Derivation of Induced Pluripotent Stem Cells from the Baboon: A Nonhuman Primate Model for Preclinical Testing of Stem Cell Therapies

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Abstract

Development of effective pluripotent stem cell-based therapies will require safety and efficacy testing in a clinically relevant preclinical model such as nonhuman primates (NHPs). Baboons and macaques are equally similar to humans genetically and both have been extensively used for biomedical research. Macaques are preferred for human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) research whereas baboons are preferred for transplantation studies because of the greater similarity of their anatomy and immunogenetic system to those of humans. We generated four induced pluripotent stem cell (iPSC) lines from skin cells of the olive baboon (*Papio anubis*). Each line shows the distinct morphology of primate pluripotent stem cells, including flat colonies with well-defined borders and a high nuclear/cytoplasm ratio. Each is positive for the pluripotency markers OCT4, SOX2, NANOG, and SSEA4. Pluripotency was confirmed in two lines by teratoma formation with representative tissues from each germ layer, whereas a third produced cells from all three germ layers following embryoid body differentiation. Three lines have a normal male karyotype and the fourth is missing the short arm of one copy of chromosome 18. This may serve as an *in vitro* model for the human developmental disorder 18p-, which impacts 1 in 50,000 births/year. These iPSC lines represent the first step toward establishing the baboon as a NHP model for developing stem cell-based therapies.

Introduction

THE DISCOVERY IN 2006 (Takahashi and Yamanaka 2006) of induced pluripotent stem cells (iPSCs) afforded a novel approach to the production of pluripotent stem cell lines from each individual that can potentially be used in patient-specific, cell-based therapeutic procedures to treat otherwise intractable diseases, injuries, or debilitating conditions without the need to immunosuppress patients receiving the transplanted cells (Dimos et al., 2008; Hanna et al., 2007; Nishikawa et al., 2008). Nevertheless, significant challenges remain regarding the practical implementation of clinical protocols involving these cells. These include questions about: (1) Specific routes of delivery of differentiated cells derived from iPSCs to specific organ or tissue targets, (2) persistence and/or proliferation of these cells *in situ* once delivered, (3) the extent to which these autologously derived cells remain

nonimmunogenic upon transfer back into patients, (4) expression of genes associated with the desired differentiated cell type in these cells *in vivo*, (5) the ability of these cells to mitigate the targeted disease or debilitation, and (6) assurance that delivery of these cells will not elicit undesirable secondary conditions such as cancer or other side or off-target effects. Each potential application of an iPSC-based therapeutic protocol will present a unique set of challenges to implementation in human patients. These concerns mandate the need for clinically relevant animal model systems that will facilitate optimization of the efficacy and safety of each potential therapeutic protocol before clinical trials are initiated.

Many of the diseases or debilitations likely to be targeted by stem cell-based therapeutic approaches represent complex conditions or physiologies unique to mammals and manifest predominantly in humans. Nonhuman primates (NHPs) afford the most clinically relevant model system for

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biomedical studies of such conditions. The establishment of NHP models for preclinical studies of stem cell-based therapeutic approaches will provide uniquely valuable resources for studies designed to optimize the efficacy and safety of these methods. Such models will accelerate the discovery, application, and clinical translation of novel therapeutic approaches involving pluripotent stem cells, while minimizing the risk of undesirable side effects.

Several NHP species have been developed as models for biomedical research. Chimpanzees have the advantage that they, along with the other great apes, are most closely related to humans (~2% different at the genomic level) (Fujiyama et al., 2002). The chimpanzees used in biomedical research are extremely costly, their numbers are limited, and there are significant restrictions on the procedures that can be performed on these animals. Rhesus monkeys are the most commonly used NHP species in biomedical research. However, availability of these animals is limited by the demand for their use in studies of human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS) (Pandrea et al., 2009; Shedlock et al., 2009). Baboons, which are equally distant from humans at the genomic level as are rhesus monkeys (both are 7–8% different from humans, but only 2% different from each other), are not in demand for HIV/AIDS studies and do not harbor many of the background viruses found in macaques such as herpes B virus (VandeBerg et al., 2009), but are more similar to humans in size and anatomy as well as in the use of bipedal locomotion. They are the most used NHP species for transplantation studies because they possess an immunogenetic system that is more like that of humans than is that in macaques (Damian et al., 1972). Several complex human diseases have been modeled in the baboon (for review, see Vandeberg et al., 2009), including endometriosis, neonatal lung disease, dental development, cardiovascular disease, fetal–maternal interactions, nutrition disorders, alcoholic liver disease, neurological defects, stroke, osteoporosis and osteoarthritis, craniofacial defects, diabetes and obesity, and drug abuse, and others that can be mimicked by various treatments. Thus, the baboon represents an attractive NHP model species in which to develop and optimize stem cell-based protocols prior to their use in humans.

Our long-term goal is to establish the baboon as a model NHP species for testing and optimizing the efficacy and safety of stem cell-based therapeutic approaches in a context that most accurately mimics the human. The baboon is a well-developed model for biomedical research in general (VandeBerg et al., 2009). To extend the utility of this model to studies of iPSC-based therapeutic applications, we have derived iPSC cells from baboons and characterized these cells to show that they resemble human pluripotent stem cells. In the future, such lines, together with access to matched, living baboons from which each iPSC line was derived, will provide a comprehensive NHP model system for testing patient-specific stem cell-based therapeutics.

Materials and Methods

Animal Ethics Statement

All authors are committed to the ethical use of animals in research. All experiments involving animals were conducted in compliance with the Animal Welfare Act and the Health Research Extension Act of 1985. Baboon skin samples were

obtained from the Southwest National Primate Research Center at the Texas Biomedical Research Institute under the oversight of that institution's Institutional Animal Care and Use Committee (IACUC). Procedures involving teratoma formation in mice were carried out at the University of Texas Health Science Center at San Antonio under the guidance of that institution's IACUC.

Biopsies of skin from fetal baboons

Skin fibroblasts were derived from fetal baboons recovered following cesarean section. These fetal baboons were recovered for an unrelated project, and skin biopsies were taken from material that would otherwise have been discarded as surgical waste. Two iPSC lines were derived from each of two different male baboon fetuses—one recovered at 90 days of gestation (dga) and the other recovered at 160 dga. In each case, a 1- to 2-cm² piece of abdominal skin was recovered under sterile surgical conditions. The skin tissue was then minced and digested with collagenase Type IV, and plated in a 10-cm dish in baboon fibroblast culture medium [bFCM, which is Dulbecco's modified Eagle medium (DMEM), 10% fetal bovine serum (FBS)+1×penicillin-streptomycin] and allowed to grow until confluent. The cells were grown for up to three passages prior to the production of iPSCs.

Production of iPSC lines

We followed the standard Yamanaka method (Takahashi et al., 2007) with some modifications. Early-passage baboon fibroblasts were plated in bFCM at 30,000 cells/well of a six-well dish and transduced the following day with 1 mL of human embryonic stem cell (hESC) medium (WiCell) containing 0.5 μ L each of high-titer retrovirus [10^8 – 10^9 infectious units (ifu)/mL] encoding human KLF4, SOX2, and OCT4, plus 0.05 μ L of virus encoding c-MYC, in the presence of 10 μ g/mL Polybrene and 10 μ M Y-27632 ROCK inhibitor (Calbiochem) per well as described (Boulting et al., 2011; Dimos et al., 2008; Mekhoubad et al., 2012). After 12–16 h, an additional 1 mL of hESC medium was added to each well, and 2–3 days after addition of virus the medium was changed to pure hESC medium. Putative iPSC colonies were transferred onto cultures of γ -irradiated mouse embryonic fibroblast (MEF) feeder cells and maintained in hESC medium preconditioned by MEFs as described previously (Varum et al., 2009; Xu et al., 2001). Colonies with morphology characteristic of human and NHP pluripotent cells (flat with distinct borders and containing cells with high nuclear/cytoplasmic ratio and prominent nucleoli) were recovered manually using a fine glass needle, expanded under the same conditions, and routinely passaged upon reaching confluency. Beginning at approximately passage six, a portion of the cells were banked in liquid nitrogen storage and this was repeated every 5–10 passages thereafter. All work involving viruses was compliant with the National Institutes of Health Guidelines for Recombinant DNA and was conducted using protocols approved by the University of Texas at San Antonio Institutional Biosafety Committee.

Characterization of each biPSC line

All characterizations of baboon induced pluripotent stem cells (biPSC) lines were performed after passage 20

and included colony morphology, immunocytochemistry (ICC), and reverse transcription polymerase chain reaction (RT-PCR) to detect expression of pluripotency factors, analysis of karyotype, and analysis of teratoma formation. Colony morphology was assessed under phase-contrast optics. Baboon iPSCs were assayed by ICC for expression of the pluripotency factors OCT4, NANOG, and SOX2 as well as the surface marker stage-specific embryonic antigen-4 (SSEA4). Colonies were grown on plastic coverslips and fixed using 2% paraformaldehyde (Electron Microscopy Services, Hatfield, PA) in phosphate-buffered saline (PBS) for 40 min or in absolute methanol (Sigma) for 10 min at -20°C (Navara et al., 2007). Primary antibodies were diluted in PBS+0.1% Triton X-100 (PBS-Tx) and incubated with fixed cells overnight at 4°C . Appropriate fluorescently labeled secondary antibodies were then applied for 1 h at 37°C . Coverslips were inverted onto Vectashield containing 4',6-diamidino-2-phenylindole (DAPI) for the detection of DNA and sealed to the slide with nail polish. Fluorescence staining was analyzed using a Personal DV deconvolution microscope (Applied Precision, Seattle WA, USA), and images were prepared for publication using Photoshop (Adobe, Mountain View, CA, USA).

RT-PCR was used to detect expression of the *SOX2*, *NANOG*, and *OCT4* genes. Total cellular RNA was extracted from biPSCs using TRIzol (Invitrogen) and DNase-treated with the DNA-free kit (Ambion, Austin, TX, USA) following the manufacturer's directions for stringent conditions. RNA was dialyzed for 30 min against RNase/DNase-free distilled water. cDNA was synthesized using the GeneAmp RNA PCR kit from Applied Biosciences (Fisher). Primers for the individual PCR reactions were: *OCT4*, forward, GTGCCG TGAAGCTGGAGAAGGA, reverse, ACCTTCCCAAATAG AACCCCCAGG; *SOX2*, forward, TCAGGAGTTGTCAA GGCAGAG, reverse, CGCCGCCGATGATTGTTATTA; and *NANOG*, forward, CAGCCTCCAGCAGATGCAAGAAC, reverse, GAGGCCTTCTGCGTCACACCA. Expression of exogenous viral constructs was determined using the forward primers for *OCT4*, *SOX2*, *KLF4*, and *cMYC* with a reverse primer specific for the viral sequence CAAATTTT GTAATCCAGAGGTTG. Each reaction contained 100 ng of cDNA and was amplified using the following parameters, 94°C for 2 min, followed by 35 cycles of 94°C for 20 sec, 60°C for 20 sec, and 70°C for 40 sec, followed by a single step of 70°C for 5 min. Amplification products were separated on a 3% agarose gel preloaded with 200 ng/mL ethidium bromide. Gels were imaged using a Bio-Rad Gel Doc EZ imager.

Teratoma analysis was carried out at the University of Texas Health Science Center at San Antonio (UTHSCSA) using protocols approved by the UTHSCSA Institutional Animal Care and Use Committee. A total of 2×10^6 biPSCs in 100 μL of 50% Matrigel/culture medium were injected intramuscularly into the flanks of immunodeficient mice (*RAG2*^{-/-}, *γc* ^{-/-}) (Wu et al., 2010). Mice were sacrificed after 8 weeks. Tumors were excised, fixed in 4% paraformaldehyde, and processed for conventional histology. Histology and analysis of the contribution of biPSCs to the three germ layers was conducted by the Histology core at Magee Womens Research Institute (<http://www.mwrf.org/119>).

Embryoid body (EB) formation was carried out as follows. Baboon iPSC colonies were grown to passaging and then manually cut into small sections of 1000–2000 cells each that

were lifted from the dish with the aid of 1 mg/mL collagenase IV. These sections were then transferred to ultra-low adherence six-well dishes in hESC medium. To determine differentiation into each of the three germ lineages, EBs were collected on day 11 of differentiation and RNA was collected as described above. RT-PCR was performed with the following primers: *NESTIN* (ectoderm), forward TGGCGC ACCTCAAGATGTCCCTC, reverse, GCTCCAGCTTGGGG TCCTGAAAG; *T* (Brachyury, mesoderm), forward, GGACG ACAACGGCCACATTA, reverse, GGTCTGGTAGGCAG TGACC; and *SOX17* (endoderm), forward, CAAGATGCT GGGCAAGTCGT, reverse, CAAGATGCTGGGCAAGTCGT, as described above. Primers were designed using Primerblast and Primer3 (Untergasser et al., 2012; Ye et al., 2012).

Baboon iPSCs were sent to Cell Line Genetics (Madison WI) for karyotype analysis by G-banding.

Results

A total of four different biPSC lines were derived and characterized—two each from each of two different fetal male baboons at 90 (biPS-90-11 and biPS-90-25) and 160 (biPS-160-2 and biPS-160-5) dga, respectively. Approximately 15–30 putative colonies were identified/30,000 fetal fibroblasts (0.05–0.1% efficiency). Colony morphology appeared similar among lines (Fig. 1A–D) and resembled that reported for human iPSCs (Takahashi et al., 2007; Yu et al., 2007), human ESCs (Thomson et al., 1998), and baboon ESCs (Simerly et al., 2009), including large flat colonies, with well-defined borders and a high nuclear/cytoplasm ratio.

