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Reprogramming the diseased brain

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Direct conversion of astrocytes to dopamine neurons *in vivo* offers fresh optimism for the development of improved Parkinson's therapies.

A study by Rivetti di Val Cervo *et al.*[1] in this issue heralds an exciting advance in the rapidly developing technology for direct neural reprogramming of adult somatic cells, with clear therapeutic potential in Parkinson's disease. The team reports a combination of genes and soluble factors that induces effective, efficient and stable reprogramming of cultured astrocytes into midbrain-like dopamine neurons, and shows that the same four genes used *in vitro* reprogram astrocytes in the depths of the mouse brain. The reprogrammed neurons exhibit morphological, phenotypic and electrophysiological features of normal dopamine neurons, and alleviate spontaneous, as well as drug-induced, symptoms in parkinsonian mice.

Parkinson's disease has long provided an attractive target in regenerative medicine for the development of a wide array of novel gene and cell therapies (**Fig. 1**), although none yet provide a practical therapy ready for widespread adoption. One reason for the sustained interest is that conventional treatments such as dopamine-replacement drugs and deep brain stimulation confer significant therapeutic benefit even though they do not restore neuronal signalling in dopaminergic circuits or alter the course of the underlying disease. The lack of regulated specificity common to these approaches may explain the declining potency and side effects that often emerge with long-term use, but their relative efficacy also suggests that the demands on a cell-based therapy may be less challenging in Parkinson's disease than in diseases that affect more precisely organized point-to-point systems of the brain.

Indeed, transplantation of dopamine-rich fetal ventral mesencephalon has proved remarkably effective at alleviating symptoms for many years in small numbers of Parkinson's disease patients[2]. These results are proof of the therapeutic potential of cell replacement for this condition. However, fetal-tissue grafting is implausible as a widespread medical treatment owing to the lack of standardization inherent in this cell source, as well as ethical concerns.

The bottleneck in donor-tissue supply has stimulated exploration of pluripotent stem cells (PSCs) — both embryonic stem cells and induced pluripotent stem cells—as an alternative source of dopamine neurons for transplantation. Clinical-grade PSCs are being expanded, banked, and characterized, while advances in our understanding of embryonic development have led to reliable protocols for differentiating PSCs into fetal ventral midbrain-like dopaminergic precursors capable of improving deficits in rodent models of Parkinson's disease[3,4]. A recent single-cell RNA-seq analysis revealed that dopamine neurons derived from both mouse and human PSCs represent many, although not all, subtypes found in the fetal ventral midbrain of the two species[5], suggesting that further work may be needed to refine differentiation protocols. Additional challenges for clinical application of these cells include achieving suitable purity and yield; demonstrating safety, especially given the potential tumorigenicity of any pluripotent cells remaining in differentiated populations; and addressing immunological and inflammatory responses to allogeneic donor cells, notwithstanding the partial immunologic privilege of the brain environment.

Direct reprogramming of somatic cells across lineage divisions into mature 'induced neurons' bypasses the pluripotent state and thus largely circumvents the risk of tumorigenesis. Mouse and human skin fibroblasts have already been converted to midbrain-like dopamine neurons that alleviate simple motor symptoms after transplantation into parkinsonian mice or rats[6,7]. Rivetti di Val Cervo *et al*.[1] now propose direct reprogramming *in vivo* in order to redirect astrocytes in the brain away from their original glial or striatal cell fates into becoming midbrain dopamine neurons.

The authors started by systematically modifying a differentiation protocol (transfection of ASCL1, LMX1A and NR4A2) previously used to reprogram fibroblasts to midbrain-like dopamine neurons. Application of this protocol to an immortal human astrocyte line generated a small proportion of cells that expressed tyrosine hydroxylase (TH), the critical enzyme in the dopamine pathway, but that did not have a neuronal phenotype. However, addition of the chromatin remodeling cofactor ascorbic acid, as well as TGFβ and BMP4 inhibitors (dual-SMAD inhibition), alongside sonic hedgehog and a GSK3β inhibitor, improved the conversion to a more neuronal-like dopamine phenotype (as judged by TH and β –tubulin expression), although the cells still lacked markers of neuronal maturity.

Next, NR4A2, found to be limiting, was dropped, and NEUROD1, known to improve neural reprogramming in human cells, and microRNA218, a factor in the regulation of midbrain DA development, were added. **[AU: OK?]** This led to the highest proportion of midbrain-like dopamine neurons and also resulted in expression of more mature neuronal markers. However, the neurons still exhibited a relatively simple neuronal morphology and lacked excitable properties of typical midbrain dopamine neurons. Finally, the authors preceded the patterning protocol above with TGFβ1 and the chromatin remodeling agents valproic acid and decitabine, on the basis that chromatin remodeling has been shown to facilitate reprogramming to induced pluripotent stem cells. Although the numbers of TH^+ cells generated was lower with this approach, the quality of the differentiation was improved as shown by markers of mature neurons and a midbrain-like dopamine neuronal phenotype.

