GLIMPSE INTO CELL REPROGRAMMING: EMERGING ROLE OF PROGESTERONE RECEPTOR IN PRIMING POTENCY OF INDUCED PLURIPOTENT STEM CELLS (iPSCs)

Michele Manganelli[1] - Mattia Bugatti[2] - Rosalba Monica Ferraro[1] - Elena Laura Mazzoldi[1] - Marta Parigi[1] - Ginetta Collo[3] - Gabriele Stocco[4] - William Vermi[5] - Luigi Mori[6] - Silvia Giliani[1]

[1] "Angelo Nocivelli" Institute for Molecular Medicine, Department of Molecular and Translational Medicine, University of Brescia, Italy, ASST Spedali Civili, Brescia, Italy, 25123, Italy.
[2] ASST Spedali Civili, University of Brescia, Brescia, 25123, Italy.
[3] Division of Farmacology, Department of Molecular and Translational Medicine, University of Brescia, Brescia, 25123, Italy.
[4] Department of Life Sciences, University of Trieste, Trieste, I-34127, Italy.
[5] Department of Molecular and Translational Medicine, University of Brescia, Italy, ASST Spedali Civili, Brescia, Italy, 25123, Italy.
[6] Department of Clinical and Experimental Sciences (DSCS), University of Brescia, Brescia, 25123, Italy.

m.manganelli@unibs.it

Introduction. Reprogramming technologies enable cells to enter an embryonic pluripotent stem cell (ESC)-like state, resulting in the generation of Induced Pluripotent stem cells (iPSCs). iPSCs share many key properties with ESCs as pluripotency, self-renewal, embryoid bodies (EBs) formation and similar gene expression profile. Steroids hormone-related receptors as estrogen (ERα) and progesterone receptor (PR) are expressed in blastocyst. In particular, progesterone is essential for the differentiation of ESCs during human embryonic development. Interestingly, the DNA-repair tumor suppressor protein BRCA1 interacts with and regulates ERα and PR transcriptional activation. We explored the expression pattern of ERα and PR in iPSCs generated with four different independent reprogramming methods (Table 1) and corresponding EBs.

Table 1. List of iPSCs cell lines used in this study.

iPSCs cell line	Parental cell	Reprogramming Method
Episomal (cat. n. A18945)	cord blood-derived CD34+ progenitors	Episomal Vector
BJ	human foreskin fibroblasts	CytoTune-iPS 2.0 Sendai Reprogramming Kit
253-G1	human fibroblasts	Retroviral trasduction
F3	human fibroblasts	Lentiviral trasduction

Results. As assessed by real-time PCR (qPCR), ERα and PR mRNA were low expressed in the iPSCs cells (Fig.1), showing expression downregulation ($p<0.001$) during 8 days EBs differentiation (Fig.2) into the three embryonic germ layers (PAX6: ectoderm, α-SMA: mesoderm and GATA4: endoderm). We strongly highlighted by immunohistochemistry (Fig.3) and immunofluorescent (Fig.4) staining the expression of PR protein in the nucleous of all the different iPSCs, while ERα was not detected. Fibroblasts parental cells (Fig.5), as well as CD34+ hematopoietic stem and progenitor cells (HSPCs) lack the expression of ERα and PR proteins. Indeed, the expression of PR in iPSCs differed of 8-fold increase compared to CD34+ HSPCs (Fig.6).

