Contrasting features of embryonic and induced pluripotent stem cells in rabbit

Marielle Afanassieff, Yann Tapponnier, Pierre Osteil, Suzy S. Markossian, Murielle Godet, Agnieszka Bernat, Pierre Savatier

Rediscovering Pluripotency: from Teratocarcinomas to Embryonic Stem Cells

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PROGRAMME & ABSTRACTS

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National Museum Cardiff, Wales
10-12 October 2011

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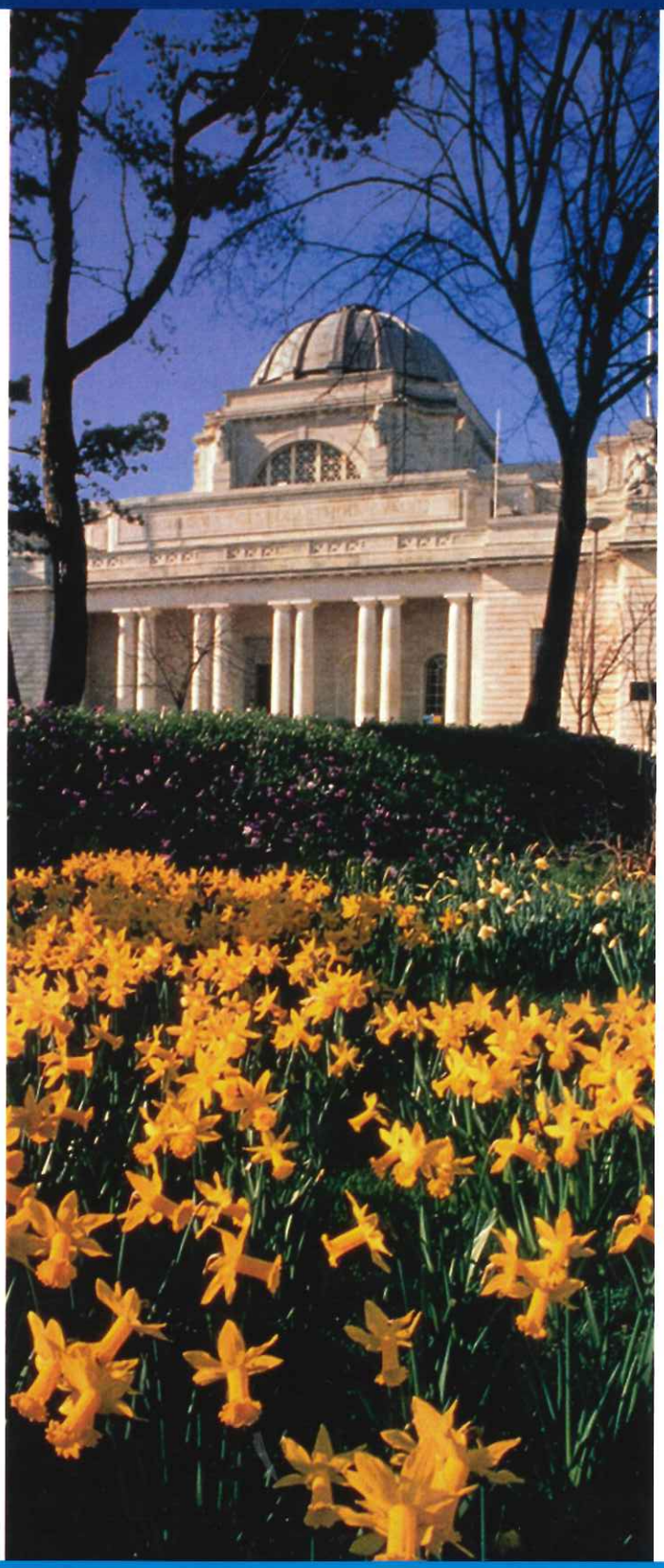

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Poster Abstracts

Poster 1

Contrasting features of embryonic and induced pluripotent stem cells in rabbit

Afanassieff, M.*, Tapponnier, Y., Osteil, P., Markossian, S., Godet, M., Bernat, A., Savatier P.

