed every day. The ES cells were passaged before ES cell colonies are contacting each other.

Induce Differentiation by Withdrawal of LIF

To induce differentiation, ES cells were trypsined and $1\times10^3\sim1\times10^4$ cells were seeded into 6-well cell culture plate, which pretreated with 0.1% gelatin for 2 hr at room temperature, and cultured at 5% CO_2 incubator for overnight. Mouse embryonic stem (ES) cells were culture with ES medium (20% ES Cell qualified fetal bovine serum (Gibco, Grand Island, NY)-DMEM supplemented with 10^{-4} M β-mercaptoethanol, 1× MEM nonessential amino acid (Gibco, Grand Island, NY) and 100 mM of sodium pyruvate (Gibco, Grand Island, NY) no LIF. The ES medium was changed with fresh ES medium and medium was changed every day and check after 4 days.

RESULTS

Phenotype of ES Cell and Feeder

ES cells grown on the feeder and made a colony formation (Fig. 1. upper left), stained with DAPI (Fig. 1. upper right). To determine whether cells were differentiated or not, we confirmed using an Oct4 expression. Oct4 population was close to 100% on ES cell and about 65% on MEF cells (Fig. 1 down).

The ES Cell Culture on the Feeder Free Condition

First, we demonstrate various kinds of ES cell lines were cultured on feeder free condition. TT2 cells have the most spreading phenotype and E14 cells have sufficient phenotype to culture on feeder free condition. Others were not enough to culture for a longtime without feeder (Fig. 2). Therefore E14 is good enough to culture on the feeder free condition.

The ES Cell Induce Differentiation on the Feeder Free Condition

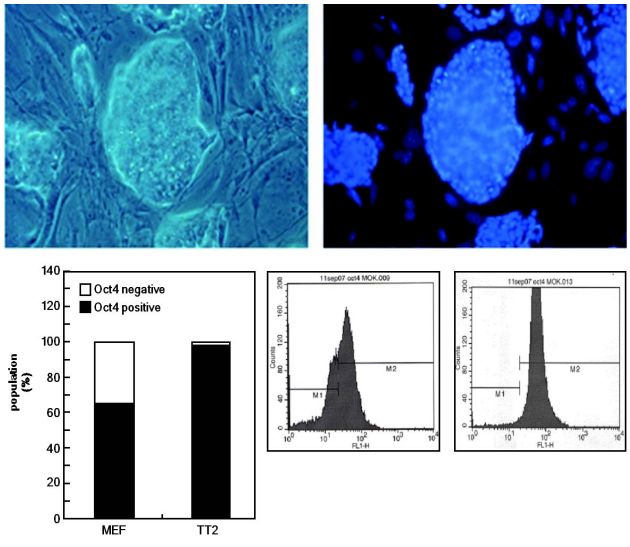

Fig. 1. Morphologic analysis of ES cell on the feeder. ES cells grown on the feeder and made a colony formation (upper left), stained with DAPI (upper right). To determine whether cells were differentiated or not, we confirmed using an Oct4 expression. Oct4 population was close to 100% on ES cell and about 65% on MEF cells (down).

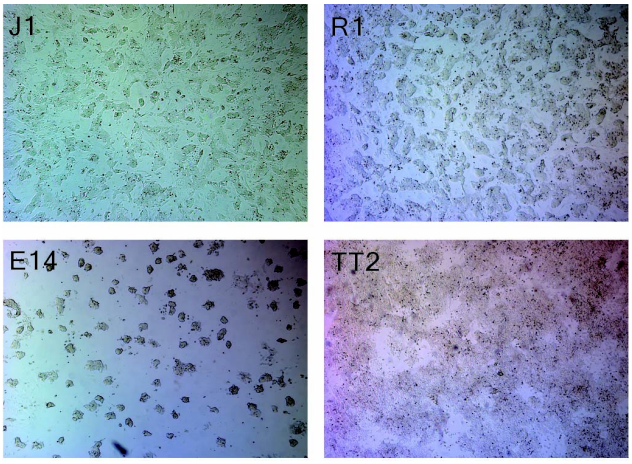

Fig. 2. Various kinds of ES cell lines were cultured on feeder free condition. TT2 cells have the most spreading phenotype and E14 cells have sufficient phenotype to culture on feeder free condition.

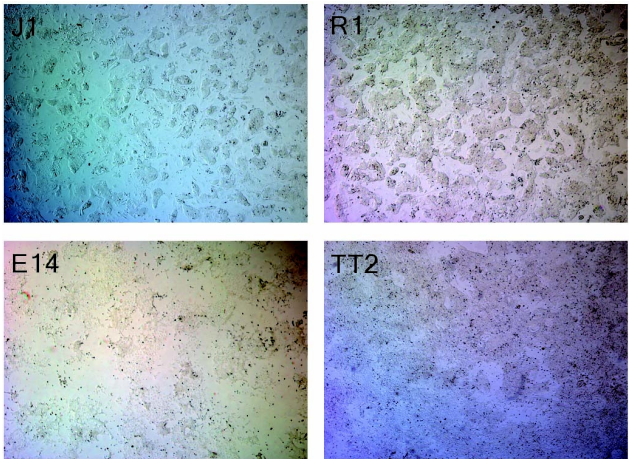

Fig. 3. Various kinds of ES cell lines were induced differentiation on feeder free condition. Mouse embryonic stem (ES) cells were culture with ES medium except LIF to induce differentiation for 4 days. E14 cell line was shown a significant changing compare with LIF (+) condition.

Various kinds of ES cell lines were induced differentiation on feeder free condition. Mouse embryonic stem (ES) cells were culture with ES medium except LIF to induce differentiation for 4days. E14 cell line was shown a significant changing compare with LIF (+) condition (Fig. 3). but others (J1, R1, and TT2) were not shown any morphological changing compare with LIF included condition.

DISCUSSION

Different ES cell lines have a different character. We use four different mouse stem cell lines for feeder free culture and induction of differentiation. When we culture ES cells on feeder free condition, E14 has best phenotype (Fig. 2) with compact and spheroid colonies induce differentiation on feeder free culture condition. J1 and R1 ES cell lines were shown not bad phenotype on feeder free condition with LIF (Fig. 2). But these cell lines had no changing in the LIF (−) condition and these cell lines can possible to culture on feeder free condition (Fig. 3). In case of TT2 cell line, it is not enough to culture on this condition because they will be changed morphologic phenotype and will be loose self-renew activity for a long time culture.

Taken together, these results will be basic information to study about differentiation of mouse ES cell and whole area of ES cell experiment. We specially suggest using E14 cell to see differentiation on the feeder free condition. Furthermore, this condition potentially useful to iPS (induced pluripotent stem) cell.

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(Received: 21 December 2012/ Accepted: 24 December 2012)