SHORTCUT ACROSS CELLS' FATE

A MANUAL FOR TRANSDIFFERENTIATION APPLIED TO DIABETES' TREATMENT

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INTRODUCTION

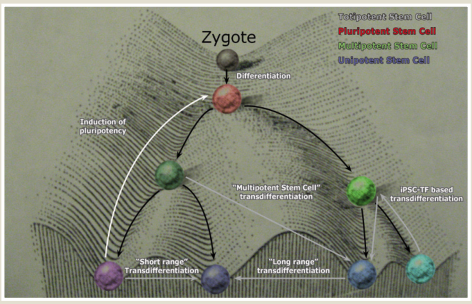
An attractive strategy is arising in the field of cell reprogramming. It consists in the direct conversion or transdifferentiation of terminally differentiated somatic cells into other adult cells, bypassing the pluripotent state. This strategy is useful to side-step recent controversial data derived from the use of *induced Pluripotent Stem Cells*. Although this technology is relatively recent, it stands as a potential method to be applied in the field of regenerative medicine, once having solved the challenges it faces. The aim of this bibliographic research is to develop an updated review of transdifferentiation, highlighting advantages and disadvantages of this method, its achievements, the multiple procedures to transdifferentiate an adult cell and its application in β-cell generation in order to treat type I Diabetes Mellitus.

MATERIALS AND METHODS

In this review, the most recent publications, either reviews or conventional articles, related to transdifferentiation were studied.

- Firstly, a search of the terms *transdifferentiation*, *reprogramming*, *direct conversion* and *β-cell* was held in *Pubmed* and *Sciencedirect*, and the articles found were ordered by preference and impact.
- Secondly, the most important and recent articles were read and summarized, and their bibliography extensively analyzed.
- Finally, high-impact journals were periodically examined in the search of new publications.

GENERAL ASPECTS OF CELL REPROGRAMMING



Waddington's epigenetic landscape is an interesting schematic representation of how differentiation takes place *in vivo* (Fig. 1, black arrows). C. H. Waddington gave emphasis to those points or bifurcations which he named *chreodes*, where a cell must choose a path and therefore become irreversibly committed, in a natural state. In 2006, when Yamanaka turned upside down the concept of reprogramming, several strategies involving transdifferentiation (Fig. 1, grey arrows) were unearthed, such as:

- Short-range transdifferentiation
- Long-range transdifferentiation
- **induced Pluripotent Stem Cell-transcription factor based transdifferentiation**
- Transdifferentiation from multipotent stem cells

Transdifferentiation avoids several problems that induced Pluripotent Stem Cells (iPSC) display, such as, tumorigenesis or difficulty to be maintained iPSC in culture. However, transdifferentiation also faces his own drawbacks, such as formation of unnatural intermediaries[1].

Figure 1. *Image representing Waddington's landscape where different reprogramming techniques are illustrated.*

APPLICATIONS

Successfully reprogrammed cells have three main applications[2], independently of the method by which they have been derived:

- Cell replacement therapy and organogenesis
- Drug discovery and toxicology
- Developmental biology understanding

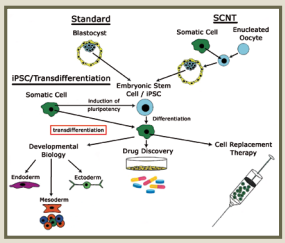


Figure 2. *Schematic representation of regenerative medicine applications.*

ACHIEVEMENTS

Few of the most relevant achievements of transdifferentiation are summed up in Table 1[2,3,4,5], except for those in β-cells, which will be discussed below.

Target cell	Origin cell	Reprograming factor	In vivo or ex vivo	Species
Short range or intralineage transdifferentiation				
Myoblasts	Fibroblasts	*MyoD*	*Ex vivo*	Mouse, rat, human, chicken
Macrophages	B-lymphocytes	*CEBPα&δ*	*Ex vivo*	Mouse
Cardiomiocytes	Fibroblasts	*Gata4, Mef2c, Tbx5*	*In vivo*	Mouse (t)
Long range or interlineage transdifferentiation				
Dopaminergic neurons	Fibroblasts	*Mash1, Nurr1, Lmx1a*	*Ex vivo*	Mouse and human
Transdifferentiation from multipotent stem cells				
Schwann cells	MDSC	*PDGF, NT-3, IGF2*	*Ex vivo*	Mouse
iPSC-TF based transdifferentiation				
Cardiomiocytes	Fibroblasts	iPSC-TF, *BMP4*	*Ex vivo*	Mouse
Neural SC	Fibroblasts	iPSC-TF, *FGF4*	*Ex vivo*	Mouse

Table 1. *Some of the most important achievements of transdifferentiation. MDSC: Muscle-derived stem cells, iPSC-TF: induced pluripotent stem cell transcription factors.*

GENERAL STRATEGIES

A broad understanding of the elements involved in determination and differentiation of lineage-specific stem cells into unipotent cells *in vivo* is the key point to develop a reliable stratey to convert any kind of somatic cell into the target cells (Table 2).