Stem Cell and Brain Research Institute, INSERM U846, Bron, France

In order to develop the induced Pluripotent Stem Cell (iPSC) technology in rabbits, we generated four rabbit iPSC lines, making use of retroviral vector that express human Oct4, Sox2, Klf4 and c-Myc to reprogram ear adult fibroblasts. The overall efficiency of iPSC derivation was estimated to 5.10^{-4}. All four lines expressed the cardinal markers of pluripotent stem cells: (*i*) they were positive for alkaline phosphatase activity; (*ii*) they expressed the pluripotency-associated Oct4 and Nanog transcription factors, as well as the SSEA-1, SSEA-4, Tra1-60 and E-Cadherin cell surface markers; (*iii*) they displayed a normal karyotype (44XX), and (*iv*) they could form teratomas containing tissues of ectodermal, mesodermal and endodermal origin upon injection under the kidney capsule in SCID mice. After 25 passages, expression of all four transgenes was fully repressed in three lines out of the four analysed, indicating complete reprogramming of fibroblasts into iPSCs. Self-renewal of rabbit iPSCs was dependent on FGF2 signaling. We compared the rabbit iPSC lines with the rabbit Embryonic Stem Cell (ESC) lines derived in the laboratory (see accompanying poster by Osteil et al.). Contrary to rabbit ESCs, rabbit iPSCs could be regularly passaged by trypsine dissociation in single cell suspension. Like mouse ESCs and rabbit ICM cells, rabbit iPSCs showed strong fluorescence after infection with EOS, a lentiviral vector expressing the Green Fluorescent Protein (GFP) under the control of the naïve state-specific distal enhancer of the mouse *Pou5f1* (*Oct4*) gene, whereas rabbit ESCs did not. Moreover rabbit iPSCs displayed cell-cycle features characteristics of pluripotent stem cells, including a short G1 phase, and the lack of DNA damage checkpoint in G1 phase like murine and primate ESC lines. In contrast, rabbit ESCs exhibited a dramatically longer G1 phase. Moreover, a fraction of the SSEA-positive ESC population constantly exhibited a DNA damage checkpoint in G1 like somatic cells. Altogether, these results indicate that rabbit iPSCs self-renew in a slightly more primitive state of pluripotency, as compared to their embryonic counterpart. Preliminary experiments indicate that rabbit iPSCs have the capacity to colonize the rabbit pre-implantation, albeit at a low frequency, suggesting that some rare iPSCs display the features of naïve pluripotency.

