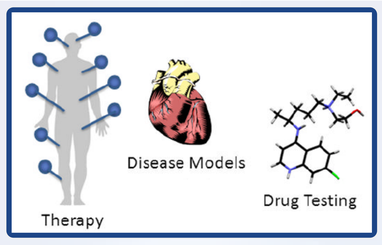
iPSC vs ESC: A tale of two pluripotent cells

Gerard Llimós Aubach

Universitat Autònoma de Barcelona

INTRODUCTION

Induced pluripotent stem cells (iPSC) are obtained from somatic cells by transcription factor reprogramming: Oct4, Sox2, Klf4 and c-Myc (Yamanaka's cocktail).



They have similar molecular and functional properties to embryonic stem cells (ESC).

But , are they identically?

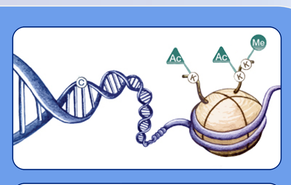
Unfortunately, there are some variations between these two cell types that can affect the functionality of iPSC. Areas where we can find differences are morphology, proteome, transcriptome, epigenetics, genome and differentiation potential. The aim of this work is to discuss the most relevant variations reported recently and which are suposed to be the main causes of these changes.

DIFFERENTIAL CHARACTERISTICS

Epigenetics

Source cell memory

Expression of tissue specific genes: unmethylated CpG, H3Ac and H3K4m3 (transcriptionally active).



Silencing of other genes: methylated CpG and H3K27m3 (repressor factors).

- Memory loss needs culture passages (p10-16).
- Important bottleneck of reprogramming and directly related to **differentiation potential.**

Genome mutations

Aneuploidy

Chr 12, 8 and X (Human)
Chr 8 and 11 (Mouse)

- Growth advantage.
- Causes: source cell and in vitro culturing.

Single nucleotid variation

>1000 SNV/iPSC line (Human)
>100 SNV/iPSC line (Mouse)

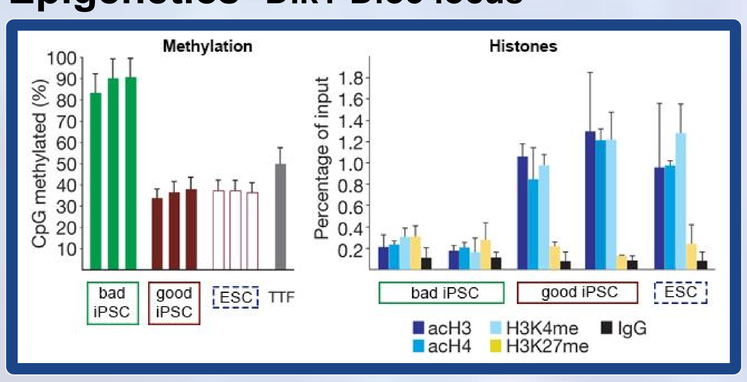
- Mutation in exons: <12/iPSC line.
- Little important feature.
- Inherited from source cell.

Copy number variation

200 CNV/line. Most common around Nanog (Chr 12) and DNMT3B (Chr 20) (Human). Deletion of suppressor genes.

- Mutation by NHEJ and selected in vitro propagation (growth advantage).
- Causes: mainly source cell. 2% CNV due to in vitro culturing and replication stress.
- Stable and independent of reprogramming methodology.
- Differentiation potential not altered.

Epigenetics Dlk1-Dio3 locus



MOUSE

- This locus is regulated by imprinting.
- Its silencing impedes mice development.
- Causes: in vitro culturing and reprogramming.
- Prevention: ascorbic acid in medium.