Mechanisms to alter cell fate
Master regulatory factors
RNA interference
Cell cycle regulators
Global activators or repressors
Reprogramming molecules
Chromatin remodelers

Table 2. *Brief list of mechanisms that can be used to determine cell fate.*

Overexpression of transcription factors

Overexpression of a single or a group of transcription factors (TF) is the most utilized method in transdifferentiation. TF that occupy the very top of a regulatory hierarchy, which therefore are not under regulation of other TF, are known as ***master regulatory factors***[6] (MRF), for example, MyoD (Fig. 3). MRF are very useful because, with only one transduction of a viral vector carrying the MRF, a cell can be fully reprogrammed. When more than one TF need to be transferred, it is important to introduce them hierarchically, simulating natural expression, in order to improve efficiency.

Recently, some authors demonstrated that cells could be temporally **co-cultured with iPSC-transcription factor (iPSC-TF) and lineage-specific TF** in order to transdifferentiate cells faster and more efficiently[1,2], compared to classical transdifferentiation, and also allowing clonal expansion. In this case, the brief stimulation with **iPSC-TF** is not sufficient to generate iPSC. It has been hypothesized that **iPSC-TF** erase the epigenetic identity of the starting cell. This technology has been used to generate cardiomiocytes, neural progenitors and definitive endoderm[2].

Figure 3. *Crystal structure of MyoD.*

"DNA-free" strategies

These strategies consist in utilization of small chemical soluble molecules involved in methylation/demethylation of DNA and hystones, heterochromatin regulators or interfering RNA (iRNA)[2]. Some "DNA-free" strategies have become a substitute to iPSC-TF, and that is why these molecules could be applied in **iPSC-TF based transdifferentiation**.

Selection of the origin cell

Defining the origin cell is as crucial as the election of differentiating elements. Accessible cells, such as fibroblasts, might be more suitable for translational applications. It is relevant that cells which do not share a common progenitor are characterized by a more complex molecular mechanism[1] underlying cellular transitions (Fig. 4).

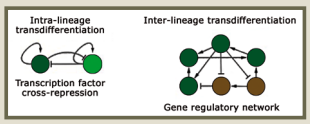


Figure 4. *Binary or network interactions of transcription factors in cell fate decisions depending on their epigenetic distance (represented by colors).*

DIRECT CONVERSION OF β-CELLS

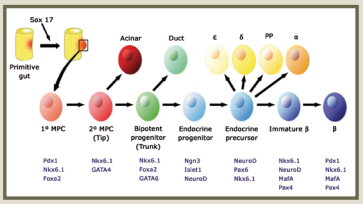


Figure 5. *Transcription factors involved in differentiation of β-cells from pancreatic multipotent progenitors (MPC).*

Type I Diabetes Mellitus (T1D) is characterized by hyperglycemia episodes caused by an autoimmune destruction of β-cells. A suitable cure for T1D must consider two aspects: supression of immune response against β-cells and replacement of cell loss.

An extensive understanding of organogenesis is essential to develop new reprogramming strategies (Fig. 5). In order to determine if a cell has been reprogrammed into a β-like cell, Glucose Stimulated Insulin Secretion (GSIS) tests or *in vivo* alleviation of hyperglycemia experiments are held[7].

Short-range or intralineage transdifferentiation of pancreatic β-cells

There are three relevant studies on transdifferentiation using definitive endoderm-derived cells as origin cells:

- The first trial ever carried out in transdifferentiation into β-cells consisted in the reprogramming of hepatocytes into insulin and glucagon-secreting cells. Hepatocytes were transduced *in vivo* by viral vectors containing Pdx1[8].
- D. A. Melton successfully identified three TF (*Pax1*, *Ngn3* and *MafA*) that could efficiently transdifferentiate β-, α-, and δ-cells from acinar cells *in vivo*[9] by adenoviral vector transduction (Fig. 6).
- Pax4 overexpression can reprogram α-cells into β-cells[10]. α-cells have bivalent chromatin modifications at genes which are active in β-cells (*Pdx1* and *MafA*).

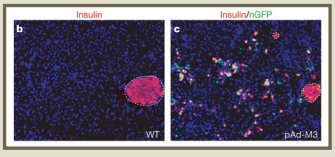


Figure 6. *Microscopic images showing transduced acinar cells which where converted into insulin-secreting cells (yellow). (From reference 9).*

Long-range direct conversion from fibroblasts to β-cells

A combination of Pdx1 and small chemical molecules (5-Azacytidine and Romidepsin) that modify the histone code has been used to transdifferentiate human fibroblasts into β-cells for the first time[11].

iPSC-TF based transdifferentiation

The most inspiring achievement in β-cell direct conversion was published in 2014. In this study, Definitive Endoderm-Like Cells (DELC) were derived from transgenic mice embryonic fibroblasts using a combination of **iPSC-TF** co-cultured with Activin A and Lithium Chloride. After that, DELC were cultured with retinoic acid and other small molecules in order to obtain functional β-cells[12].

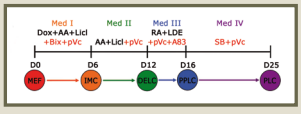


Figure 7. *Schematic representation of β-cell reprogramming from fibroblasts using **iPSC-TF** based transdifferentiation. (Edited from reference 12).*

CONCLUDING REMARKS

- All experiments fulfilled in this field represent a proof-of-concept that transdifferentiation might be a vital tool in regenerative medicine.
- Improvements in "DNA-free" strategies and culture methods are essential for a translational approach in humans.
- Regenerative medicine, together with immunomodulation, are the only potential method not to treat, but to cure T1D.
- **iPSC-TF based transdifferentiation** might become the keystone to regenerative medicine.

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