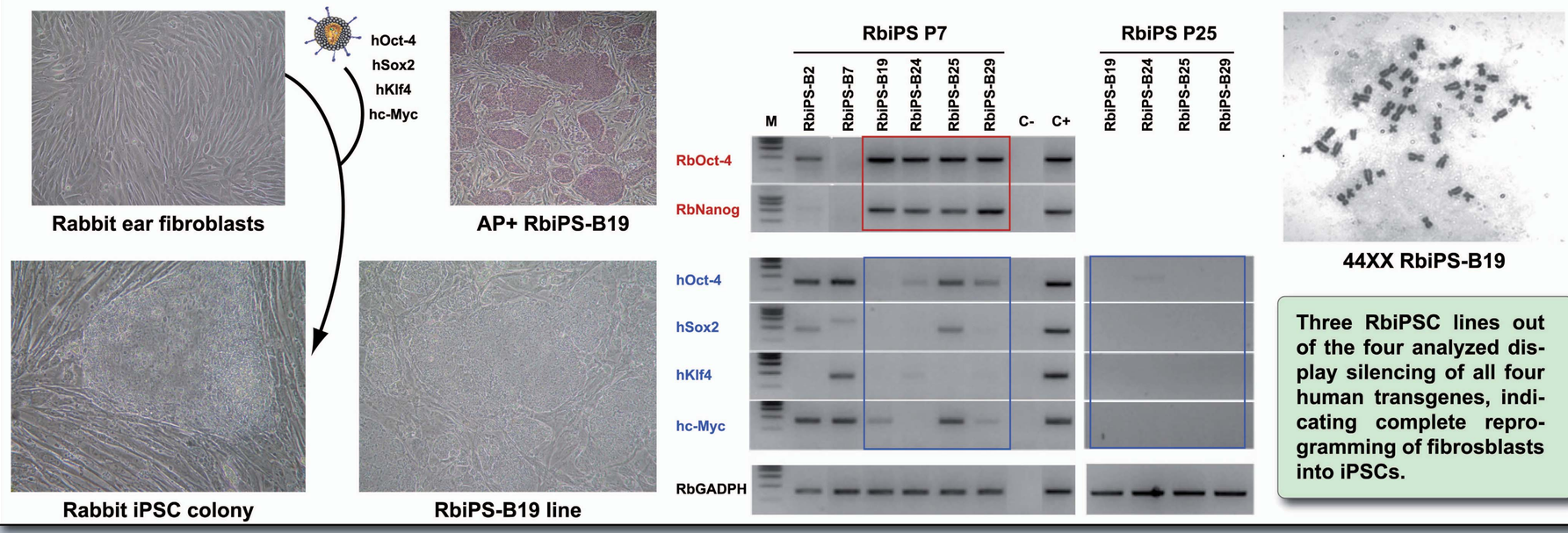
CONTRASTING FEATURES OF EMBRYONIC AND INDUCED PLURIPOTENT STEM CELLS IN RABBIT

Marielle AFANASSIEFF, Yann TAPPONNIER, Pierre OSTEIL, Suzy MARKOSSIAN
Murielle GODET, Agnieska BERNAT, Thierry JOLY & Pierre SAVATIER
Stem Cell and Brain Research Institute, INSERM U846, Bron, France

INTRODUCTION

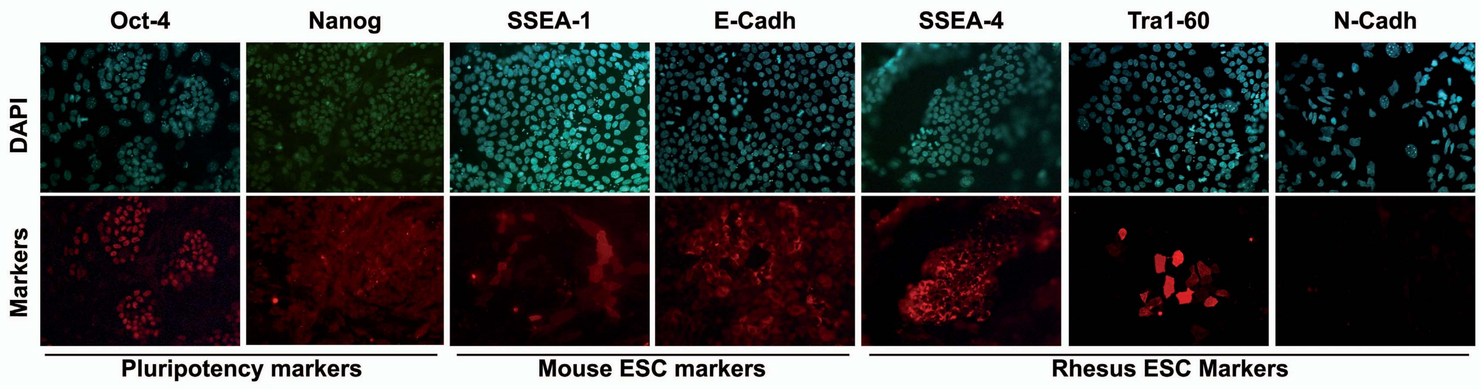
In order to develop the induced Pluripotent Stem Cell (iPSC) technology in rabbits, we generated four rabbit iPSC lines, making use of retroviral vector that express human Oct-4, Sox2, Klf4 and c-Myc to reprogram ear adulte fibroblasts. The overall efficiency of iPSC derivation was estimated to 5.10^{-4}. All four lines expressed the cardinal markers of pluripotent stem cells. Self-renewal of rabbit iPSC lines was dependent of FGF2 signaling and rabbit iPSCs could be regularly passaged by trypsine dissociation in single cell suspension. The goal of this study was to compare the rabbit iPSC lines with the rabbit Embryonic Stem Cell (ESC) lines derived in the laboratory (see accompanying poster by Osteil et al.).

1/ Generation of four rabbit iPSC lines

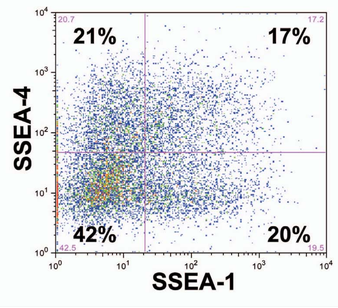


Three RbiPSC lines out of the four analyzed display silencing of all four human transgenes, indicating complete reprogramming of fibrosblasts into iPSCs.

2/ Pluripotency and cell surface markers

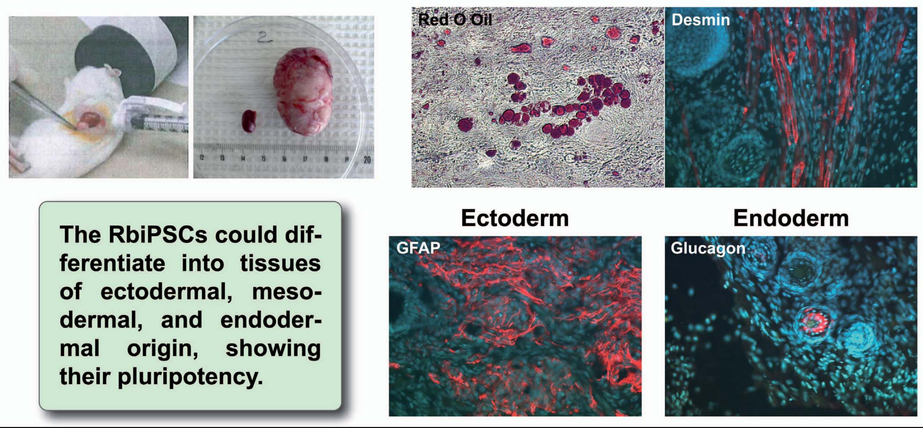


Lines	mESC	RhESC	RbESC	RbF	RbiPS-B19	RbiPS-B24	RbiPS-B29
Oct-4	+	+	+	-	+	+	+
Nanog	+	+	+	-	+	+	+
SSEA-1	+	-	+	-	+	+	+
E-Cadh	+	+	+	-	+	+	+
SSEA-4	-	+	-	-	+	+	+
Tra1-60	-	+	-	-	+	+	+
N-Cadh	-	+	+	-	-	-	+