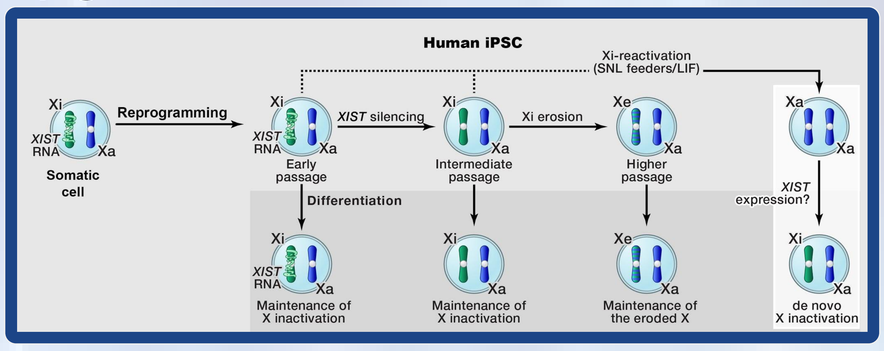
◄ *Stadtfeld M et al. Nature 2010; 465:175–181.*

HUMAN

- In vitro culturing is tha main problem of X chr inactivation.

Papp B and Plath K. Cell 2013; 152:1324-1343. ►

Epigenetics X Chromosome

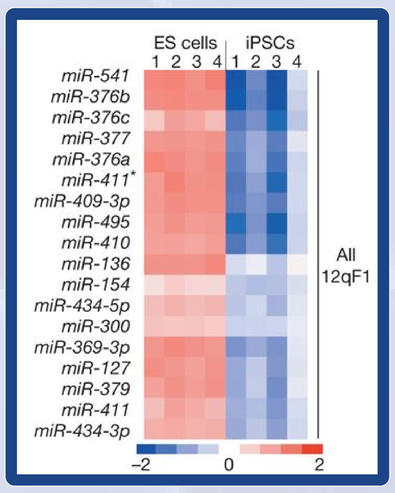


Transcriptome

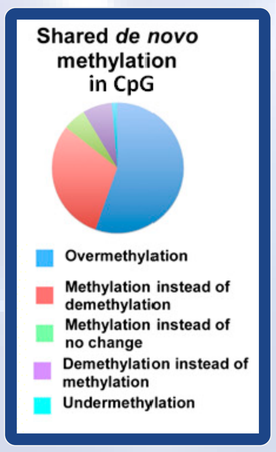
miRNA 106a~363 and 290~295 clusters + miR-200c-3p

- Few differences in transcriptome.
- miRNA profile can distinguish the state of partially reprogrammed lines.
- Causes: reprogramming and in vitro culturing.
- More expressed in mESC than miPSC.
- Their downregulation is associated with low efficiency.
- miRNAs can be good pluripotency markers.

Dlk1-Dio3 cluster



▲ *Stadtfeld M et al. Nature 2010; 465:175–181.*



Aberrant DNA methylation and histone modification

- Hypermethylation in CpG & CpG islands. Regions related to binding sites of TF KLF4 and FOXL1.
- Large-scale hotspots (100kb-1'3Mb) in subtelomeric regions caused by incomplet 5-hydroxymethylation. Culture induces the repressive histone modification H3K9m3 that blocks de novo methylation. Generally is a random process.
- Culture passages increase aberrant methylation in imprinted genes. Hypermethylation brings total silencing and hypomethylation produces loss of imprinting (LOI).

◄ *Ruiz S et al. PNAS 2012; 109:16196–16201.*

Transcriptome

TMEM132C TMEM132D TCERG FZD10 DPP6 FAM19A5

- These genes commonly have different expression between hiPSC and hESC.
- Causes: culture and reprogramming.

CONCLUSION

There are differences between ESC and iPSC. They can have distinct origins: inherited from donor somatic cells, induced or selected by the reprogramming process or acummulated during culture passages. A lot of changes are not specific of iPSC because are also seen in ESC (all related to in vitro culturing). This suggests that reprogramming efficiency could rapidly increase with the improvement of medium cultures. Certain alterations can change iPSC properties and their derivatives:

Tumorigenesis **Immunogenicity** **↓ Differentiation potential**

These changes can be used to select bona fide iPSC which have undergone a perfect reprogramming. So, it is important to:

- **Detect and monitor variations.**
- **Optimize reprogramming strategy and culture conditions.**

STATE OF THE ART

Omics

- Genome-wide DNA sequencing
- RNA-seq microarray
- Bisulphite pirosequencing
- ChIP-chip and ChIP-seq
- Immunocytochemistry

Pluripotency fingerprints

iPSC screening

Database (like Pluritest for gene expression)

Bioinformatic tools

Select good quality iPSC

Fast
Exhaustive
Reliable
Cheap