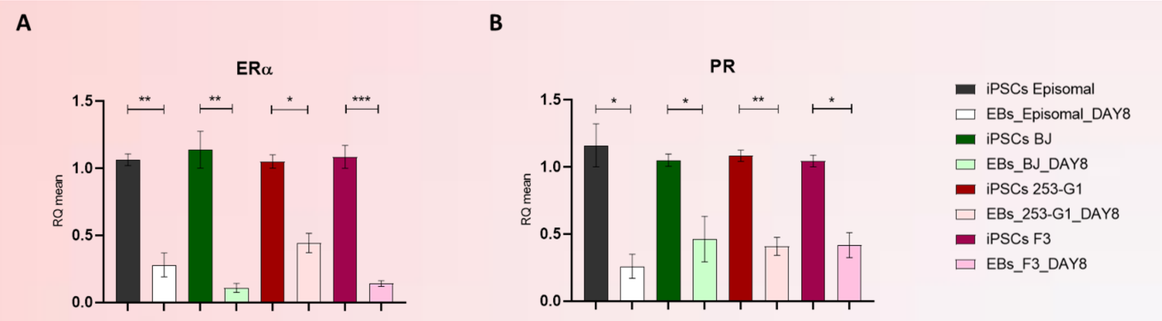

Figure 1. Expression profile of ERα and PR in iPSCs and EBs. A) ERα mRNA was significantly downregulated following EBs generation compared to their parental iPSCs cell lines. B) PR mRNA was significantly downregulated during EBs generation compared to their parental iPSCs cell lines. Student's t-test. *p<0.05, **p<0.01. ***p<0.001. Histograms represent relative quantification (RQ) of three independent experiments, while error bars represent ± SEM.

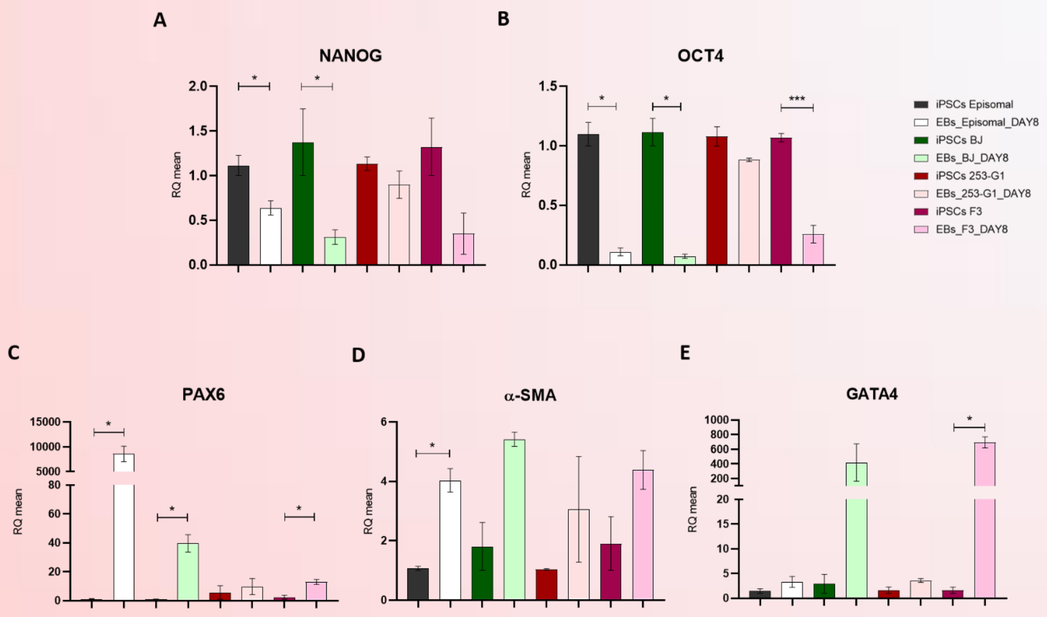

Figure 2. Expression profile of pluripotency and germ layers markers in iPSCs and corresponding EBs. Pluripotency markers A) NANOG and B) OCT4 were downregulated, while markers of C) ectoderm (PAX6), D) mesoderm (α-SMA) and E) endoderm (GATA4) showed a trend of upregulation following EBs formation compared to their parental iPSCs cell lines. Student's t-test. *p<0.05, **p<0.01, ***p<0.001. Histograms represent relative quantification (RQ) of three independent experiments, while error bars represent ± SEM.

