Cell Research (2014) 24:141-142.

www.nature.com/cr

RESEARCH HIGHLIGHT

Regenerative medicine: Transdifferentiation *in vivo*

Cell Research (2014) **24**:141-142. doi:10.1038/cr.2013.165; published online 17 December 2013

A major challenge in regenerative medicine is the generation of functionally effective target cells to replace or repair damaged tissues. Transdifferentiation *in vivo* is a novel strategy to achieve cell fate conversion within the native physiological niche; this technology may provide a time- and cost-effective alternative for applications in regenerative medicine and may also minimize the concerns associated with *in vitro* culture and cell transplantation.

Since its establishment, cell reprogramming of somatic cells into induced pluripotent stem cells (iPSCs) *in vitro* has provided a fantastic tool for disease modeling studies as well as presented potential applications in regenerative medicine [1]. However, in practice, the reprogramming of patient cells into iPSCs and subsequent redifferentiation into desired target cells is often an inefficient and time-consuming process. As an alternative approach, transdifferentiation directly converts one cell type into another, which bypasses many of the safety concerns associated with the pluripotent cell state. If this method can be applied in a safe and efficient manner *in vivo*, it can further help to eliminate the undesired issues that may arise during the *in vitro* culture and cell transplantation processes.

The idea of *in vivo* transdifferentiation is not a completely novel notion in the stem cell field and it has been developing rapidly in recent years due to the rise in the discovery of transcription factors that induce cell reprogramming. A particularly remarkable advance in this field was the direct reprogramming of adult pancreatic exocrine cells into β-cells in 2008 [2]. Utilizing a cocktail, the authors introduced different combinations of nine β-cell development-related transcription factors into the pancreas of adult mice by adenovirus. They found three factors (Pdx1, Ngn3, and Mafa) that were required to convert up to 20% of infected exocrine cells into insulin-producing β-cells. Lineage tracing experiments further demonstrated the exocrine cell origins of the induced β-cells. The absence of BrdU-labeled cells during the process excluded the possibility of a dedifferentiation stage. The induced β-cells were characteristically similar to the endogenous islet β -cells in cell morphology, ultrastructure, lineage marker expression and insulin secretion. However, the majority of the induced β-cells remained scattered or in small clusters without efficient integration into existing islets, which presumably resulted in their limited capacity to fully restore glucose homeostasis after chemically induced pancreatic injury. Nonetheless, this report provided an encouraging proof-of-principle for *in vivo* transdifferentiation and stimulated further studies. Using a similar method, Qian *et al*. [3] generated induced cardiomyocyte-like cells from cardiac fibroblasts *in vivo* by retroviral delivery of three transcription factors (Gata4, Mef2c, and Tbx5) into the myocardium based on a previous *in vitro* experiment [4]; Torper *et al*. [5] reported the successful conversion of resident astrocytes into mature neurons *in situ* by forced expression of Ascl1, Brn2a, and Myt11 in the adult mouse striatum; and other studies also reported the successful *in vivo* transdifferentiation [6-8] (listed in Table 1).

Although the methodologies were similar, the mechanisms involved in these *in vivo* transdifferentiation studies appeared to be different. In some cases, it seemed to be a direct cellular conversion, such as from exocrine cells to β-cells, but in other cases, the initial cells might need to dedifferentiate into an intermediate precursor stage before final conversion to a new fate. For instance, Zhang and colleagues reprogrammed astrocytes in the adult mouse striatum to neuroblasts, which are neural precursor cells [9]. In their study, they found that Sox2 alone was sufficient to reprogram the endogenous quiescent astrocytes to neuroblasts through a proliferative stage. The induced neuroblasts could subsequently differentiate into neurons with exogenous expression of Brain-derived neurotrophic factor (BDNF) and Noggin, or with VPA (a histone deacetylase inhibitor) treatment. Though the induced neuroblasts were proliferative, no tumor formation was detected during this study. More excitingly, neurons derived from induced neuroblasts exhibited an elaborate neural morphology, possessed functional voltage-gated sodium channels, and formed synapses with the endogenous neurons, a sign of efficient integration into the local neural network.

