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Embryonic Stem Cells in Domestic Animals

Derivation of cat embryonic stem-like cells from *in vitro*-produced blastocysts on homologous and heterologous feeder cells

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Abstract

The domestic cat is a focal mammalian species that is used as a model for developing assisted reproductive technologies for preserving endangered cats and for studying human diseases. The generation of stable characterized cat embryonic stem cells (ESC) lines to use as donor nuclei may help to improve the efficiency of interspecies somatic cell nuclear transfer for preserving endangered cats and allow the creation of knockout cell lines to generate knockout cats for studying function of specific genes related to human diseases. It will also enable the possibility of producing gametes *in vitro* from ESC of endangered cats. In the present study, we report the generation of cat embryonic stem-like (cESL) cells from blastocysts derived entirely *in vitro*. We generated 32 cESL cell lines from 331 *in vitro* derived blastocysts from which inner cell masses were isolated by immunosurgery or by a mechanical method. Inhibition of cat dermal fibroblast (CDF) proliferation after exposure to mitomycin-C was both dose and time dependent, where doses of 30 to 40 μg/mL for 5 h were most efficient. These dosages were higher than that required to inhibit cell proliferation of mouse fetal fibroblasts (MFF; 10 μg/mL for 2.5 h). Mitomycin-C did not significantly increase necrosis of cells from either species, and had an anti-proliferative effect at concentrations below cytotoxicity. A clear species-specific relationship between feeder layers and derivation of cESL cell lines was observed, where higher numbers of cESL cell lines were generated on homologous cat feeder layers (n = 26) than from those derived on heterologous mouse feeder layers (n = 6). Three cESL cell lines generated from immunosurgery and cultured on CDF maintained self-renewal and were morphologically undifferentiated for nine and twelve passages (69–102 days). These lines showed a tightly packed dome shaped morphology, exhibited alkaline phosphatase activity and immuno-expression of the pluripotent marker *OCT-4* and surface marker SSEA-1. Primary colonies at P0 to P3 and cat blastocysts expressed transcription factors *OCT-4*, *NANOG* and *SOX-2* and the proto-oncogene *C-MYC*. However, expression was at levels significantly lower than *in vitro* produced blastocysts. During culture, cESL colonies spontaneously differentiated into fibroblasts, cardiomyocytes, and embryoid bodies. Development of techniques to prevent differentiation of cESL cells will be essential for maintaining defined cell lines.

Keywords: Cat ESC; Feeder cells; Mitomycin-C; Inner cell mass isolation

Contents

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1. Introduction

Mammalian embryonic stem cells (ESC) derived from the inner cell mass (ICM) of pre-implantation blastocysts are pluripotent cells with the ability to undergo differentiation into all cell lineages. Stem cell research provides an effective approach to the study of the cell biology of embryogenesis, cell reprogramming and therapeutic applications for regenerative medicine. The domestic cat is a focal mammalian species that has been used as a model for developing assisted reproductive technologies for preserving endangered cats [1–3] and for the study of human diseases [4]. The generation of stable characterized cat ESC lines to use as donor nuclei may help to improve the efficiency of interspecies somatic cell nuclear transfer (SCNT) for preserving endangered cats. In fact, there is evidence that less differentiated cell types can increase SCNT efficiencies as compared to terminally differentiated cell types because they are more easily reprogrammed. In the mouse and bovine, nuclear transfer using ESC was more efficient than when done with differentiated cells [5–7]. Since ESCs can be genetically modified [8], the generation of cat ESC may allow the creation of knockout cell lines to generate knockout cats for studying function of specific genes related to human diseases. Moreover, it will enable the possibility of producing gametes *in vitro* from ESC of endangered cats, as recently demonstrated in mice [9–13].

Authentic ESC cell lines must exhibit specific characteristics of morphology, unlimited self renewal, expression of specific molecular markers, maintenance of a normal karyotype and retain the ability to differentiate into multiple cell types representing all primitive embryonic germ layers. To date, stable and fully characterized mouse [14,15] and human [16,17] ESC lines have been generated. Although efforts have been made to establish ESCs from such domestic mammalian species as porcine [18], bovine [6,19,20], equine [21,22], ovine [23], canine [24–26] and feline [27], the success at producing stable characterized ESC lines has been variable.

Continuous proliferation of ESCs requires optimal *in vitro* conditions to maintain self-renewal and pluripotent characteristics. ESCs from different mammalian species have been derived from the ICM by immunosurgery [28] or by plating the whole embryo after removal of the zona pellucida [16]. However, it is not clear if the presence of trophectoderm cells (TE) after plating the whole embryo or remaining TE cells from