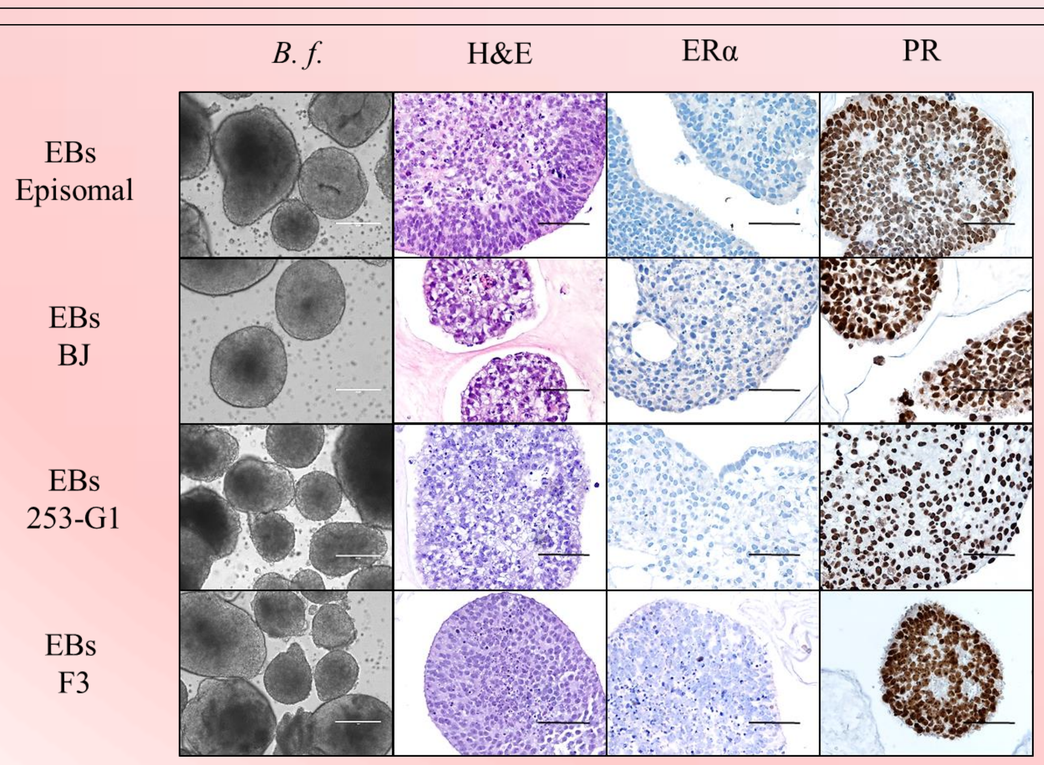

Figure 3. H&E and IHC stain on EBs at day 8. Bright field (*B.f.*), H&E and IHC staining of the EBs of the different iPSCs cell lines. ERα protein was not expressed in EBs, while PR protein was expressed. Bright field scale bar is 200µm, while IHC scale bar is 100µm.

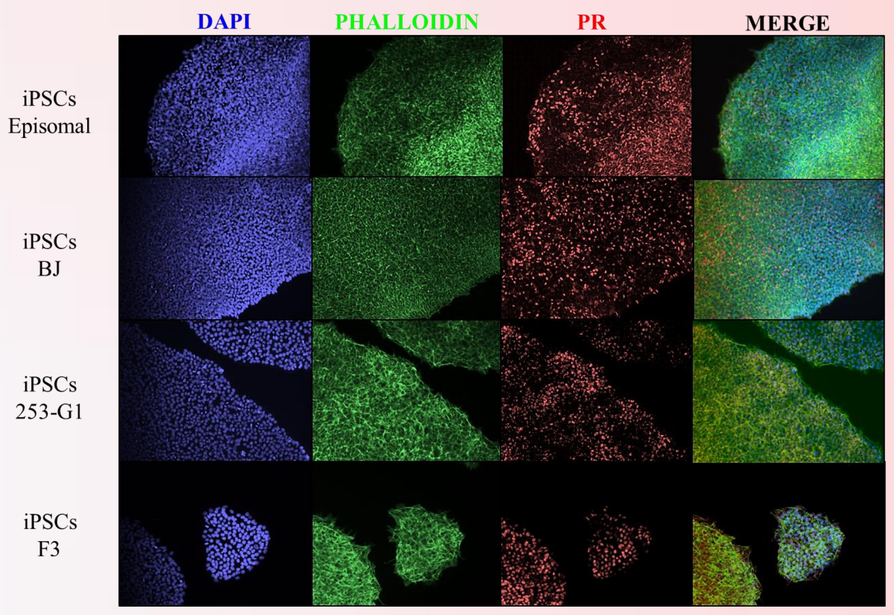

Figure 4. Detection of PR in iPSCs cell lines. Immunofluorescent staining for the detection of PR among the different iPSCs cell lines. Nuclei were counterstained in blue (DAPI), while cytoskeleton in green (phalloidin-488) and PR in red (Alexa-647). Magnification 10X.

