



Molecular and functional characterization of rabbit embryonic stem cells

Pierre Osteil, Suzy S. Markossian, Murielle Godet, Thierry Joly, Pierre Savatier, Marielle Afanassieff

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Rediscovering Pluripotency: from Teratocarcinomas to Embryonic Stem Cells

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PROGRAMME & ABSTRACTS

VENUE & DATE:

National Museum Cardiff, Wales
10-12 October 2011

INVITED SPEAKERS:

- Peter Andrews
- Philip Avner
- Ivan Damjanov
- Sir Martin Evans
- Rolf Kemler
- Barbara Knowles
- Gail Martin
- Virginia Papaioannou
- Martin Pera
- Janet Rossant
- Austin Smith
- Davor Solter
- Peter Stern

ORGANIZERS:

Peter Andrews and Abcam

The event will be supported by:



The European
Cancer Stem Cell
Research Institute



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Poster Index

- Poster 1 Marielle Afanassieff
Contrasting features of embryonic and induced pluripotent stem cells in rabbit
- Poster 2 Sharmini Alagaratnam
Pluripotency in malignancy: differential gene expression between embryonal carcinomas and embryonic stem cells
- Poster 3 Juan Aréchaga
Transplant of ES cells into seminiferous tubules as a model to study germ cell tumor invasion mechanisms
- Poster 4 Ivana Barbaric
Survival of the fittest: single-cell behaviour of normal and culture-adapted human embryonic stem cells
- Poster 5 Vladimir Botchkarev
p63 Regulates satb1 to control tissue-specific chromatin remodelling in epidermal progenitor cells during development
- Poster 6 Pierre-Yves Bourillot
KLF4 and KLF5 play specific roles in the inhibition of ES cell differentiation into extra-embryonic endoderm and mesendoderm
- Poster 7 Mihaela Culmes
Inhibition of G9a histone methyltransferase is positively influencing the reprogramming of adipose derived mesenchymal stem cells into cells with endothelial features
- Poster 8 Miho K Furue
Development of a novel drug screening system using human iPS cells in a defined culture system
- Poster 9 Terri Gaskell
Development of robust, scalable, and synthetic systems for the maintenance of pluripotency and subsequent differentiation
- Poster 10 Vincent Giudice
Contrasting patterns of pluripotency in embryonic stem cells overexpressing KLF4 and KLF5
- Poster 11 David Harley
Role of DLK-1 in the adaptation of human embryonic stem cells
- Poster 12 Jean Harrington
The complexity of translation
- Poster 13 Neil J Harrison
The role of COPS3 in human embryonic stem cell fate
- Poster 14 Kate Hawkins
The role of E-cadherin in mouse embryonic stem cell self-renewal
- Poster 15 Anne Jørgensen
Vitamin D induces differentiation in testicular germ-cell tumour-derived cell lines
- Poster 16 Zoya Katarova
Expression regulation of GAD forms in mouse ES and P19 cells
- Poster 17 Shalinee Khadun
Support of undifferentiated human embryonic stem cell lines by human cell lines

- Poster 18 Andrijana Klajn
SOX2-overexpressing NT2/D1 cell clones: establishment and initial characterization
- Poster 19 Alison Kraft
The blood stem cell, c. 1945-1995: A biography
- Poster 20 Michaela Kunova
Efficient and reversible high-density monolayer culture of human pluripotent stem cells increases rate of teratoma formation
- Poster 21 Pierre Osteil
Molecular and functional characterization of rabbit embryonic stem cells
- Poster 22 Luke Piggott
Suppression of c-FLIP expression selectively sensitizes breast cancer initiating cells to TRAIL/APO2-L-induced killing independently of hormone receptor status
- Poster 23 Pierre Savatier
The brevity of the G1 phase is an intrinsic determinant of the naive state of pluripotency
- Poster 24 Jill L. Shepherd
Stemformatics: a portal to bridge the gap between stem cell research and bioinformatics
- Poster 25 Abhishek Sinha
The OCIA domain protein Asrij is required to maintain embryonic stem cell pluripotency
- Poster 26 Mike Storm
Zscan4 regulation and function in murine embryonic stem cells
- Poster 27 Yann Tapponnier
Fluorescent ubiquitination-based cell cycle indicator (FUCCI) applied to the study of the pluripotent stem cell cycle
- Poster 28 Sebastian Vencken
Mechanisms of expression and regulation of SOX2 and its targets in two embryonal carcinoma cell lines
- Poster 29 Patompon Wongtrakoongate
Reprogramming nullipotent state by induced pluripotency

Poster 21

Molecular and functional characterization of rabbit embryonic stem cells

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In order to obtain Embryonic Stem Cell (ESC) lines suitable for transgenesis in Rabbits, we isolated 180 Inner Cell Masses (ICMs) from New Zealand GFP-transgenic blastocysts and plated them onto growth-inactivated murine embryonic fibroblasts in a medium supplemented either with FGF2, LIF, sodium butyrate (SB), Kenpaullone (KP), SB + LIF, or KP + LIF. Four lines were derived in the presence of FGF2 (16% of plated ICMs), and two lines were derived in the presence of LIF (5% of plated ICMs). All six lines displayed a flattened morphology and were positive for alkaline phosphatase expression. They all expressed the pluripotency markers Oct4 and Nanog. They were also positive for both SSEA1 and E-cadherin, which are expressed in mouse ES cells, as well as for SSEA4, Tra-1-60 and N-cadherin, which are expressed in primate ES cells and mouse EpiSCs. Noteworthy, the percentage of SSEA1-positive cells varied between ESC lines, ranging from 5% in LIF-dependent ESC lines to 50% in some FGF2-dependent lines. SSEA4 was only expressed in FGF2-dependent lines. All ESC lines were able to make teratomas after injection beneath the kidney capsule in SCID mice, and to differentiate into ectodermal, mesodermal, and endodermal derivatives. No sign of ICM colonization by GFP-positive cells was evidenced after injection of FGF2-dependent ESCs into rabbit pre-implantation embryos. By contrast, mouse ESCs injected into rabbit 8-cell stage embryos efficiently colonized the blastocyst (15 positive embryos out of 15), indicating that the rabbit embryo is permissive to colonization by ESCs that self-renew in the naïve state of pluripotency. LIF-dependent rabbit ESCs readily differentiated upon LIF withdrawal, indicating that LIF signaling stimulates self-renewal. Of note, LIF-dependent ESC lines displayed a low rate of growth and a high rate of spontaneous differentiation. The capacity of these LIF-dependent ESCs to colonize the rabbit pre-implantation embryo is currently being examined.

In a second step, we attempted to generate rabbit ESCs that self-renew in a state closer to the ground state of pluripotency. To this aim, 330 eight-cell stage rabbit embryos were cultured to the blastocyst stage in the presence of the MEK inhibitor PD0325901, followed by ICM isolation by immunosurgery, and plating onto various substrates (growth-inactivated mouse fibroblasts, gelatin, fibronectin) in N2B27 or DMEM/F12 medium supplemented with PD0325901 and CHIR99021 (2i+LIF). Most ICMs plated and gave rise to primary outgrowths, which could be passaged once. No difference was observed between outgrowths that originated from PD0325901-treated and untreated embryos. However, none of these outgrowths survived beyond passage 1. These results indicate that the pluripotent stem cells of the rabbit ICM are unable to sustain self-renewal in the ground state of pluripotency, using the culture conditions previously described in rodents.

Notes: