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Reshaping the brain: direct lineage conversion in the nervous system

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Abstract

During embryonic development, cells in an uncommitted pluripotent state undergo progressive epigenetic changes that lock them into a final restrictive differentiated state. However, recent advances have shown that not only is it possible for a fully differentiated cell to revert back to a pluripotent state, a process called nuclear reprogramming, but also that differentiated cells can be directly converted from one class into another without generating progenitor intermediates, a process known as direct lineage conversion. In this review, we discuss recent progress made in direct lineage reprogramming of differentiated cells into neurons and discuss some of the therapeutic implications of the findings.

Introduction

During embryonic development, cells in an uncommitted pluripotent state undergo progressive epigenetic changes that lock them into a final differentiated state. Famously, Conrad Hal Waddington likened this process to a marble traveling down a downward slope and ending up in one of the many valleys surrounded by impassable hills [1]. This epigenetic landscape model has often been used to explain the restrictive nature of the differentiated cell state.

However, somatic cell nuclear transfer experiments, cellular fusion experiments and, more recently, induced pluripotent stem cell (iPSC) technology have shown that it is possible for a fully differentiated cell to revert back to a pluripotent state, a process called nuclear reprogramming [2-7]. Moreover, it has become clear that not only is it possible for the marble to travel up-slope but it can also jump from valley to valley, a mechanism known as direct lineage reprogramming. In this process, overexpression of various factors can alter cellular identity from one differentiated state into another. Focusing on the central

nervous system (CNS), we specifically review and discuss recent progress made in directing the lineage reprogramming of differentiated cells into neurons.

As a gateway for both cell replacement therapy and *in vitro* disease modeling, direct lineage reprogramming of differentiated cells has the potential to provide a large number of patient-derived, class-specific neurons [8]. To this end, directed differentiation of iPSCs also remains promising; however, there are key differences to consider. Reports demonstrate that nuclear reprogramming to an iPSC state involves a transient tumorigenic pluripotent state that is also susceptible to chromosomal aberrations [9,10]. These concerns are less likely to apply to direct reprogramming of differentiated cells. Additionally, because direct reprogramming has been achieved between different cell types, it is reasonable to postulate that a terminal, new cell state may be more readily achieved when the starting population shares some common features or the same developmental origin. Direct lineage reprogramming, of course, comes at a price, since compared with the near-full erasure of epigenetic memory

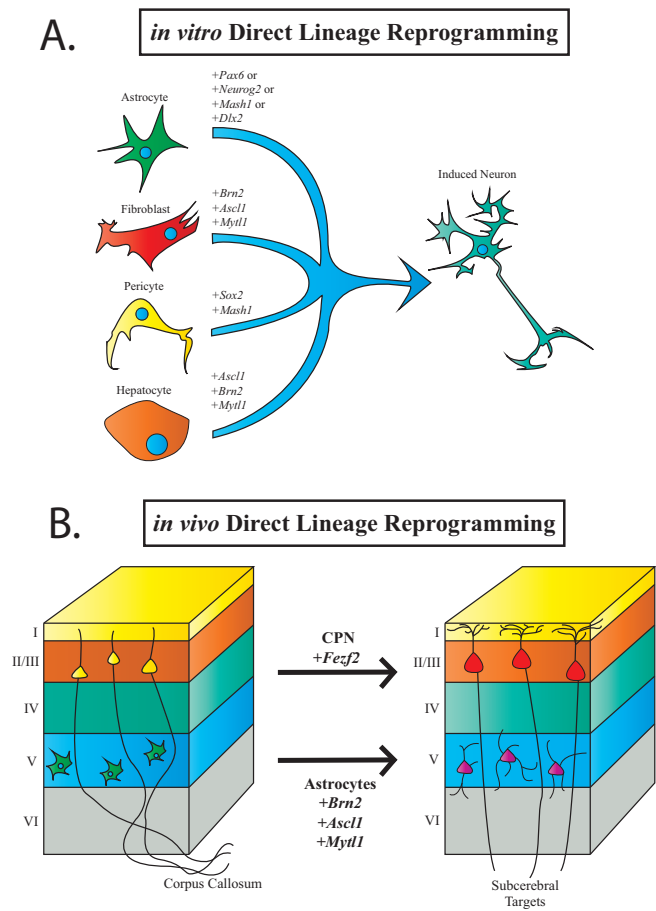
during the process of generating iPSCs (and their cell derivatives), neurons obtained by direct lineage reprogramming often maintain transcriptional and epigenetic traces (a “memory”) of the cell of origin [11]. Finally, while iPSC generation and differentiation occurs *in vitro*, recent progress in direct lineage reprogramming indicates that this process could be induced *in vivo*, bypassing the need to transplant the end product [12-14]. For these reasons, there is great interest in pursuing this avenue as an alternative to iPSC directed differentiation.