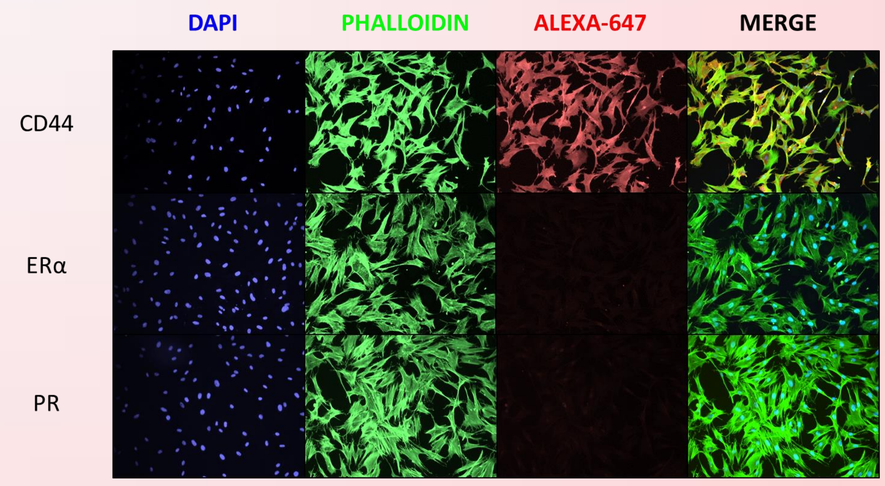

Figure 5. Immunofluorescent staining for the detection of CD44, ERα and PR in BJ human foreskin fibroblast. Nuclei were counterstained in blue (DAPI) and cytoskeleton in green (phalloidin-488), while CD44, ERα and PR in red (Alexa-647). Magnification 10X.

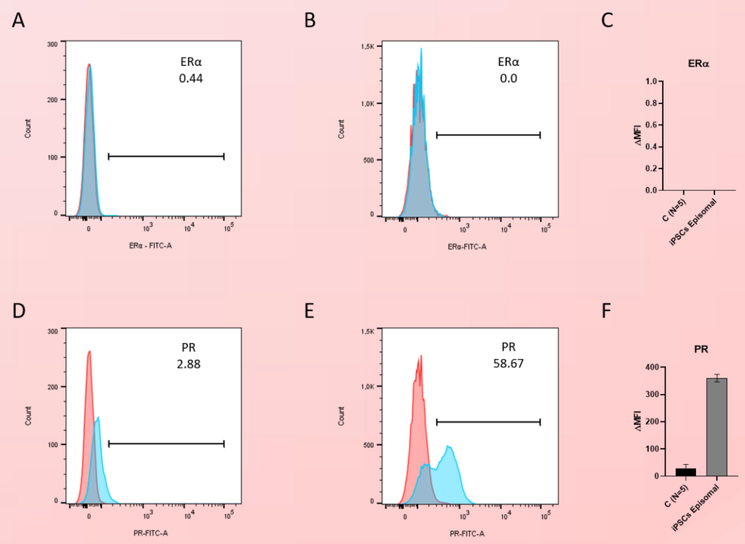

Figure 6. Flow cytometry analysis for the detection of ERα and PR in mobilized-PB compared to iPSCs. A) Representative image of the expression of ERα gated on CD34 in G-CSF mobilized-PB (C=control); B) Representative image of the expression of expression of ERα in episomal iPSCs, red indicated aspecific fluorescence, while blue indicated labelled-FITC target; C) Histogram representing ΔMFI= mean fluorescence intensity of ERα between PB compared to episomal iPSCs, error bars represent ± SEM from at least three independent experiments; D) Expression of PR gated on CD34 in PB; E) Representative image of the expression of PR in episomal iPSCs, red indicated aspecific fluorescence, while blue indicated labelled-FITC target; F) Histogram representing ΔMFI= mean fluorescence intensity of PR between PB compared to episomal iPSCs, error bars represent ± SEM from at least three independent experiments.

Conclusion. Our results demonstrated for the first time the presence of PR in iPSCs, underling their close relation to ESCs, and suggesting a possible role of PR in priming pluripotency, a state that immediately precedes germ layer specification and differentiation.