These fascinating results from the recent progress in *in vivo* transdifferentiation brought us new hopes and also raised new questions. For instance, does transdifferentiation go through a dedifferentiation stage or does it occur directly? What is the master regulator in the *in vivo* cell fate conversion process? Undisputedly, transcription factors play key roles during this process in which they build up a transition bridge between initial and target cells. It has been reported that using the same initial

Table 1 Examples for *in vivo* transdifferentiation (TFs: transcription factors)

Year	Animal	Initial cells	Target cells	TFs	Delivery	Ref.
2008	Mouse	Pancreatic exocrine cells	β-cells	Pdx1, Ngn3, Mafa	Adenovirus	[2]
2012	Mouse	$Sox9^+$ cells in liver	Insulin-secreting ducts	Pdx1, Ngn3, Mafa	Adenovirus	[8]
2012	Mouse	Non-myocytes (primarily cardiac fibroblasts)	Cardiomyocyte-like cells	Gata4, Mef2c, Tbx5	Retrovirus	[3]
2012	Mouse	Non-myocytes	Cardiomyocyte-like cells	Gata4, Hand2, Mef2c, Tbx5	Retrovirus	[6]
2013	Mouse	Astrocytes	Neuron	Ascl1, Brn2a, Myt1l	Lentivirus	[5]
2013	Mouse	Astrocytes	Neuroblast	Sox2	Lentivirus	[9]
2013	Mouse	Embryonic and early post-natal callosal projection neurons in layer II/II	Corticofugal projection neurons in layer V/VI	Fezf2	Plasmid	[7]

cells, astrocytes could be converted to neuroblasts and mature neurons by Sox2 alone [9] and by combination of Ascl1, Brn2a, Myt11 [5], respectively. With the appropriate combination of transcription factors, even terminally differentiated cells can be directly converted into another terminally differentiated cell type. For example, Rouaux *et al*. [7] demonstrated that early post-mitotic callosal neurons could be converted into corticofugal neurons *in vivo*. Moreover, the type of initial cells selected also appears to have significant impact on transdifferentiation ability *in vivo*. The developmental origin of the initial and target cells may directly influence the efficiency and complexity of the transdifferentiation process. This is particularly evident in the transdifferentiation of pancreatic exocrine cells to β-cells, which occurred rapidly and efficiently [2]. This conversion benefited substantially from the few epigenetic differences between the two lineages as they share a common precursor.

On the other hand, as the underlying mechanisms of transdifferentiation remain largely unknown, a step-wise methodology for transdifferentiating cells cannot be defined. Meanwhile, the safety issues related to *in vivo* transdifferentiation have never been extensively investigated. A recent study by Serrano and colleagues detected iPSCs derived from hematopoietic cells or non-hematopoietic cells in the blood stream through overexpression of OSKM *in vivo* [10]. Those iPSCs migrate to different tissues via the blood stream and differentiate on site or form teratomas. Certainly these tumor-forming cells would not be desired targets in any *in vivo* transdifferentiation study. Therefore, the scale and extent of the dedifferentiation process need to be carefully monitored.

Considering the clinical advantages of *in vivo* transdifferentiation, the associated safety and efficacy issues are well worth careful investigation. Can the induced target cells maintain their new properties and functions in the long run? Can the unnecessary damages to native tissue or non-targeted cells be minimized in induced transdifferentiation? Do infected but un-reprogrammed target cells pose a safety concern? In order to meet the criteria of potential clinical application, it is also important to replace viral vectors without jeopardizing the efficacy of transdifferentiation. Screening small compounds that mimic the role of transcription factor overexpression is certainly a promising substitute for the use of virus. Furthermore, the recent report from Abad *et al*. suggested that *in vivo* cell reprogramming may also take place in the kidney, intestine, intracranial, stomach and other tissues [10]. Therefore, if the safety concerns can be solved, *in vivo* transdifferentiation may open a new avenue to generate various cell types for different clinical purposes.

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