Because pluripotent stem cells frequently develop aneuploidies in culture (Draper et al., 2004; Mitalipova et al., 2005), karyotype analysis was performed on each line to verify normal chromosome numbers (Fig. 1E–H). Three lines (biPS-90-25, biPS-160-2, and biPS-160-5) showed normal male karyotypes (42XY, Fig. 1E–G). The fourth line, biPS-90-11, showed a clonal abnormality (42XY, 18p–, Fig. 1H), lacking the short arm of one copy of chromosome 18. Interestingly, baboon chromosome 18 is syntenic with human chromosome 18, so this line may be an ideal model system for studies of developmental defects associated with human 18p–. This syndrome (18p–) is a developmental disorder estimated to affect about 1:50,000 liveborn human infants (for review, see Turleau, 2008). It is characterized by a wide spectrum of dysmorphic changes in neurological, cardiac, and other tissues or organs. In less frequent cases, holoprosencephaly (fused right and left halves of the brain) is present. The precise molecular etiology of these defects is largely unknown. Therefore, the biPS-90-11 cell line may provide a useful “disease-in-a-dish” model for gaining insight into the genetic or molecular mechanisms associated with this syndrome, with the added attribute afforded by the baboon model that studies of these cells in culture can be augmented with studies of cells transplanted into living hosts.

Characterization of pluripotency was performed on all three karyotypically normal cell lines. Each cell line was positive for expression of the core pluripotent transcription factors NANOG (Fig. 2A, I, Q), OCT4 (Fig. 2B, J, R), and SOX2 (Fig. 2C, K, S) when assayed by ICC (Fig. 2) or RT-PCR (Fig. 3). Importantly, each of these transcription factors localized primarily to the nucleus of the biPSCs (Fig. 2).

FIG. 1. Morphology and karyotype of baboon iPSC lines. (A–D) biPSC lines grown on a MEF feeder layer formed flat colonies with well-defined borders. (E–H) Karyotype analysis revealed that three lines had a normal male baboon karyotype (42XY, E–G) whereas the fourth line (biPS-90-11, H) had a clonogenic absence of the short arm of one copy of chromosome 18 (arrow). Bar, 200 μ m.

Additionally, each cell line was positive for the cell-surface marker SSEA4 (Fig. 2D, L, T).

The gold standard for confirmation of pluripotency of a cell line is teratoma formation in immunocompromised mice, demonstrating contributions of the cells to tissues representative of all three germ layers. Baboon iPSCs were injected intramuscularly into immunocompromised mice. Putative teratomas were collected 8 weeks later and out-sourced for analysis. Two of the lines (biPS-90-25 and biPS-90-11) demonstrated clear differentiated cell types or tissues representative of each of the three germ layers. These included gastrointestinal and glandular tissue (endoderm, Fig. 4B, F), muscle (mesoderm, Fig. 4C, G), and neuronal tissue (ectoderm, Fig. 4 D, H). The other two cell lines demonstrated early lineage differentiation but had not yet differentiated enough to confirm each lineage. To further characterize the differentiation capacity of these latter two lines, we performed EB differentiation. We included one of the lines (90-11) in which pluripotency was confirmed by teratoma analysis. After 11 days of differentiation, RNA was collected and analyzed for expression of NESTIN (ectoderm), T (Brachyury, mesoderm), and SOX17 (endoderm). As shown in Figure S1 (Supplementary Data are available at www.liebertpub.com/cell/), EBs differentiated from biPS-90-11 and biPS-160-2 expressed all three lineage markers, indicating differentiation into all three germ layers. biPS-160-5 was positive for NESTIN and SOX17 but did not express T.

To further characterize expression of the pluripotency factors in these cell lines and to distinguish expression from endogenous and exogenous pluripotency genes, we used RT-PCR including a reverse primer specific for viral sequences. In fetal fibroblasts at 11 days posttransduction, expression of each of the four factors from exogenous viral constructs was detected (Fig. S2). No exogenous expression was detected in either biPS-90-11 or biPS-160-2. However, persistent expression of c-MYC from the viral construct was observed in biPS-160-5, perhaps explaining the differentiation deficiencies observed for this line. Endogenous NANOG was used as a

positive control in the biPSC lines and was negative in the transduced fetal fibroblasts at 11 days posttransduction.

Discussion

Pluripotent (ESCs and/or iPSCs) stem cells have now been derived from at least six different NHP species: Rhesus monkeys (ESCs and iPSCs; Chan et al., 2011; Liu et al., 2008; Mitalipov et al., 2006; Navara et al., 2007; Pau and Wolf 2004; Thomson et al., 1995), marmosets (ESCs and iPSCs; Muller et al., 2009; Sasaki et al., 2005; Thomson et al., 1996; Wu et al., 2010), cynomolgus monkeys (ESCs and iPSCs; Suemori and Nakatsuji 2006; Suemori et al., 2001), pigtail macaques (iPSCs; Gori et al., 2012), drills (iPSCs; Ben-Nun et al., 2011), and baboons (ESCs, Chang et al., 2011; Simerly et al., 2009). Here we describe the first derivation of iPSCs from the baboon. This represents the first step toward our goal to develop the baboon as a clinically relevant, NHP resource for testing and optimizing the efficacy and safety of iPSC-based therapeutic approaches to patient-specific, regenerative tissue engineering, or cell-based treatments of diseases or injuries.

The baboon iPSC lines we describe here are fundamentally similar to human iPSCs in all aspects examined, which supports our contention that these cells will provide an accurate model of human iPSCs. Indeed, we observed no obvious differences between the biPSCs derived in our study and human iPSCs described in previously published reports (Takahashi et al., 2007; Yu et al., 2007).

ESCs represent an established standard of pluripotent cells, and banks of approved ESC lines may be used therapeutically in the United States and elsewhere (Carpenter et al., 2009). However, iPSCs afford the unique potential to provide pluripotent stem cell lines specific to each individual as so-called “patient-specific” stem cells that will theoretically elicit little or no immune response upon transplantation back into the individual from whom the cell line was originally derived, and thus require little or no immunosuppression accompanying any transplantation procedure.

FIG. 2. ICC analysis of pluripotency in biPSC lines. All three karyotypically normal biPSC lines (biPS-90-25, **A–H**; bi-PS-160-5, **I–P**; bi-PS-160-2, **Q–X**) were positive for expression and nuclear localization of the three core pluripotency factors NANOG (**A, I, Q**), OCT4 (**B, J, R**), and SOX2 (**C, K, S**). Each line also labeled positively for the cell-surface marker SSEA4 (**D, L, T**). Bar, 100 μ m; DNA (DAPI, **E–H, M–P, U–X**).

Indeed, there is ever broadening evidence that mouse or human iPSC- or ESC-derived cells can engraft, survive, and provide functional benefit when transplanted into appropriate tissues in living rodents (Boulting et al., 2011; Goldring et al., 2011; Lindvall et al., 2012). However, there is considerably less experience regarding transplantation of such cells into adult primates. Therefore, it is less well understood whether grafts of stem cell-derived somatic cell types will

survive following transplantation into a primate, how quickly they will colonize the engraftment site, how long it will take for transplanted cells to establish proper interactions with surrounding cells, and ultimately whether they can restore functionality to damaged or diseased tissues or organs. In particular, there is no information regarding these questions in the context of transplantation of autologous primate cells into an isogenic recipient. For these reasons,

FIG. 3. RT-PCR analysis of expression of pluripotency factors. A baboon ESC cell line and each biPS cell line expressed the three pluripotency genes *OCT4*, *NANOG*, and *SOX2* at the RNA level.

one or more well-developed NHP model systems are needed to facilitate preclinical optimization of the efficacy and safety of stem cell-based therapeutic protocols prior to initiation of clinical trials in humans.

Derivation of baboon ESCs was previously accomplished by Simerly et al. (2009). These authors noted several advantages of the baboon as a model NHP system for studies of stem cell-based therapeutic applications. These included the fact that “baboons represent a preclinical research resource that is readily available and relatively cost-effective to complement the nearly inexhaustible demands on macaques”

(Simerly et al., 2009). In addition, these authors noted that the size and relative docility of baboons is ideal for noninvasive magnetic resonance imaging (MRI) studies for tracking the fate of transplanted cells. Finally, they pointed out that baboons have been extensively used in transplantation research because their physiology, size, and organ compatibilities more closely resemble human conditions (Simerly et al., 2009). We believe these characteristics are of critical importance in selecting an NHP model for stem cell-based therapeutic approaches. Although smaller NHP species might be less costly or cumbersome to house and may require fewer cells for transplantation, these economies of scale may lead to key differences in the manner in which a recipient will respond to any cell-based therapeutic intervention. In contrast, an NHP species that is genetically, anatomically, and physiologically similar to humans, and is of a similar overall size and uses a similar bipedal mode of locomotion, will afford the most accurate, and, hence, most clinically relevant model for testing a wide variety of potential cell-based therapeutic applications.

One primary target for stem cell-based treatment is neurodegenerative disease. Baboons afford particular advantages over macaques for this application. Thus, baboons show greater similarities to humans with respect to: (1) The size and anatomy of the overall brain in general and the frontal lobe in particular, including the ratio of white matter to gray matter in the frontal lobe (Schoenemann et al., 2005), (2) the relative size of structures within the basal ganglia (Hardman et al., 2002), (3) neuronal/brain metabolism as measured by glucose and oxygen use per neuron (Herculano-Houzel, 2011), (4) the presence of an occipital/marginal venous system in the brain, an adaptation associated with bipedalism that is not found in rhesus macaques (Aurbonyawat et al., 2007), (5) the occurrence of age-related tauopathy resembling that seen in humans with Alzheimer's disease (Schultz et al., 2000), (6) the extent of vascular collaterals in the brain as measured by radiolabeled Xe inhalation (D'Ambrosio et al., 2004), (7) cerebral development

FIG. 4. Histological analysis of teratomas formed from biPS-90-25 and biPS-90-11. Teratomas contained diverse tissues (A, E, Overview), including endodermally derived gastrointestinal tissue, (arrow, B, F), mesodermally derived smooth muscle (arrow, C, G), and ectodermally derived neuronal tissue (arrow, D, H).

in utero (Inder et al., 2005), and (8) age-related dendritic and spine changes in corticocortically projecting neurons (Duan et al., 2003).

Thus, among NHP species commonly used in biomedical research, baboons offer numerous advantages as a model system for final preclinical testing and development of stem cell-based therapeutic protocols prior to initiating clinical trials in patients. The results reported here extend the utility of this model to investigations involving the use of iPSCs, representing a promising new area of application for the baboon model.

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Author Disclosure Statement

The authors declare that no conflicting financial interests exist.

References

- Aurboonyawat, T., Suthipongchai, S., Pereira, V., Ozanne, A., and Lasjaunias, P. (2007). Patterns of cranial venous system from the comparative anatomy in vertebrates. Part I, introduction and the dorsal venous system. *Interv. Neuroradiol.* 13, 335–344.
- Ben-Nun, I.F., Montague, S.C., Houck, M.L., Tran, H.T., Garitaonandia, I., Leonardo, T.R., Wang, Y.C., Charter, S.J., Laurent, L.C., Ryder, O.A., et al. (2011). Induced pluripotent stem cells from highly endangered species. *Nat. Methods* 8, 829–831.
- Boulting, G.L., Kiskinis, E., Kroft, G.L., Amoroso, M.W., Oakley, D.H., Wainger, B.J., Williams, D.J., Kahler, D.J., Yamaki, M., Davidow, L., et al. (2011). A functionally characterized test set of human induced pluripotent stem cells. *Nat. Biotechnol.* 29, 279–286.
- Carpenter, M.K., Frey-Vasconcells, J., and Rao, M.S. (2009). Developing safe therapies from human pluripotent stem cells. *Nat. Biotechnol.* 27, 606–613.
- Chan, A.W., Cheng, P.H., Neumann, A., and Yang, J.J. (2011). Reprogramming huntington monkey skin cells into pluripotent stem cells. *Cell. Reprogram.* 12, 509–517.
- Chang, T.C., Liu, Y.G., Eddy, C.A., Jacoby, E.S., Binkley, P.A., Brzyski, R.G., and Schenken, R.S. (2011). Derivation and characterization of novel nonhuman primate embryonic stem cell lines from in vitro-fertilized baboon preimplantation embryos. *Stem Cells Dev.* 20, 1053–1062.
- D'Ambrosio, A.L., Sughrue, M.E., Mocco, J., Mack, W.J., King, R.G., Agarwal, S., and Connolly, E.S., Jr. (2004). A modified transorbital baboon model of reperfused stroke. *Methods Enzymol* 386, 60–73.
- Damian, R.T., Luker, M.F., Greene, N.D., and Kalter, S.S. (1972). The occurrence of baboon-type IgG subclass antigenic determinants within the order primates. *Folia Primatol. (Basel)* 17, 458–474.
- Dimos, J.T., Rodolfa, K.T., Niakan, K.K., Weisenthal, L.M., Mitsumoto, H., Chung, W., Croft, G.F., Saphier, G., Leibel, R., Goland, R., et al. (2008). Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. *Science* 321, 1218–1221.
- Draper, J.S., Smith, K., Gokhale, P., Moore, H.D., Maltby, E., Johnson, J., Meisner, L., Zwaka, T.P., Thomson, J.A., and Andrews, P.W. (2004). Recurrent gain of chromosomes 17q and 12 in cultured human embryonic stem cells. *Nat. Biotechnol.* 22, 53–54.
- Duan, H., Wearne, S.L., Rocher, A.B., Macedo, A., Morrison, J.H., and Hof, P.R. (2003). Age-related dendritic and spine changes in corticocortically projecting neurons in macaque monkeys. *Cereb. Cortex* 13, 950–961.
- Fujiyama, A., Watanabe, H., Toyoda, A., Taylor, T.D., Itoh, T., Tsai, S.F., Park, H.S., Yaspo, M.L., Lehrach, H., Chen, Z., et al. (2002). Construction and analysis of a human-chimpanzee comparative clone map. *Science* 295, 131–134.
- Goldring, C.E., Duffy, P.A., Benvenisty, N., Andrews, P.W., Ben-David, U., Eakins, R., French, N., Hanley, N.A., Kelly, L., Kitteringham, N.R., et al. (2011). Assessing the safety of stem cell therapeutics. *Cell Stem Cell* 8, 618–628.
- Gori, J.L., Chandrasekaran, D., Kowalski, J.P., Adair, J.E., Beard, B.C., D'Souza, S.L., and Kiem, H.P. (2012). Efficient generation, purification, and expansion of CD34+ hematopoietic progenitor cells from nonhuman primate induced pluripotent stem cells. *Blood* 120, e35–e44.
- Hanna, J., Wernig, M., Markoulaki, S., Sun, C.W., Meissner, A., Cassady, J.P., Beard, C., Brambrink, T., Wu, L.C., Townes, T.M., et al. (2007). Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin. *Science* 318, 1920–1923.
- Hardman, C.D., Henderson, J.M., Finkelstein, D.I., Horne, M.K., Paxinos, G., and Halliday, G.M. (2002). Comparison of the basal ganglia in rats, marmosets, macaques, baboons, and humans: Volume and neuronal number for the output, internal relay, and striatal modulating nuclei. *J. Comp. Neurol.* 445, 238–255.
- Herculano-Houzel, S. (2011). Scaling of brain metabolism with a fixed energy budget per neuron: implications for neuronal activity, plasticity and evolution. *PLoS One* 6, e17514.
- Inder, T., Neil, J., Kroenke, C., Dieni, S., Yoder, B., and Rees, S. (2005). Investigation of cerebral development and injury in the prematurely born primate by magnetic resonance imaging and histopathology. *Dev. Neurosci.* 27, 100–111.
- Lindvall, O., Barker, R.A., Brustle, O., Isacson, O., and Svendsen, C.N. (2012). Clinical translation of stem cells in neurodegenerative disorders. *Cell Stem Cell* 10, 151–155.
- Liu, H., Zhu, F., Yong, J., Zhang, P., Hou, P., Li, H., Jiang, W., Cai, J., Liu, M., Cui, K., and others. (2008). Generation of induced pluripotent stem cells from adult rhesus monkey fibroblasts. *Cell Stem Cell* 3, 587–590.
- Mekhoubad, S., Bock, C., de Boer, A.S., Kiskinis, E., Meissner, A., and Eggan, K. (2012). Erosion of dosage compensation impacts human iPSC disease modeling. *Cell Stem Cell* 10, 595–609.
- Mitalipova, M.M., Rao, R.R., Hoyer, D.M., Johnson, J.A., Meisner, L.F., Jones, K.L., Dalton, S., and Stice, S.L. (2005). Preserving the genetic integrity of human embryonic stem cells. *Nat. Biotechnol.* 23, 19–20.
- Mitalipov, S., Kuo, H.C., Byrne, J., Clepper, L., Meisner, L., Johnson, J., Zeier, R., and Wolf, D. (2006). Isolation and characterization of novel rhesus monkey embryonic stem cell lines. *Stem Cells* 24, 2177–2186.
- Muller, T., Fleischmann, G., Eildermann, K., Matz-Rensing, K., Horn, P.A., Sasaki, E., and Behr, R. (2009). A novel embryonic stem cell line derived from the common marmoset monkey (*Callithrix jacchus*) exhibiting germ cell-like characteristics. *Hum. Reprod.* 24, 1359–1372.
- Navara, C.S., Redinger, C., Mich-Basso, J., Oliver, S., Ben-Yehudah, A., Castro, C., and Simerly, C. (2007). Derivation and

