

# STEM CELLS®

## **Canine Embryo-Derived Stem Cells—Toward Clinically Relevant Animal Models for Evaluating Efficacy and Safety of Cell Therapies**

Marlon R. Schneider, Heiko Adler, Joachim Braun, Beate Kienzle, Eckhard Wolf and Hans-Jochem Kolb

*Stem Cells* 2007;25;1850-1851

DOI: 10.1634/stemcells.2006-0357

**This information is current as of July 22, 2007**

The online version of this article, along with updated information and services, is located on the World Wide Web at:

<http://www.StemCells.com/cgi/content/full/25/7/1850>

STEM CELLS®, an international peer-reviewed journal, covers all aspects of stem cell research: embryonic stem cells; tissue-specific stem cells; cancer stem cells; the stem cell niche; stem cell genetics and genomics; translational and clinical research; technology development.

STEM CELLS® is a monthly publication, it has been published continuously since 1983. The Journal is owned, published, and trademarked by AlphaMed Press, 318 Blackwell Street, Suite 260, Durham, North Carolina, 27701. © 2007 by AlphaMed Press, all rights reserved. Print ISSN: 1066-5099. Online ISSN: 1549-4918.

 **AlphaMed Press**

# Canine Embryo-Derived Stem Cells—Toward Clinically Relevant Animal Models for Evaluating Efficacy and Safety of Cell Therapies

MARLON R. SCHNEIDER,<sup>a</sup> HEIKO ADLER,<sup>b</sup> JOACHIM BRAUN,<sup>c</sup> BEATE KIENZLE,<sup>c</sup> ECKHARD WOLF,<sup>a</sup> HANS-JOCHEM KOLB<sup>b</sup>

<sup>a</sup>Institut für Molekulare Tierzucht und Biotechnologie, Genzentrum der LMU München, Munich, Germany;

<sup>b</sup>Klinische Kooperationsgruppe Hämatopoetische Zelltransplantation, GSF-Nationales Forschungszentrum für Umwelt und Gesundheit GmbH, und Medizinische Klinik III der LMU München, Munich, Germany; <sup>c</sup>Chirurgische und Gynäkologische Kleintierklinik, Tierärztliche Fakultät der LMU München, Munich, Germany

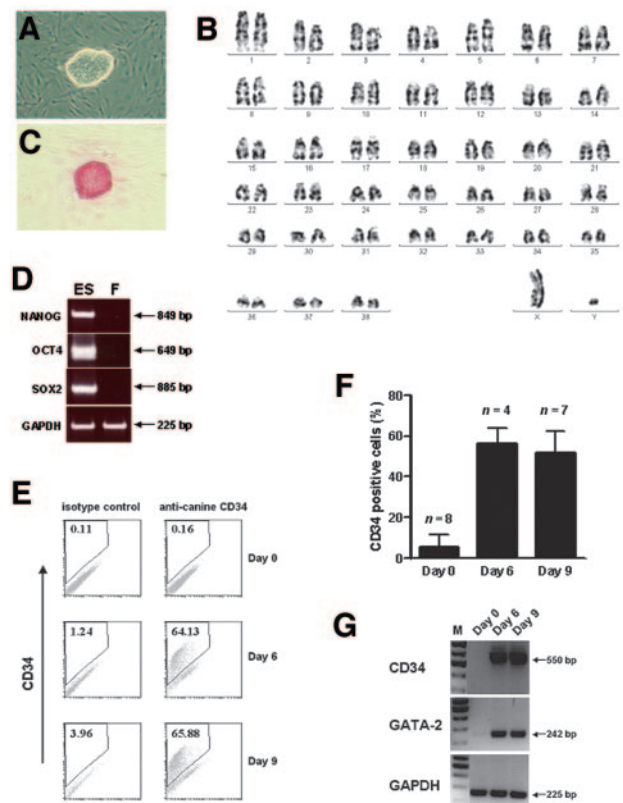
**Key Words.** Embryonic stem cells • In vitro differentiation • Dog • Hematology

Pluripotent embryonic stem (ES) cells, which are available in mouse [1, 2] and human [3], are permanent cell lines that can differentiate into cell types of all three germ layers. Therefore, ES cells have a remarkable potential for both basic research and clinical applications toward replacement of degenerated or malignant cells. From the perspective of hematology, where stem cell therapies are most advanced, ES cells have a number of advantages over conventional sources of transplantable material. They can be expanded indefinitely in vitro, and, more importantly, they can be obtained from a bank representing major haplotype combinations [4] or may even be derived by reprogramming somatic cells from individual patients. Proof of principle for this “therapeutic cloning” concept has been provided in the mouse [5]; however, translation of ES cell-based therapeutic strategies to clinical application requires larger animal models for predictive efficacy and safety studies.

