Derivation of induced pluripotent stem cells from an infertile boar carrying a reciprocal translocation

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New insights in pig pluripotency by two approaches of cell reprogramming

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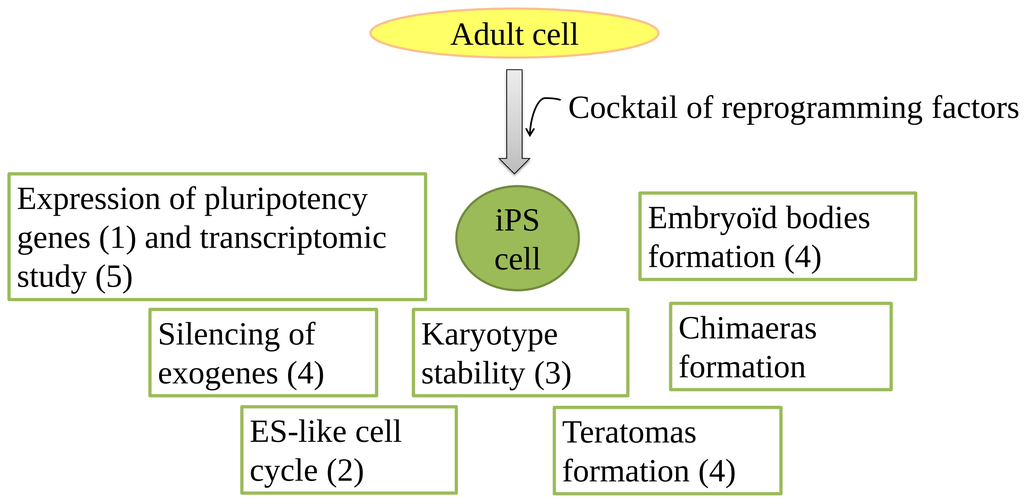
INTRODUCTION

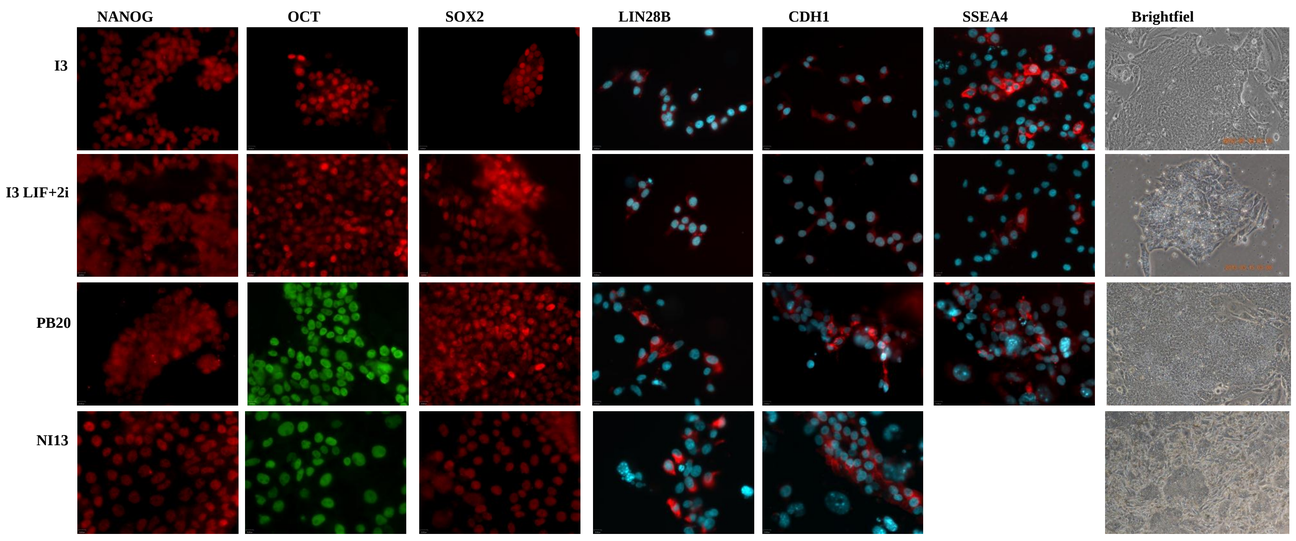
Derivation of germ cells from embryonic (ES) or induced (iPS) pluripotent stem cells in mouse and human is a great advance for the study of germ cell biology in the earlier step of development and provide an *in vitro* model for the analysis of new mechanisms leading to infertility.

