Instituto de Medicina Molecular

Master Project Proposal

Title: Comprehensive study of the stability of genomic imprinting during iPSC reprogramming and differentiation

State of the art

For a long time, it was thought that an epigenetic barrier would impede the conversion of a differentiated cell into an undifferentiated one. However, Yamanaka and colleagues (2006) were able to surpass that barrier and reprogram differentiated cells all the way to the pluripotent state generating the so-called induced pluripotent stem cells (iPSCs) [1]. This was also a medical breakthrough since it gave the unprecedented possibility to reprogram any patient cell to model diseases and increase the hope for personalized regenerative cell therapies.

The challenge of reprogramming is to switch off the differentiated cellular program and reset it into the pluripotent state. Despite the great advances in reprogramming technology, the process remains inefficient and prone to epigenetic errors [2]. Indeed, genome-wide studies revealed substantial epigenetic changes between iPSCs and embryonic stem cells (ESCs), their natural counterpart [2, 3]. The source of errors are several, from failed epigenetic remodeling of the differentiated program to mistakes inadvertently introduced during the process which often impact on the process of genomic imprinting (imprinting for short) [2], an important read-out of successful reprogramming.

Imprinting is a process that causes a few clustered genes to be expressed from only one of the two parental alleles. This is determined by differential epigenetic marking of the two parental alleles (*e.g.*, at the level of DNA methylation), which is faithfully inherited in somatic cells [4]. Imprinting errors causes several developmental disorders such as the Angelman syndrome (AS), only inherited when the maternal allele of the UBE3A gene is affected [5]. Imprinting errors occur frequently during iPSC reprogramming and they impact negatively on the pluripotent capacity of the iPSCs [6, 7]. The stability of imprinting during iPSC reprogramming has not been systematically evaluated neither in mouse or human. The preservation of the imprinting status inherited from the donor cell is a requirement for the safe use of iPSCs in disease modeling and regenerative medicine. This is particularly important in the case of iPSCs derived from patients with disorders associated with genomic imprinting, whereby imprinting status and allele-specific gene expression patterns inherited from the donor cell should be maintained.

Objectives

In this master project proposal, we will evaluate the stability of imprints during both mouse iPSC reprogramming and during neuronal differentiation of human iPSCs derived from Angelman patients. It has two specific aims:

1. Monitor the stability of imprinting status during mouse iPSC reprogramming

2. Monitor the stability of imprinting status during neuronal differentiation of iPSCs derived from AS patients

Plan and Methods

1. Imprinted gene expression is often disturbed in mouse iPSCs, being a read-out for incorrect reprogramming [6, 7]. To better appreciate the status of imprints during reprogramming is necessary to start with a donor cell harboring DNA polymorphisms to

distinguish the two parental alleles and have a system where reprogramming can be well controlled. For this purpose, we will use a mouse model with a doxycycline (DOX)-inducible cassette encoding the four pluripotent reprogramming factors inserted on its genome (called i4F-B mouse) [8]. The i4F-B mouse will be crossed to the Mus Musculus Castaneus mouse strain to generate a polymorphic i4F-B mouse harboring DNA sequence polymorphisms to distinguish the two parental alleles for expression and epigenetic studies. Embryonic fibroblasts from this mouse will be collected and reprogramming will be induced *in vitro* in a controlled fashion. This system will allow us to determine the number, nature and timing of imprinting errors.

Techniques involved:

- *Tissue culture*: culture of primary cells, iPSC reprogramming;
- *Molecular biology*: FACS sorting, RNA/DNA extractions, DNA methylation analysis (bisulfite sequencing), allelic expression analysis (RT-PCR, RT-qPCR, RNA FISH)

2. The advent of the iPSCs gives the unprecedented possibility to reprogram any patient cell to model diseases and increase the hope for personalized regenerative medicine. In this second objective, we will reprogram cells from patients with AS, a neuro-developmental imprinting disorder [5]. The generated iPSCs will then be differentiated into neurons, the cell type affected by the disorder to model the disease *in vitro*. Since this is an imprinting disease, imprinting status of the locus affected by the disease will be monitored in human iPSCs and also after neuronal differentiation to make sure that the pattern received from the donor cell is maintained.

Techniques involved:

- *Tissue culture*: human iPSC culture, neuronal differentiation
- *Molecular biology*: RNA/DNA extractions, DNA methylation analysis (bisulfite sequencing), allelic expression analysis (RT-PCR, RT-qPCR, RNA FISH)

Expected outcomes and perspectives

This project offers the first comprehensive analysis of the stability of genomic imprinting during mouse iPSC reprogramming. We will describe the common errors and when they are likely to happen. This will open prospects on interventions, such as epigenetic modulation on reprogramming, to overcome the frequency of imprinting errors.

We will also monitor imprinting on human iPSCs and during neuronal differentiation from AS patients. These are essential steps to guarantee the establishment ofthis *in vitro* system to model this disease and as a platform to screen for therapeutic drugs.

Supervisor: *Simão Rocha, MCFonseca Lab, simaoteixeiradarocha@medicina.ulisboa.pt*
Webpage of the group: https://imm.medicina.ulisboa.pt/en/investigacao/labs/carmo-fonseca-maria-lab/

Remunerated or volunteer training: Volunteer training

Bibliography:

1. Takahashi, K. & S. Yamanaka, Cell, 2006. **126**(4): p. 663-76.
2. Robinton, D.A. and G.Q. Daley, **481**(7381): p. 295-305.
3. Kim, K., et al., Nature, 2010. **467**(7313): p. 285-90.
4. da Rocha, S.T. and A.C. Ferguson-Smith, Curr Biol, 2004. **14**(16): p. R646-9.
5. Dagli, A.,et al., Mol Syndromol, 2012. **2**(3-5): p. 100-112.
6. Stadtfeld, M., et al., Nature, 2010. **465**(7295): p. 175-81.
7. Sun, B., et al., 2012. **30**(2): p. 161-8.
8. Abad, M., et al., Nature, 2013. **502**(7471): p. 340-5.