DEVELOPMENT

Parthenogenetic Stem Cells in Nonhuman Primates

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Parthenogenesis is the process by which an egg can develop into an embryo in the absence of sperm. This process has been characterized to some extent in nonhuman primates (*1*, *2*); however, to date, no primate parthenogenetic embryonic stem (ES) cell lines have been derived. Although attempts to obtain full-term mouse and bovine parthenogenetic individuals have failed (*3*–*5*), chimeras of parthenogenetic cells with biparentally derived embryonic tissues have generated apparently normal offspring (*6*).

Here we demonstrate broad differentiation capabilities of primate pluripotent stem cells derived by parthenogenesis. After in vitro maturation for 36 hours [with media supplemented with pregnant mare serum (10 IU/ml) and hCG (10 IU/ml); Sigma], 28 out of 77 primate eggs (*Macaca fascicularis*) reached metaphase II. Eggs were parthenogenetically activated with protocols previously described (*2*). Four out of 28 eggs (14%) developed to the blastocyst stage (Fig. 1A). Inner cell masses (ICM) were isolated by immunosurgery, and 1 week after plating, cell proliferation was observed in three ICMs and one stable cell line (Cyno-1) was obtained. Morphologically, the cells had a small cytoplasmic/nuclear ratio and numerous and prominent nucleoli and cytoplasmic lipid bodies. These cells could be extensively propagated in vitro (10 months) while maintaining their undifferentiated state. They tested positive for markers of primate ES cells with the exception of SSEA-3. High telomerase activity seen in the undifferentiated cells was lost completely after 2 weeks of differentiation, suggesting that telomerase activity is regulated in Cyno-1 cells and is not constitutive, as is observed in many tumor cell lines. In addition, karyotyping revealed 40+2 chromosomes, as is expected for *Macaca fascicularis*. Typing for sequence repeats and micro SSPTM generic human lymphocyte antigen class II DNA performed in Cyno-1 cells and somatic cells from donor animal were indistinguishable and therefore should be considered autologous.

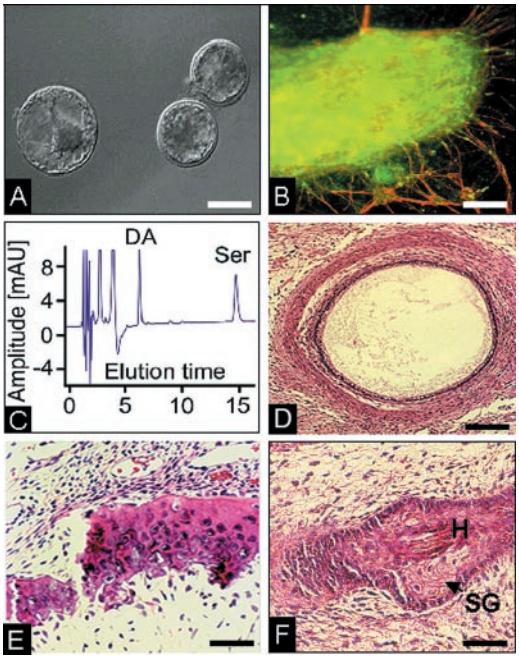

Fig. 1. Characterization of primate parthenogenetic embryos and derived cell lines. (**A**) Parthenogenetically activated eggs at day 8 of development before ICM isolation. (**B**) Upon induced differentiation, up to 25% of TUJ1$^+$ cells coexpressed tyrosine-hydroxylase (rabbit antibody to TH 1:250; PelFreez) exhibiting complex neurites. (**C**) Neuronal function was assessed by reverse-phase HPLC with electrochemical detection of the neurotransmitters dopamine (DA) and serotonin (Ser). (**D** to **F**) In vivo differentiation-teratoma. (D) Gut, (E) bone, and (F) hair follicle complex with hair (H) and sebaceous gland (SG). Scale bars: (A), 100 μm; (B), (E), and (F), 20 μm; (D) 50 μm.

Neural differentiation of Cyno-1 cells was induced with a multistep culture procedure (*7*), and astrocytes and neurons were obtained. Up to 25% of dopaminergic neurons could be obtained as judged by immunocytochemical criteria (Fig. 1B). Neuronal identity and function were confirmed by high-performance liquid chromatography (HPLC) analysis, which showed in vitro release of the neurotransmitters dopamine and serotonin (Fig. 1C). By modifying culture conditions, a large variety of specialized cell types could be generated in vitro, including spontaneously beating cardiomyocyte-like cells, smooth muscle cells, adipocytes, and beating ciliated epithelium, among others.

The capacity of Cyno-1 cells to differentiate was also tested in vivo by injecting them into the peritoneal cavity of immunocompromised severe combined immunodeficiency disease (SCID) mice (C.B-17 SCID; Charles River). Teratomas were isolated 8 and 15 weeks after injection and subjected to histological analysis. Derivatives of all three germ layers were observed, including cartilage, muscle and bone (mesoderm), neurons, melanocytes, skin and hair follicles (ectoderm), and intestinal and respiratory epithelia (endoderm) (Fig. 1, D to F). The presence of mature tissues and low frequency of mitotic figures in these tumors indicated their benign nature.

The in vitro differentiation of these cells to well-characterized dopaminergic neurons is of particular interest, because of their potential to replace lost neurons in Parkinson's disease. Thus far, dopaminergic neurons have been derived from mouse ES cells in vitro (*7*) but not from primate ES cells.

The proposal of human therapeutic cloning (HTC) describes the generation of autologous ES cells through somatic cell nuclear transfer (*8*). This study suggests an alternative to HTC. Differentiated cell types derived in vitro by parthenogenesis eliminate the requirement to produce or disaggregate a normal, competent embryo and may circumvent the ethical concerns voiced by some, positively impacting the debate in stem cell research.

References and Notes

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9. We wish to thank N. Sawyer, J. Balise, N. Krieger, D. Wolf, J. Robl, P. Andrews, B. Banner, T. Clarkson, M. Anthony, and A. Kiessling. Experiments were supported by Advanced Cell Technology and grants P50-AA11997 and T32-AA07565 from the National Institute on Alcohol Abuse and Alcoholism.

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