Direct lineage conversion into neurons

One of the first studies to show that direct reprogramming is feasible in the CNS employed astrocytes of the cerebral cortex as the starting cell type. Overexpression of *Pax6* was sufficient to reprogram early postnatal GFAP⁺ astrocytes to Tuj1⁺ neurons [15]. Further characterization and improvements from the Götz and Berninger groups have subsequently successfully produced electrophysiologically active glutamatergic and GABAergic neurons from astrocytes, *in vitro* [15-18]. In addition, endogenous astrocytes have been directly reprogrammed to neuroblasts and neurons upon injury combined with *Olig2* repression or via overexpression of the BAM factors (*Ascl1*, *Brn2* and *Myt1l*), respectively [14,19]. These astrocyte-to-neuron studies underscore how minimal manipulation of transcription factor signaling may be sufficient to directly reprogram the identity of differentiated cells of the CNS into neurons.

Collecting a large amount of astrocytes is not always feasible, especially from humans. Thus, several groups have focused on reprogramming more lineage-distant, yet easily obtainable, skin fibroblasts into neurons. In 2010, the Wernig group first demonstrated that transduction of three genes – *Ascl1*, *Brn2*, and *Myt1l* (BAM) – was sufficient to directly reprogram primary postnatal mouse fibroblasts into induced neurons [20] (Fig. 1). These induced neurons were electrophysiologically active, formed synapses *in vitro*, and expressed markers of postmitotic neurons. Subsequent studies have shown that induced neurons can be made from human fibroblasts, with different combinations of neuronal developmental genes, miRNAs, small molecule inhibitors, and inhibition of a splicing factor [21-25]. Remarkably, the Brüstle group has attained a conversion yield of 200% (two neurons for every fibroblast plated) [26]. Since then, direct lineage reprogramming of induced neurons has been achieved from other cell types, including pericytes and, notably, from terminally differentiated cells of a different embryonic lineage, like hepatocytes [27,28]. Given that neurons are highly specialized, these groundbreaking studies underscore the vast potential of direct lineage reprogramming to generate even complex cell types.

Figure 1. Direct lineage reprogramming of somatic cells into neurons



(A) Specific transcription factors can directly reprogram astrocytes, fibroblasts, pericytes, and hepatocytes into induced neurons *in vitro*. (B) Examples of *in vivo* direct lineage reprogramming include conversion of callosal projection neurons into corticospinal motor neuron-like cells by overexpression of *Fezf2*, and astrocytes into induced neurons by overexpression of BAM factors.

These exciting results have now paved the way for the ultimate challenge of generating specific classes of neurons to recreate the heterogeneity of neurons resident in the mammalian brain and to meet the need for replacement of different classes of neurons in distinct diseases.

In order to produce differentiated neurons that are specifically lost in neurodegenerative disorders like Amyotrophic Lateral Sclerosis, Parkinson’s disease, and Alzheimer’s disease, several groups have added additional factors to the original BAM cocktail. Mouse and human fibroblasts have been directly reprogrammed into neurons that resemble midbrain dopaminergic neurons, a population that degenerates in Parkinson’s disease

[11,29-31]. The resulting neurons display electrophysiological properties and share some markers of endogenous dopaminergic neurons. Interestingly, the Jaenisch group has shown that upon ectopic transplantation in the striatum, these induced-dopaminergic neurons are able to at least partly integrate into the local circuitry and produce dopamine [29]. Similarly, the Eggan group successfully reprogrammed mouse and human fibroblasts directly into lower motor neurons [32]. Not only were these neurons electrophysiologically active and expressed fewer motor neuron-specific markers but they also integrated into the ventral horn of the developing chick spinal cord upon transplantation. As demonstrated in these studies, the generation of neurons carrying defined, class-specific features might be feasible and especially valuable in the context of disease-related research and therapy.