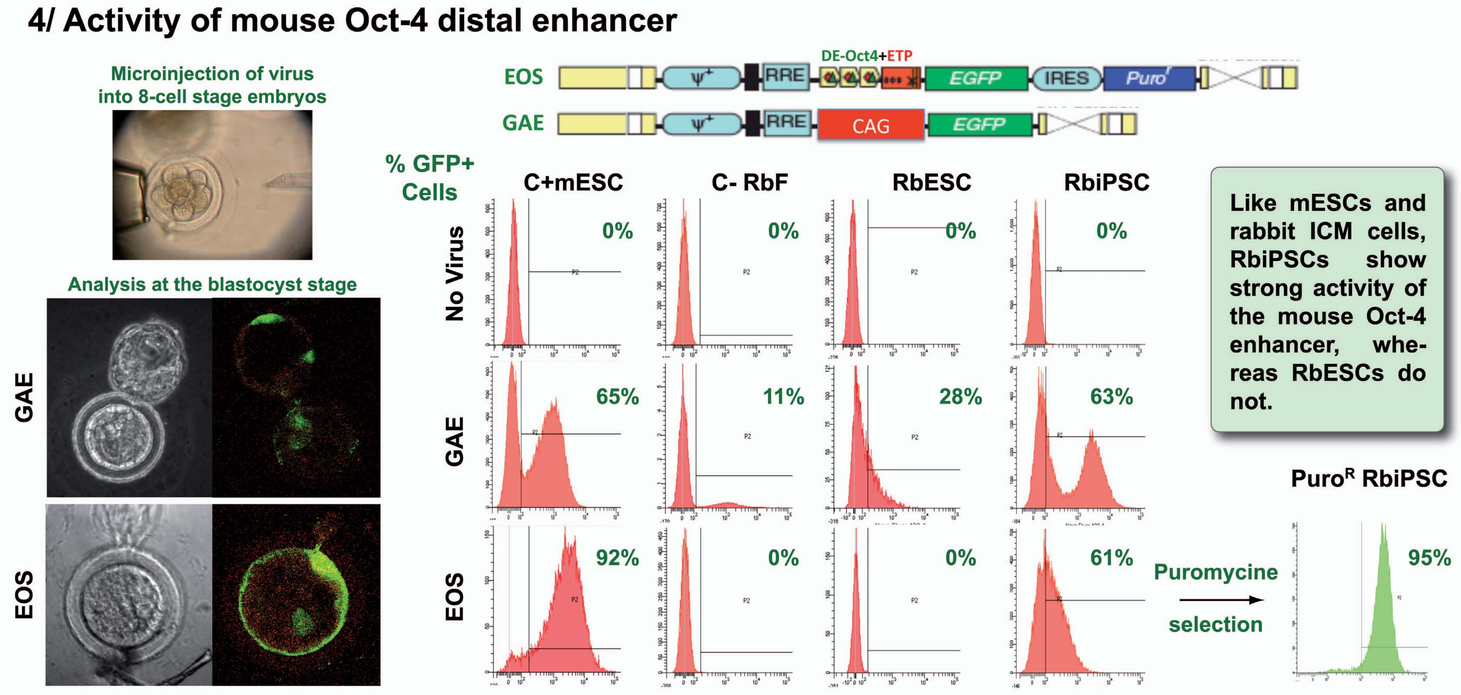
Contrary to RbESC lines, RbiPSC lines express both SSEA-1 and SSEA-4 markers.

3/ Teratoma formation



The RbiPSCs could differentiate into tissues of ectodermal, mesodermal, and endodermal origin, showing their pluripotency.

4/ Activity of mouse Oct-4 distal enhancer



Like mESCs and rabbit ICM cells, RbiPSCs show strong activity of the mouse Oct-4 enhancer, whereas RbESCs do not.

5/ Cell cycle and DNA damage checkpoints

RbiPSCs display cell-cycle features characteristics of pluripotent stem cells, including a short G1 phase and the lack of DNA damage checkpoint in G1 phase. In contrast, RbESCs exhibit a dramatically longer G1 phase and a G1 checkpoint.

6/ Colonization of the pre-implantation embryo by SSEA-1+ RbESC and RbiPSC

RbiPSCs have the capacity to colonize the rabbit pre-implantation embryo, albeit at low frequency. RbESCs do not show the same capacity.

CONCLUSIONS

1. Rabbit ESCs display molecular and functional properties that resemble those of primate ESCs. By contrast, rabbit iPSCs seem closer to mouse ESCs (activity of the distal enhancer of Oct-4, resistance to single cell dissociation with trypsine).
2. Rabbit iPSCs have the capacity to colonize the pre-implantation embryo, albeit at a low frequency, suggesting that some rare iPSCs display the features of naive pluripotency.

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