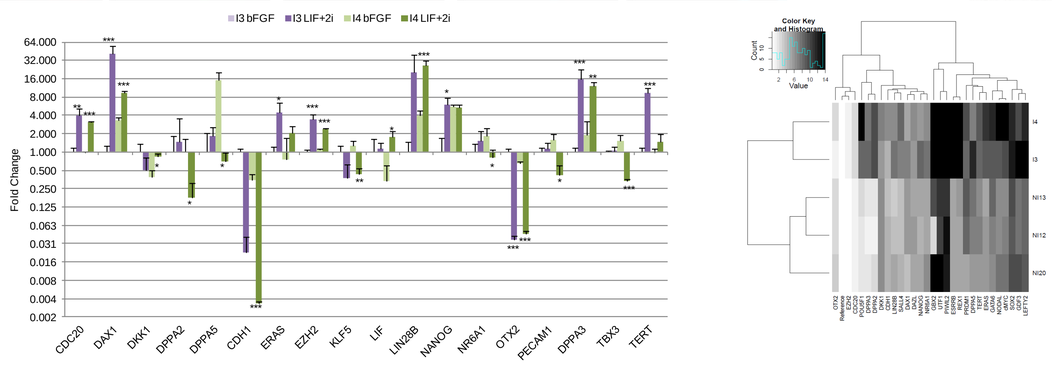
Based on recent advances in pig iPS production, we propose here t**o create a library of porcine iPS derived from fibroblasts of infertile boars carrying chromosomal rearrangements**. Using two systems of cell reprogramming, we produced and characterized iPS-like cell lines from fibroblasts with a t(Y;14) reciprocal translocation. Gene expression analysis, differentiation assays and cytogenetic analysis were used to assess the reprogramming efficiency of both protocols and the pluripotency level of the cell lines. This study gives new insights on pig pluripotency of which mechanisms have not been completely solved yet.



Two approaches :
Integrative viral infection → translocated lines I3 and I4 and control line PB20
Non-integrative viral infection → translocated lines NI12, NI13 and NI20

1. EXPRESSION OF PLURIPOTENCY GENES



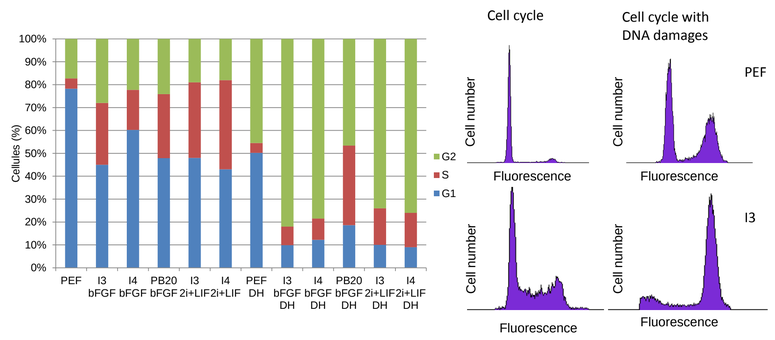


• Both I and NI lines express the core pluripotency genes.

• Integrative lines are maintained in LIF+2i culture media which promotes induction of naïve pluripotency.

