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Induced pluripotent stem cells as genetic disease models

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Abstract

Rare diseases (together representing 1 in 8000) provide a valuable model to study cellular mechanisms involved in health and disease. However, a major obstacle in the study of pathogenic mechanisms and evaluation of new therapeutic approaches is the accessibility of the target cells. Rare diseases of lysosomal accumulation (Lysosomal storage Disorders, LSDs) are multisystemic and exhibit different degrees of involvement, often presenting target cells that can only be obtained through very invasive procedures. The loss of lysosomal integrity influences the entire cellular environment and leads to impairment of cellular function. A few LSD therapies exist aiming at increasing the clearance of lysosomal burden. Through the development of specific and versatile cellular models, we hope to contribute to increasing the choices in terms of therapeutic interventions/correction in cases of rare or common diseases. The set-up of new technologies, with cross-interest in health, is also one of t he aims of the ongoing work.
We can generate induced pluripotent stem cell lines (iPSCs) and induce them into the disease target cells. This approach is most valuable since the genetic background of the patient is maintained and the disease cell targets are obtained through minimally invasive procedures.
Although in the beginning, it is a costly and time-consuming task to achieve, later it becomes easier to carry out. Once the biological material is obtained, vectors are chosen, and conditions are established, obtaining iPSCs becomes a delicate but feasible task. The importance of iPSCs as experimental models is vast since they provide an optimal platform for investigation pathological mechanisms.

Introduction

From Genetics of disease to models and pathogenesis.

Genetics and the genetic study of patients and populations, provided important data concerning the cause of inherited diseases and the risk of specific populations. Carrier screening in specific populations provides the foundations for more effective approaches to precision medicine (1). Lysosomal storage diseases have long been the focus of our group (2). Few lysosomal storage diseases (LSDs) have efficient treatments. Nevertheless, enzyme replacement therapy (ERT, regular supplementation of the defective enzyme) is the most common and effective treatment used to clear the accumulated substrates in patient cells. However, this is a costly life-long treatment with many limits to its cost-effective application. The lack of good models, that mimic the human cell target of the disease, hinders R&D and the understanding of the human pathophysiologic mechanisms. Through iPSC (3) it seems feasible to create promising cell models (Table 1).

LSD	Common mutations in Portugal	Clinical expression-cell targets
Fabry disease	several	Cardiac, renal , cerecrovascular; skin
Metachromatic Leukodystrophy	c.465+1G>A, (IVS2DS+1G>A	Neuronal
GM2 Gangliosidosis	c.533G>A, p.178H	Neuronal

Table 1: Possible LSD targets for iPSC and CRISPR/Cas9.

Main objectives:

1 – Establish hiPSCs models that can be used for better comprehension of the disease and of therapeutic approaches.
2 - Apply CRISPR/Cas9-mediated gene editing: to make disease specific cellular models in order to test therapeutic approaches; to correct causal mutations; or to examine the potential effects of mutations in the cell.

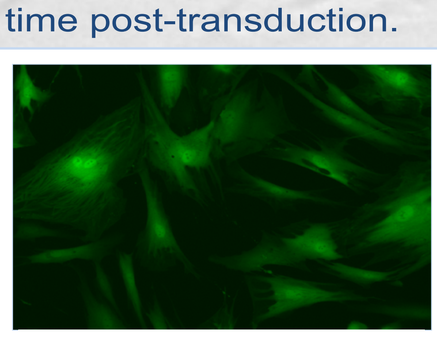
Materials and Methods

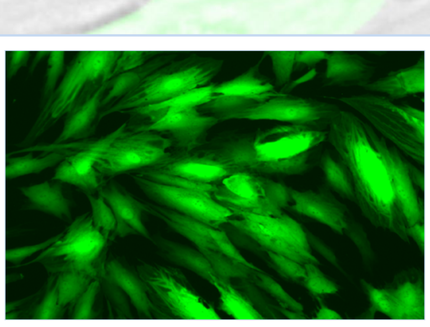
The biological material of choice was human skin fibroblasts. These cells were acquired from commercial suppliers (Thermo Scientific) or obtained with all necessary procedures from a cell bank (Gaslini Institute). After several experiments, the method for delivery of the Yamanaka factors, chosen for forcing the fibroblasts into stem-like cells, was the Sendai virus (Thermo Scientific).

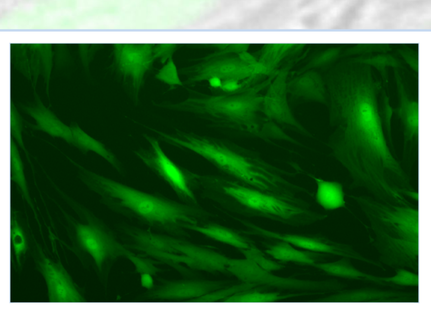
Results

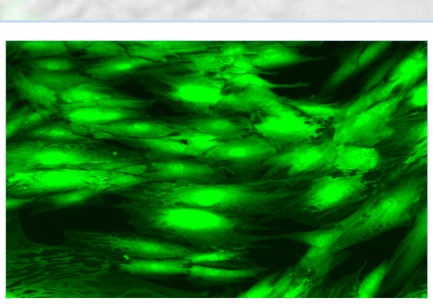
The cells become infected at low MOI (Figure 1) and with minimum vector footprint. The methods pertaining to this work, namely, cell cultures, Sendai infection and other assays were carried out using standard methods and. In the case of kits, the manufacturer's instructions were followed.

Figure 1: HDFa cells transduced with the CytoTune-EmGFP Sendai Fluorescence Reporter at multiplicity of transfection (MOI) 2 and 4. Cells are shown (460X of optical magnification) at MOI 2 or 4 and at the indicated time post-transduction.


HDFa transduced at MOI 2 after 24 hours


HDFa transduced at MOI 2 after 48 hours


HDFa transduced at MOI 4 after 24 hours


HDFa transduced at MOI 4 after 48 hours

Conclusion: hiPSCs have already been used to study some diseases, including LSDs and the results show great potential (4). Hence our work is likely to provide new insights into these disorders.

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Affiliation and Funding

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