- characterization of nonhuman primate embryonic stem cells. *Curr. Protoc. Stem Cell Biol.* Chapter 1, Unit 1A 1.
- Nishikawa, S., Goldstein, R.A., and Nierras, C.R. (2008). The promise of human induced pluripotent stem cells for research and therapy. *Nat. Rev. Mol. Cell Biol.* 9, 725–729.
- Pandrea, I., Silvestri, G., and Apetrei, C. (2009). AIDS in african nonhuman primate hosts of SIVs: A new paradigm of SIV infection. *Curr HIV Res* 7, 57–72.
- Pau, K.Y., and Wolf, D.P. (2004). Derivation and characterization of monkey embryonic stem cells. *Reprod. Biol. Endocrinol.* 2, 41.
- Sasaki, E., Hanazawa, K., Kurita, R., Akatsuka, A., Yoshizaki, T., Ishii, H., Tanioka, Y., Ohnishi, Y., Suemizu, H., Sugawara, A., et al. (2005). Establishment of novel embryonic stem cell lines derived from the common marmoset (*Callithrix jacchus*). *Stem Cells* 23, 1304–1313.
- Schoenemann, P.T., Sheehan, M.J., and Glotzer, L.D. (2005). Prefrontal white matter volume is disproportionately larger in humans than in other primates. *Nat. Neurosci.* 8, 242–252.
- Schultz, C., Hubbard, G.B., Rub, U., Braak, E., and Braak, H. (2000). Age-related progression of tau pathology in brains of baboons. *Neurobiol. Aging* 21, 905–912.
- Shedlock, D.J., Silvestri, G., and Weiner, D.B. (2009). Monkeying around with HIV vaccines: using rhesus macaques to define 'gatekeepers' for clinical trials. *Nat. Rev. Immunol.* 9, 717–728.
- Simerly, C.R., Navara, C.S., Castro, C.A., Turpin, J.C., Redinger, C.J., Mich-Basso, J.D., Jacoby, E.S., Grund, K.J., McFarland, D.A., Oliver, S.L., et al. (2009). Establishment and characterization of baboon embryonic stem cell lines: An Old World Primate model for regeneration and transplantation research. *Stem Cell Res.* 2, 178–187.
- Suemori, H., and Nakatsuji, N. (2006). Generation and characterization of monkey embryonic stem cells. *Methods Mol. Biol.* 329, 81–89.
- Suemori, H., Tada, T., Torii, R., Hosoi, Y., Kobayashi, K., Imahie, H., Kondo, Y., Iritani, A., and Nakatsuji, N. (2001). Establishment of embryonic stem cell lines from cynomolgus monkey blastocysts produced by IVF or ICSI. *Dev. Dyn.* 222, 273–279.
- Takahashi, K., and Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126, 663–676.
- Takahashi, K., Tanabe, K., Ohnuki, M., Narita, M., Ichisaka, T., Tomoda, K., and Yamanaka, S. (2007). Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 131, 861–872.
- Thomson, J.A., Kalishman, J., Golos, T.G., Durning, M., Harris, C.P., Becker, R.A., and Hearn, J.P. (1995). Isolation of a primate embryonic stem cell line. *Proc. Natl. Acad. Sci USA* 92, 7844–7848.
- Thomson, J.A., Kalishman, J., Golos, T.G., Durning, M., Harris, C.P., and Hearn, J.P. (1996). Pluripotent cell lines derived from common marmoset (*Callithrix jacchus*) blastocysts. *Biol. Reprod.* 55, 254–259.
- Thomson, J.A., Itskovitz-Eldor, J., Shapiro, S.S., Waknitz, M.A., Swiergiel, J.J., Marshall, V.S., and Jones, J.M. (1998). Embryonic stem cell lines derived from human blastocysts. *Science* 282, 1145–1147.
- Turleau, C. (2008). Monosomy 18p. *Orphanet J. Rare Dis.* 3, 4.
- Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B.C., Remm, M., and Rozen, S.G. (2012). Primer3—new capabilities and interfaces. *Nucleic Acids Res.* 40, e115.
- VandeBerg, J.L., Williams-Blangero, S., and Tardif, S. (2009). *The Baboon in Biomedical Research*. New York, NY: Springer, p. 391.
- Varum, S., Momcilovic, O., Castro, C., Ben-Yehudah, A., Ramalho-Santos, J., and Navara, C.S. (2009). Enhancement of human embryonic stem cell pluripotency through inhibition of the mitochondrial respiratory chain. *Stem Cell Res.* 3, 142–156.
- Wu, Y., Zhang, Y., Mishra, A., Tardif, S.D., and Hornsby, P.J. (2010). Generation of induced pluripotent stem cells from newborn marmoset skin fibroblasts. *Stem Cell Res.* 4, 180–188.
- Xu, C., Inokuma, M.S., Denham, J., Golds, K., Kundu, P., Gold, J.D., and Carpenter, M.K. (2001). Feeder-free growth of undifferentiated human embryonic stem cells. *Nat. Biotechnol.* 19, 971–974.
- Ye, J., Coulouris, G., Zaretskaya, I., Cutcutache, I., Rozen, S., and Madden, T.L. (2012). Primer-BLAST: A tool to design target-specific primers for polymerase chain reaction. *BMC Bioinformatics* 13, 134.
- Yu, J., Vodyanik, M.A., Smuga-Otto, K., Antosiewicz-Bourget, J., Frane, J.L., Tian, S., Nie, J., Jonsdottir, G.A., Ruotti, V., Stewart, R., et al. (2007). Induced pluripotent stem cell lines derived from human somatic cells. *Science* 318, 1917–1920.

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