So how similar were the differentiated cells to *bona fide* midbrain dopamine neurons? *In vitro* immunohistochemistry showed a morphology consistent with midbrain dopamine neurons and expression of a range of cellular markers typical of these cells. Analysis of gene expression revealed down-regulation of glial genes, up-regulation of genes characteristic of midbrain dopamine neurons, and an overall profile closer to that of human fetal midbrain (at a gestational age of cells known to be capable of improving function in patients after transplantation) than of the original astrocyte line. The gene expression profiles **[AU: of what?]** were not identical, which could indicate that the reprogrammed cells lacked a genuine midbrain dopamine phenotype, but could also reflect other factors, such as the generation of cells at different developmental stages not necessarily matching those of the fetal tissue.**[AU: OK?]** Application of the protocol to primary human embryonic astrocytes led to similar results, as well as improved electrophysiological data suggestive of a *bona fide* midbrain dopamine neuron fate.

Evidence that astrocytes in the living adult mouse brain can be converted into midbrain-like dopamine neurons came from experiments using the standard mouse model of Parkinson's disease. In this model, administration of 6-OHDA selectively destroys midbrain dopamine neurons and deprives the striatum of its normal dopaminergic input. **[AU: shortening OK?]** Rivetti di Val Cervo *et al*.[1] induced striatal astrocytes to adopt a dopaminergic neuron–like fate using the four reprogramming genes alone, under the control of the GFAP promoter to ensure reprogramming of astrocytes only. Reprogramming striatal, rather than midbrain, astrocytes means that the induced cells are already in their target area. Thus, although they are ectopic, this provides them with the best chance of locating their target.

Interestingly, although the *in vivo* protocol lacked the additional factors required *in vitro*, it appeared to produce cells expressing a mature dopamine neuron fate. Slice electrophysiology confirmed their ability to reliably generate action potentials, and behavioral studies demonstrated improved function across multiple (albeit not all) behavioral tests. Thus, whether or not the cells have acquired a genuine midbrain dopamine phenotype in every detail, they are sufficiently like these neurons in the intact brain to have a significant functional impact.

This strategy of Rivetti di Val Cervo *et al*.[1] offers several potential advantages. First, repair *in situ* avoids the prohibitive costs of manufacturing clinical-grade cell therapies. Second, host astrocytes, which appear to have a regional identity, may be predisposed to produce neurons of the same regional identity after neural induction[8]. Third, the approach may enable more-even dispersal and better integration of dopamine neurons in the brain compared with cell transplantation. Finally, many of the tools needed for gene therapy have already been well established and validated for human use.

Previous gene therapy trials for neurodegenerative disease have used viral vectors to genetically alter striatal cells to secrete neuroactive molecules in animal models of parkinsonism [9,10]. The two main strategies have been to introduce either enzymes (e.g. TH, AADC, GTP-cyclohydrolase) that will enable non-dopaminergic cells to synthesize dopamine [8] or trophic factors (e.g. CNTF, GDNF) that may exert neuroprotective influence on ongoing dopaminergic degeneration [9]. However, such *in vivo* gene therapies do not fundamentally alter the underlying primary phenotypes of the transfected host cells, synthesis and release of the transfected molecules is essentially unregulated, and initial clinical results have not been compelling[11]. By contrast, the present study, while using similar tools for *in situ* genetic modification, reprograms host astrocytes into fully functional, apparently regulated, dopamine neurons. This promises a fundamentally different mechanism of disease modification, offering to actually replace dopamine neurons lost to the disease, and circumventing critical immunological, safety, feasibility and ethical issues of the alternative approaches (**Fig. 1**).

There is every reason for confidence that, once a fully effective reprogramming protocol is established, induced dopamine neurons will function as well as transplanted fetal midbrain dopamine neurons. But it is also possible that other, as-yet-unidentified drawbacks will emerge. For example, will astrocytes in the treated brain region be depleted to an extent that disrupts local brain function? Will the induced dopamine neurons be integrated into the neural circuitry, and will they survive long term? Needless to say, while we are ever the optimists and are encouraged by these results, the initial report of Rivetti di Val Cervo *et al*.[1] is just the beginning.

Figure 1. Alternative strategies for regenerative medicine in Parkinson's disease. Cell therapy involves transplantation of dopamine neurons or dopamine-neuron progenitors, which can be derived from a variety of sources (right). Conventional gene therapy relies on delivery of genes that support dopamine neuron function, such as TH, AADC and growth factors (bottom left). Rivetti di Val Cervo *et al*.[1] now propose a clinical strategy in which astrocytes are converted *in vivo* to dopamine neurons by delivery of reprogramming genes (top left, red dotted arrow). In their study, they demonstrate reprogramming of human astrocytes *in vitro* and reprogramming of mouse astrocytes *in vivo* (solid red arrows). The comparative advantages and disadvantages of each strategy are shown in green (+) and orange (-), respectively. ATMP, Advanced therapy medicinal products; GMP; Good manufacturing practice; RTMP, remodeling TGFβ midbrain protocol; iPSC, induced pluripotent stem cell.

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