The dog has been used for preclinical studies of stem cell transplantation, and many techniques have been derived from canine studies [6]. Furthermore, techniques applicable to canine cells and dogs can be applied to man, taking into account known biologic properties of the dog [7]. Since the dog is the ideal preclinical model for testing new therapies for many human diseases, the availability of canine embryo-derived stem cells for in vitro differentiation studies will be of great value for the development of new therapies, especially in hematology. Moreover, nuclear transfer from canine somatic cells is possible [8], providing the opportunity to evaluate the concept of “therapeutic cloning” in a clinically relevant animal model.

In a recent report, Hatoya and colleagues [9] describe the isolation of two ES-like cell lines from canine blastocysts. The cell lines were shown to exhibit characteristic ES-like morphology and expression of pluripotency markers. Importantly, the cells formed embryoid bodies in suspension culture, which differentiated upon adhesive culture into various cell types, including neuron-like, epithelium-like, fibroblast-like, melanocyte-like, and myocardium-like cells, demonstrating that these cells are indeed pluripotent. Unfortunately, it was not possible to maintain the undifferentiated phenotype of the cell lines beyond passage 8.

We have performed similar studies that confirm the possibility of establishing canine embryo-derived cell lines and, more importantly, demonstrate for the first time that these cells can be



**Figure 1.** Canine embryo-derived stem cells. (A): Characteristic ES-like morphology. (B): Karyotype analysis indicating the presence of a Y chromosome. (C): Alkaline phosphatase activity. (D): Reverse-transcriptase polymerase chain reaction (PCR) showing mRNA expression of pluripotency markers NANOG, OCT4, and SOX2. (E, F): Fluorescence-activated cell sorting analysis showing increasing expression of canine CD34 after coculture with the murine cell line OP9. (G): Reverse-transcriptase PCR showing expression of *CD34* and *GATA2* mRNA after coculture (same cells as in [E, F]). Abbreviations: bp, base pairs; ES, canine ES-like cells; F, canine embryonic fibroblasts; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; M, marker.

Correspondence: Marlon R. Schneider, D.V.M., Institute of Molecular Animal Breeding and Biotechnology, Gene Center, University of Munich, Feodor-Lynen-Str. 25, 81377 Munich, Germany. Telephone: +49 89 218076815, Fax: +49 89 218076849, e-mail: schneider@imb.uni-muenchen.de Received June 10, 2006; accepted for publication March 29, 2007. ©AlphaMed Press 1066-5099/2007/\$30.00/0 doi: 10.1634/stemcells.2006-0357

differentiated into hematopoietic stem cells. Eight blastocysts were obtained from a Golden Retriever bitch by flushing the uterine horns after ovariectomy. After mechanical removal of the embryonic coats, the blastocysts were cultured individually on mitotically-inactivated mouse embryonic fibroblasts in 48-well plates (for further details, see supplemental online Methods). From one blastocyst, colonies exhibiting typical ES-like morphology (Fig. 1A) were obtained. At this stage, the cells could be maintained independent of mouse feeder cells but formed autologous feeders as previously described for human ES cells [10]. Cytogenetic analysis confirmed the canine origin of the cells, which contained a Y chromosome (Fig. 1B). The ES-like cells exhibited alkaline phosphatase activity (Fig. 1C) and expressed NANOG, OCT4, and SOX2, the most important pluripotency-associated transcription factors for mouse and human ES cells (Fig. 1D). Sequencing confirmed that the obtained polymerase chain reaction (PCR) products correspond to canine sequences. Furthermore, in agreement with the report by Hatoya et al. [9], the canine ES-like cells showed expression of SSEA-1 but were negative for SSEA-4 (data not shown).

