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Molecular and functional characterization of rabbit embryonic stem cells

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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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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 naive 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: