Molecular and functional characterization of rabbit embryonic stem cells

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

Rediscovering Pluripotency: from Teratocarcinomas to Embryonic Stem Cells

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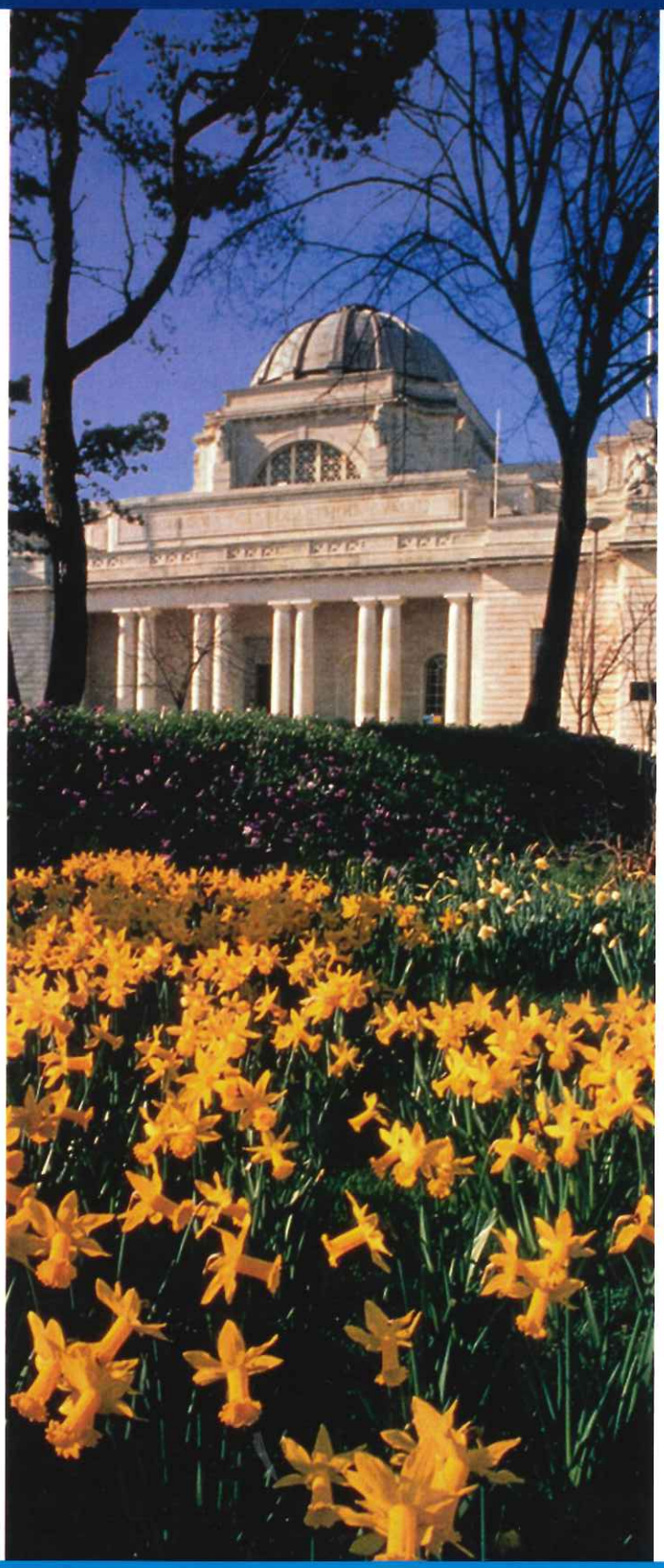

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Poster Index

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

Rediscovering pluripotency: from teratocarcinomas to embryonic stem cells conference
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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 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: