**Neuronal reprogramming for brain repair: challenges and perspectives**

Riccardo Bocchi1,2\*, Magdalena Götz1,2,3\*

**AFFILIATIONS**

1. Physiological Genomics, Biomedical Center (BMC), Ludwig-Maximilians-Universitaet (LMU), Großhaderner Str. 9, 82152 Planegg/Martinsried, Germany
2. Helmholtz Center Munich, Biomedical Center (BMC), Institute of Stem Cell Research, Großhaderner Str. 9, 82152 Planegg/Martinsried, Germany
3. SyNergy Excellence Cluster, Munich, Germany

\*Correspondence:

[riccardo.bocchi@bmc.med.lmu.de](mailto:riccardo.bocchi@bmc.med.lmu.de) (R. Bocchi), [magdalena.goetz@helmholtz-muenchen.de](mailto:magdalena.goetz@helmholtz-muenchen.de) (M. Götz)

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**ABSTRACT**

Brain injuries and neurodegenerative diseases elicit neuronal loss that persists as the adult mammalian brain lacks robust regenerative abilities. Direct reprogramming of local glial cells into neurons is a promising strategy for neuronal replacement *in vivo*. Here we discuss recent advances and future challenges in this approach for brain repair.

**TEXT**

Neuronal loss is a major hallmark of both acute brain injuries and neurodegenerative disorders. The adult mammalian brain lacks robust regenerative abilities, since persistent neurogenesis occurs only in few specialized niches [1,2]. Therefore, the crucial long-standing questions are how lost neurons could be replaced and to what extent this would be beneficial for restoring brain function. Over the past few decades, neuronal replacement therapy and regenerative medicine have mainly focused their efforts on two promising approaches using either exogenous or endogenous cell sources. The former takes advantage of different neuronal lineages as sources of donor cells for cell transplantation [1]. Its straightforward conception and possible clinical application made this approach so far the favored choice, despite some limitations, including the need for immunosuppression treatment and its use restricted to focal locus. The second strategy which overcomes these drawbacks uses endogenous local glial cells for neuronal replacement. This approach consists of forcing direct conversion of local non-neuronal cells, such as astrocytes, to a neuronal fate by introducing neurogenic factors. Upon neuronal loss, some astrocytes play a detrimental inflammatory role and are also involved in scar formation, therefore their reprogramming into neurons allows on the one hand to replace degenerated neurons and on the other hand to alleviate scar formation as well as reducing the harmful inflammatory response. Thus, this strategy harbors a huge potential for restorative brain therapy (see ref. [2] for exhaustive review).

Great progress has been made since the first report describing in vivo glia-to-neuron reprogramming after brain lesion in mice [3]. Over the past 15 years, we have witnessed the improvement of the reprogramming protocols by unveiling better performing neurogenic factors and identifying major hurdles in converting glia into neurons, to ensure a progressively higher reprogramming rate [4]. Hand in hand with this development, it has been possible to obtain neurons that increasingly mirror the endogenous ones. Indeed, the combinatorial expression of Neurogenin 2 (Ngn2, a proneuronal factor) with Bcl2 (a survival factor and antioxidant agent) driven by retroviral vectors, led to an unprecedented improvement in glial-to-neuron conversion after traumatic cortical injury in mice [5]. Reprogrammed neurons acquired the typical pyramidal cell morphology and expressed the hallmarks of mature cortical neurons. More recently, the combination of adeno-associated viruses (AAVs) carrying Ngn2 and the Nuclear Receptor Related 1 (Nurr1, a transcription factor), was also shown to induce pyramidal neurons from astrocytes in the cortex upon traumatic injury. In this case, induced neurons matured over time and acquired the precise neuronal subtype and axonal projection identities, congruently with their laminar location [6].

However, the potential of these reprogrammed neurons resides in their possible capacity to integrate into functional circuits and eventually ameliorate the phenotypic defects due to neuronal loss. Recent studies have addressed this using mouse models of Parkinson’s disease, where dopaminergic neurons were lost in the substantia nigra resulting in motor behavior deficits [7–9]. Depleting the RNA-binding protein PTB (known to inhibit neuronal differentiation) in astrocytes was apparently sufficient to *in vivo* reprogram astrocytes from the substantia nigra [7] and from the striatum [8] into dopaminergic neurons, reminiscent to those lost in Parkinson’s disease. These showed a partial re-innervation of the striatum [7], restored dopamine levels in this region and rescued the deficits in motor behavior [7,8].

If this conversion is indeed reliable, it would offer an exciting long-term perspective to replace neurons in various disease conditions. However, the ease of conversion seen in these recent studies also warrants caution. For example, only *in vitro* astrocyte-to-neuron conversion was monitored by video time lapse [5]. The *in vivo* live imaging following a single astrocyte progressing into a neuron could not only prove the conversion beyond doubts, but also provide unprecedented insights into the mechanisms how this process occurs in the injured brain. Likewise, very little is still known about the transcriptional changes during *in vivo* astrocyte-to-neuron conversion. Single-cell molecular analysis at different stages of the conversion will provide better understanding on how this is achieved and ultimately revealing the similarity between induced and endogenous neurons.

A critical point for *in vivo* reprogramming is also the gene delivery strategy. Different viral vectors have been tested so far (including lentiviruses, retroviruses and AAVs), giving rise to a broad range of reprogramming efficiencies and neuronal survival [4]. In particular, the virus-related immunogenicity, which ultimately could influence the reprogramming rate, varies depending on the virus used. Injection of AAVs in the cortex of mice showed very low levels of inflammation and a moderate reactive gliosis, as compared to lentiviral and retroviral vectors, where abundant inflammation and strong reactive gliosis was observed [6]. On the other hand, until now lentiviruses and retroviruses could target astrocytes more selectively using pseudotyped versions (e.g. pseudotyped Mokola) or due to a restricted potential of infection (retroviruses integrate their genome only in diving cells, i.e. cycling reactive glial cells). Conversely, AAVs used so far in reprogramming (e.g. serotypes 2 and 5) are less specific for glia and target also endogenous neurons. Specificity is supposedly achieved by regulating the expression of the neurogenic factors, for instance by using astrocyte-specific promoters (e.g. GFAP) or mouse lines expressing an activating gene in astrocytes (e.g. GFAP-Cre mice with Cre mediating recombination of flexed constructs). Even with these tricks, a certain degree of leakiness in endogenous neurons was observed when using AAV2/5 [6,7] and it is conceivable that the expression of neurogenic factors may activate GFAP promoter also in endogenous neurons [10]. Therefore, possible alternative explanations need to be considered for the results of the experiments with AAV2/5, such as a possible gradual increase of expression of the reporter genes in endogenous neurons, which could account for the increased amount of labelled neurons. This can be controlled by labeling endogenous neurons in order to show that the reprogrammed neurons are not pre-existing neurons [6]. An additional important control is to genetically label the glial cells in order to prove that reprogrammed neurons are derived from local glial cells [8,11]. This issue may also be tackled at a more functional level assessing if the improvement seen in behavioral studies, disappears upon ablation or silencing of the reprogrammed neurons as was also previously done for transplanted neurons [1]. Qian and colleagues used a chemogenetic approach to show that the lesion-induced motor phenotype, which disappeared two months after reprogramming, re-appears when the induced dopaminergic neurons were silenced [7]. This strongly supported the critical role of the activity of induced neurons for the phenotypic recovery. Thus, labelling endogenous neurons, tracing astrocyte conversion and reversion of behavior phenotypes should become standard in the field of reprogramming to help ensuring reliable reprogramming protocols.

Despite these caveats, AAVs represent one of the safest vectors for clinical use in patients, as they remain episomal (do not integrate into the host genome) and have a low immunogenicity. Thus, using AAVs for neuronal reprogramming offers a translational opportunity, especially since some serotypes (such as AAV9) can be systemically delivered and still be able to selectively target glial cells in the central nervous system [12]. All these advantages setup neuronal reprogramming as a promising strategy to pursue in the future, to ultimately treat neuronal loss.

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