To differentiate the canine embryo-derived stem cells into hematopoietic progenitor cells, a similar method as described for human ES cells by Kaufman et al. [11] was applied. Canine embryo-derived stem cells were cocultured with irradiated OP9 murine bone marrow stroma cells. At defined time points, the cells were harvested and subjected to fluorescence-activated cell sorting (FACS) analysis using an anti-canine CD34 monoclonal antibody or an isotype control antibody. Whereas only very few cells stained positive for CD34 at day 0, approximately 50% of the canine cells exhibited CD34 expression at days 6 and 9 (Fig. 1E, 1F). To further demonstrate differentiation toward the hematopoietic lineage, the mRNA expression of *CD34* and *GATA2*, encoding a transcription factor specific for hematopoietic progenitor cells, was investigated by reverse transcriptase-PCR analysis (Fig. 1G). Expression of both genes was hardly detectable at day 0 but increased substantially after 6 and 9 days

of coculture, consistent with the FACS data. To test the ability of the cells harvested from the cocultures to grow as colonies in colony-forming units (CFUs), the cells were cultured in 6-well plates. After 14 days, colonies were enumerated by light microscopy. Although cells from day 0 of coculture never did grow to colonies, cells from day 9 of coculture gave rise to CFU-M, CFU-E, CFU-G, and CFU-GM (0 colonies with cells from day 0 vs. 23 colonies with cells from day 9 of coculture) in one experiment. In another experiment, the responsiveness of the cells to hematopoietic growth factors was tested. Cells harvested from day 6 of coculture proliferated in response to a mixture of hematopoietic growth factors known to support the growth of canine hematopoietic progenitor cells [12], whereas much less proliferation was observed with cells harvested from day 0 of coculture (data not shown). Importantly, our in vitro differentiation results were obtained using cells at passages 10–12. Although the possibility of unlimited culture of these cells and their suitability for transplantation purposes remains to be demonstrated, we provide proof of principle of the practicability of this promising strategy.

## ACKNOWLEDGMENTS

The authors thank the Else Kröner-Fresenius-Stiftung (Bad Homburg, Germany) for financial support. The excellent technical assistance by Steffen Schiller and Gisela Werner is also acknowledged. M.R.S. and H.A. contributed equally to this work.

## DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicate no potential conflicts of interest.

## REFERENCES

- Evans MJ, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. *Nature* 1981;292:154–156.
- Martin GR. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proc Natl Acad Sci U S A* 1981;78:7634–7638.
- Thomson JA, Itskovitz-Eldor J, Shapiro SS et al. Embryonic stem cell lines derived from human blastocysts. *Science* 1998;282:1145–1147.
- Kyba M, Daley GQ. Hematopoiesis from embryonic stem cells: Lessons from and for ontogeny. *Exp Hematol* 2003;31:994–1006.
- Rideout WM 3rd, Hochedlinger K, Kyba M et al. Correction of a genetic defect by nuclear transplantation and combined cell and gene therapy. *Cell* 2002;109:17–27.
- Kolb HJ, Gunther W, Schumm M et al. Adoptive immunotherapy in canine chimeras. *Transplantation* 1997;63:430–436.
- Neff MW, Rine J. A fetching model organism. *Cell* 2006;124:229–231.
- Lee BC, Kim MK, Jang G et al. Dogs cloned from adult somatic cells. *Nature* 2005;436:641.
- Hatoya S, Torii R, Kondo Y et al. Isolation and characterization of embryonic stem-like cells from canine blastocysts. *Mol Reprod Dev* 2006;73:298–305.
- Stojkovic P, Lako M, Stewart R et al. An autogeneic feeder cell system that efficiently supports growth of undifferentiated human embryonic stem cells. *STEM CELLS* 2005;23:306–314.
- Kaufman DS, Hanson ET, Lewis RL et al. Hematopoietic colony-forming cells derived from human embryonic stem cells. *Proc Natl Acad Sci U S A* 2001;98:10716–10721.
- Weber M, Lange C, Gunther W et al. Minor histocompatibility antigens on canine hemopoietic progenitor cells. *J Immunol* 2003;170:5861–5868.



See [www.StemCells.com](http://www.StemCells.com) for supplemental material available online.

**Canine Embryo-Derived Stem Cells—Toward Clinically Relevant Animal Models for Evaluating Efficacy and Safety of Cell Therapies**  
Marlon R. Schneider, Heiko Adler, Joachim Braun, Beate Kienzle, Eckhard Wolf and Hans-Jochem Kolb  
*Stem Cells* 2007;25;1850-1851  
DOI: 10.1634/stemcells.2006-0357

**This information is current as of July 22, 2007**

**Updated Information  
& Services**

including high-resolution figures, can be found at:  
<http://www.StemCells.com/cgi/content/full/25/7/1850>

**Supplementary Material**

Supplementary material can be found at:  
<http://www.StemCells.com/cgi/content/full/25/7/1850/DC1>

 **AlphaMed Press**