Stem Cell Research (2009) 2, 178–187

available at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/scr

REGULAR ARTICLE

Establishment and characterization of baboon embryonic stem cell lines: An Old World Primate model for regeneration and transplantation research

Calvin R. Simerly [a,1], Christopher S. Navara [a,1], Carlos A. Castro [a,1], Janet C. Turpin [a], Carrie J. Redinger [a], Jocelyn D. Mich-Basso [a], Ethan S. Jacoby [a], Kevin J. Grund [a], David A. McFarland [a], Stacie L. Oliver [a], Ahmi Ben-Yehudah [a], Diane L. Carlisle [a], Patricia Frost [b], Cecilia Penedo [c], Laura Hewitson [a], Gerald Schatten [a,*]

[a] *Division of Developmental and Regenerative Medicine, Department of Obstetrics, Gynecology, and Reproductive Sciences; Cell Biology-Physiology; and Bioengineering; Pittsburgh Development Center; Magee-Womens Research Institute, University of Pittsburgh, Pittsburgh, PA 15213, USA*
[b] *Southwest National Primate Research Center (NPRC), Southwest Foundation for Biomedical Research, San Antonio, TX 78227, USA*
[c] *Veterinary Genetics Laboratory, University of California, Davis, One Sheilds Avenue, Davis, CA 95616, USA*

Received 21 October 2008; received in revised form 23 January 2009; accepted 6 February 2009

Abstract Here we have developed protocols using the baboon as a complementary alternative Old World Primate to rhesus and other macaques which have severe limitations in their availability. Baboons are not limited as research resources, they are evolutionarily closer to humans, and the multiple generations of pedigreed colonies which display complex human disease phenotypes all support their further optimization as an invaluable primate model. Since neither baboon-assisted reproductive technologies nor baboon embryonic stem cells (ESCs) have been reported, here we describe the first derivations and characterization of baboon ESC lines from IVF-generated blastocysts. Two ESCs lines (BabESC-4 and BabESC-15) display ESC morphology, express pluripotency markers (Oct-4, hTert, Nanog, Sox-2, Rex-1, TRA1-60, TRA1-81), and maintain stable euploid female karyotypes with parentage confirmed independently. They have been grown continuously for >430 and 290 days, respectively. Teratomas from both lines have all three germ layers. Availabilities of these BabESCs represent another important resource for stem cell biologists.

Abbreviations: ESCs, embryonic stem cells; ICC, immunocytochemistry; ICM, inner cell mass; iPS, induced pluripotency; MEFs, mouse embryonic fibroblasts; nhp, nonhuman primates; OWP, Old World Primate; SCNT, somatic cell nuclear transfer.

* Corresponding author. Fax: +1 412 641 2410.
E-mail address: schattengp@upmc.edu (G. Schatten).
[1] These authors contributed equally to this work.

1873-5061/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.scr.2009.02.004

Introduction

Nonhuman primates and embryonic stem cells derived from nhps (nhpESCs) have contributed significantly to the translation of mouse stem cell findings to humans. For example, the establishment of nhpESCs (Thomson et al., 1995, 1996) provided the experimental foundation and biomedical rationale for the derivations of human ESCs 3 years later (Thomson et al., 1998). Innovative discoveries with nhpESCs continue to provide experimental evidence in support of hESC investigations (Friedrich Ben-Nun and Benvensity, 2006; Shufaro and Reubinoff, 2004; Dvash and Benvenisty, 2004). Parthenogenetic nhpESCs (Cibelli et al., 2002a) preceded the generation of parthenogenetic human ESCs (Lin et al., 2007; Mai et al., 2007; Revazova et al., 2007), which are of great utility notwithstanding the findings that they are unlikely to be exact immune matches to the female donor (Dighe et al., 2008). Therapeutic cloning has now succeeded in rhesus (Byrne et al., 2007; Cram et al., 2007), inspiring encouraging advances in humans (French et al., 2008) beyond previous credible attempts (Cibelli et al., 2002b; Lavoir et al., 2005; Stojkovic et al., 2005). Now, evaluating the clinical utility of stem cells generated by induced pluripotency (iPS) as well as direct differentiation (Zhou et al., 2008) is of keen importance. Nonhuman primate ESCs are accelerating mechanistic discoveries of diseases, e.g., nhpESCs as Alzheimer's disease models (Wianny et al., 2008). Transplantation of differentiated nhpESCs into primate models of Parkinson's disease (Takagi et al., 2005) and other disorders (Hematti et al., 2005) is providing encouraging preclinical results on the feasibility of stem cell therapies in clinical regenerative medicine, as shown in rodent models (Ben-Hur et al., 2004). Macaques, which include both rhesus (Mitalipov et al., 2006; Navara et al., 2007; Thomson et al., 1995) and cynomolgus (Suemori and Nakatsuji, 2006), represent the only family of OWPs from which nhpESCs have been established [reviewed by (Wilmut and Taylor, 2007)], though ESCs from the New World marmoset have also been derived (Thomson et al., 1996; Sasaki et al., 2005).

Notwithstanding the admirable contributions of macaque ESCs to translational and fundamental biology, several crucial questions about hESC biology which could be solved with nonhuman primates have not been answered using the available nhp stem cell lines. These questions include: Are primate ESCs pluripotent when investigated using ideally intraspecific or even interspecific chimera assays? Will they contribute to the germ line? Will pluripotent primate ESCs engraft and differentiate properly when transplanted into fetal or adult primates? Will they be rejected or will they generate teratomas or other tumors? Will primate cells generated by iPS behave similar to nhpESCs derived from embryos generated by IVF or somatic cell nuclear transfer (SCNT)? There are several reasons that these questions have not yet been solved, including severe limits in resource availability, cost, generation time, and lack of pedigreed lineages, as well as possible limitations in the nhpESCs currently available.

Results

Baboon (*Papio anubis*) embryos were produced by ICSI and cultured *in vitro* for 8–12 days postinsemination. Eleven females generated 157 successfully fertilized oocytes

Table 1 Baboon ESC established line from embryos generated by intracytoplasmic sperm injection

Oocyte No. (*n*)	Zygote No.	2 cell No.	3-to-4 cell No.	6-to-8 cell No.	16-to-32 cell No.	Morula No.	Early blastocyst No.	Expanded blastocyst No.	ESC in culture No.			Baboon ESC established line(>3 months) (blst) [zygote]
									Plated (blst) [zygote]	1 week (blst) [zygote]	1 month (blst) [zygote]	
166 (13)	157 (95%)	128 (77%)	124 (75%)	103 (62%)	83 (50%)	65 (39%)	54 (33%)	19 (11%)	16 (84%) [10%]	15 (79%) [10%]	2 (11%) [1%]	2 (11%) [1%]