While these results are encouraging, an important question remains: do the induced neurons need to be functionally and molecularly exact for direct application? This young field is still struggling to define a set of guidelines and standards that could be applicable across experiments to classify cell types generated by direct reprogramming. Global gene expression analysis of induced-dopaminergic neurons and lower motor neurons reveals a cell type that resembles the endogenous population; however, it is also clear that it is not a perfect match [11,28,32]. While functionality – i.e. firing properties, neurotransmitter release, appropriate connectivity – may be enough for cell replacement therapy, perfectly matched global gene expression profiles may be necessary for reliable disease modeling. These two applications may require separate criteria to define and accept the cell population obtained by direct lineage reprogramming. Similarly, the extent of neuronal-specificity required to properly model a disease *in vitro* is disease-dependent. For example, Parkinson's disease primarily affects dopaminergic neurons of the substantia nigra pars compacta, while damages to the dopaminergic neurons of the ventral tegmental area are variable [33]. Thus, for the purpose of *in vitro* disease modeling of Parkinson's disease, it might be more useful to directly reprogram fibroblasts from patients with a familial form of Parkinson's disease into specific dopaminergic neuron classes – those that resemble more closely the neurons of the substantia nigra pars compacta. Different criteria may apply to Alzheimer's disease, a disease in which many neuronal subtypes of the cerebral cortex are affected [34]. In support of this assessment, Qiang et al. isolated fibroblast samples from human patients with Alzheimer's disease and directly reprogrammed them into a heterogeneous population of neurons [35]. While the resulting induced neurons did not appear to be of any specific class, they still recapitulated some of the cellular phenotypes observed in neurons of

Alzheimer's disease patients that were absent in fibroblasts. While the jury is clearly still out and classification criteria for reprogrammed cells are not well defined, it is clear that such guidelines are needed for the field to move forward.

In the context of neurological disorders, one theoretically plausible idea to repair the brain would be to switch a healthy neighboring neuronal subtype into neuronal classes that are lost in disease, using direct reprogramming *in vivo*, rather than in the dish. Neurons have historically been thought to be immutable, and reversal of their differentiated state impossible. However, nuclear reprogramming studies of olfactory and cortical neurons have shown that even postmitotic neurons from postnatal mice retain nuclear plasticity, as demonstrated in mice by successful generation of pluripotent stem cells via tetraploid complementation with the nucleus of a postmitotic neuron [36-39]. In agreement with this, we have recently demonstrated that it is possible to directly reprogram one neuronal subtype of the cerebral cortex into another by overexpression of a single transcription factor, *Fezf2*, *in vivo* [13]. As a master regulator of corticospinal motor neuron generation during development [40,41], *Fezf2* induced layer II/III callosal projection neurons to acquire the molecular identity and axonal connectivity of layer V corticospinal motor neuron. When expressed in cortical neurons of layer IV, *Fezf2* was also able to change their electrophysiological properties to resemble those of corticospinal motor neurons [12]. Although lineage conversion was only possible during a defined window of embryonic and postnatal life of the neurons, taken together, these studies highlight not only the possibility of *in vivo* direct lineage programming of neurons but also the importance of knowledge gained from developmental studies to inform *de novo* differentiation of specific neuronal subtypes.

Future directions

Direct lineage reprogramming in the CNS is a concept in its infancy. However, results from the last few years clearly show that this is a field with great potential, both for disease modeling and cell replacement therapy. In the upcoming years, it will be necessary to further our mechanistic understanding of the complex process by which differentiated cells can "change their mind". This knowledge will be necessary to equip researchers with additional molecular tools that facilitate the production of neurons that meet the standards and needs of transplantation, disease modeling, and chemical screening. At the cellular level, it will be important to define the discrete steps of reprogramming and whether intermediate, stable cell states do exist. Initial data show that direct lineage reprogramming to induced neurons is a direct

process [32,35]. However, it is also conceivable that intermediate cell types are present, but are difficult to identify due to their expression of “unexpected” molecular markers. Support for this hypothesis exists from one of the rare events of natural reprogramming that occurs during *Caenorhabditis elegans* development. Here, natural direct reprogramming of rectal cells into motor neurons does occur and it includes a discrete intermediate cell type that does not express markers of either rectal cells or motor neurons [42,43]. Tools have recently become available for complex molecular profiling of small populations of cells, often down to the single-cell level. These new approaches will facilitate work to shed light on the dynamics of direct lineage reprogramming. Although multiple challenges and questions remain in order to apply direct reprogramming to therapy, these pioneering studies have paved the way for further breakthroughs in what is an exciting and rapidly growing field.

Abbreviations

CNS, central nervous system; iPSC, induced pluripotent stem cell.

Disclosures

The authors declare that they have no disclosures.

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