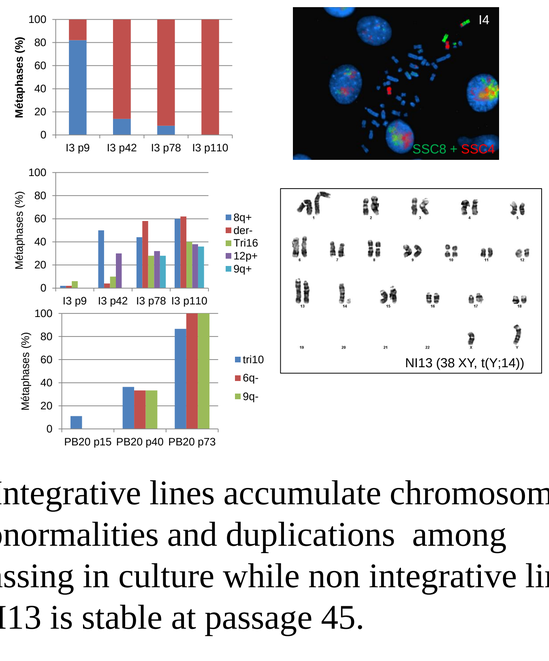
• Pluripotency genes network in non integrative lines is more activated.

2. ES-LIKE CELL CYCLE



• Integrative lines have an ES-like cell cycle characterized by a short G1 phase and absence of the G1/S DNA damage checkpoint.

3. KARYOTYPE ANALYSIS



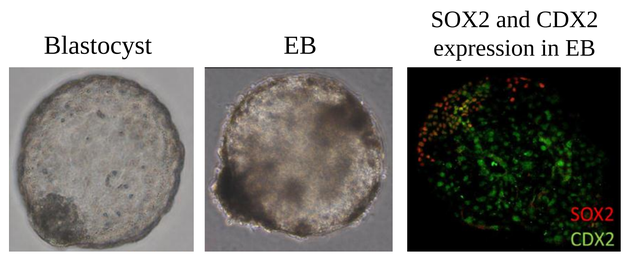
• Integrative lines accumulate chromosomal abnormalities and duplications among passing in culture while non integrative line NI13 is stable at passage 45.

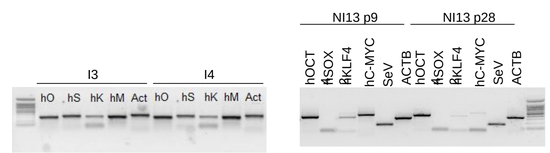
4. DIFFERENTIATION ASSAYS : EBs and teratomas formation

• Integrative lines have a poor differentiation potential : teratoma assay led to carcinoma formation.

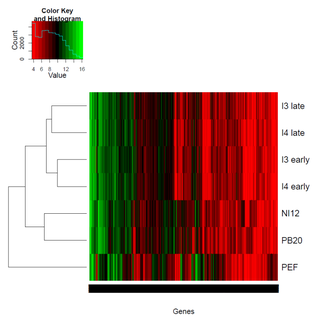
• NI lines form embryoid bodies resembling pig blastocysts in morphology and gene expression.

• Continuous transgene expression could prevent cells to complete reprogramming and blocks cell differentiation.





5. TRANSCRIPTOMIC STUDY



• Clustering of iPS-like cell lines compared to fibroblasts.

• Up-regulation of cell-cycle related genes in reprogrammed lines.

• Differential expression of genes involved in organogenesis, embryo development, cancer and pluripotency pathways between I and NI lines.

• Up-regulation of lipid metabolism in embryoïd bodies.

CONCLUSION AN PROSPECTS

We used two different reprogramming systems (I and NI) to produce iPS-like cells from t(Y;14) fibroblasts coming from an infertile boar. These cells lines share key pluripotency features with mouse and human iPS cells, like the **expression of core pluripotency genes** and the **specificity of their cell cycle**. Depending on the culture conditions, I cell lines express genes of both **primed and naïve pluripotency**. Those cells have a **poor differentiation** potential and accumulate easily **chromosomal abnormalities**.
NI lines on the contrary are **karyotypically stable** over time, show a slow **repression of exogenous reprogramming factors**, seems to deeper **activate the pluripotency network** even in primed culture conditions and form **blastocyst-like embryoïd bodies**.
Further studies are needed to break down the barriers blocking the complete reprogramming of pig somatic cells and access to ground pluripotency : epigenetic barrier, loss of transgene expression, role of different signaling pathways (lipid metabolism) in pluripotency and requirement to extrinsic factors for cell differentiation.

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