Neurosci. Bull.
https://doi.org/10.1007/s12264-021-00630-x

www.neurosci.cn
www.springer.com/12264

RESEARCH HIGHLIGHT

# Targeting PTB as a One-Step Procedure for *In Situ* Astrocyte-to-Dopamine Neuron Reprogramming in Parkinson's Disease

**Yuanyuan Jiang[1,2] · Yongjie Wang[1,2] · Zhihui Huang[1,2]**

Accepted: 20 August 2020


Parkinson disease (PD) is a progressive neurodegenerative disease characterized by the loss of dopamine neurons in the substantia nigra, resulting in striatal dopamine deficiency, hence eventually severe disability [1]. Research carried out across the >200-year history of PD has led to important discoveries [2]. Among them, efforts have been made to repair or promote regeneration using drugs, neurotrophic factors, cell-based disease modification, and cell replacement therapies in which exogenous cells are transplanted for functional improvement in neurodegenerative disorders. However, these treatment approaches are not sufficiently effective, while in recent years, the *in situ* repair strategy by trans-differentiation of certain somatic cells into functional neurons has gained great promise for the treatment of neurodegenerative diseases that feature loss of neurons [3].

The key for *in vivo* trans-differentiation of one cell type to another, also called *in vivo* reprogramming, relies on using lineage-specific transcription factors. Researchers have found that PTB, an RNA-binding protein encoded by polypyrimidine tract-binding protein 1 (*Ptbp1*) [4], suppresses a neuronal induction loop by inhibiting the dismantling of the RE1-silencing transcriptional factor (REST) complex by the microRNA miR-124. In this loop, REST silences numerous neuronal genes, including miR-124 and multiple neuron-specific transcriptional genes in non-neuronal cells [5,6] (Fig. 1A). Therefore, downregulation of *Ptbp1* can promote miR-124 to target REST, resulting in the expression of neuron-specific transcription factors for neurogenesis. On the other hand, de-repression of miR-124 further inhibits PTB, enhancing this neurogenesis loop (Fig. 1B). Studies have shown that downregulation of the expression of *Ptbp1* is sufficient to induce the trans-differentiation of cultured mouse embryonic fibroblasts into functional neurons [5].

Strikingly, when the same protocol is applied to human cells, only immature neurons are converted from human adult fibroblasts (HAFs) [6]. Further investigations found that downregulation of PTB promotes the expression of nPTB (a PTB paralog in the nervous system, encoded by *Ptbp2*) [5,6], thereby suppressing the transcription activator BRN2 (encoded by *pou3f2*) and miR-9, both of which are required for neuronal maturation. These results demonstrate that there exists a second nPTB-BRN2-miR-9 loop for neuronal maturation in human cells besides the PTB-miR124-REST loop for neuronal conversion. Interestingly, sequential knockdown of PTB and nPTB triggers the trans-differentiation of HAFs into functional neurons in the presence of glial cells [6].

These results reveal that both mouse and human fibroblasts can be efficiently converted into functional neurons when targeting PTB and nPTB *in vitro*. However, application of this somatic-to-neuronal conversion strategy to the field of regenerative medicine for the treatment of neurodegenerative diseases such as PD remains to be explored. Recently, Fu and colleagues studied the effect of PTB on astrocytes, a type of glial cell extensively present in the nervous system with high plasticity. In their research, they showed that astrocytes can be directly converted into dopamine neurons in the substantia nigra when PTB is

✉ Zhihui Huang
hzhzju021@163.com

[1] Department of Neurosurgery of The Affiliated Hospital, Holistic Integrative Pharmacy Institutes, Hangzhou Normal University, Hangzhou 311121, China

[2] Engineering Laboratory of Development and Application of Traditional Chinese Medicine from Zhejiang Province, Hangzhou Normal University, Hangzhou 311121, China

Published online: 13 January 2021

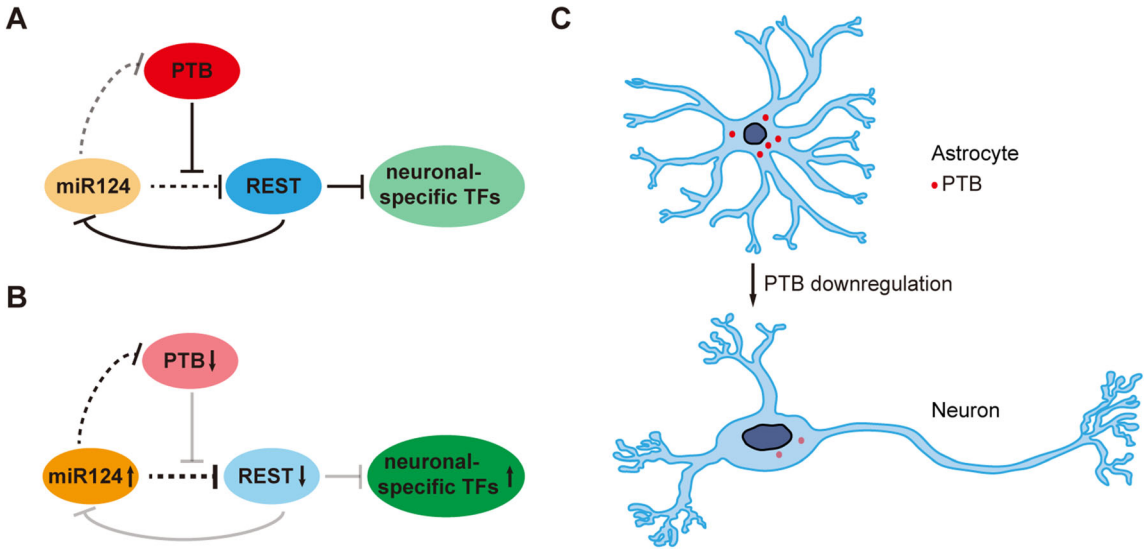


**Fig. 1** Astrocyte-to-neuron reprogramming in a one-step strategy by downregulating PTB. **A**, **B** PTB-regulated loop for neuron induction. **A** The transcriptional repressor REST is responsible for silencing a large array of neuronal genes, including multiple neuron-specific transcriptional genes and miR124 in non-neuronal cells, which in turn inhibits the REST complex. PTB is a key inhibitor of miR124, but also a substrate for miR124. **B** Downregulation of PTB promotes miR-124 to dismantle REST, thereby upregulating the expression of neuron-specific transcription factors involved in neurogenesis. The de-repression of miR-124 further suppresses PTB, thus enhancing this loop. **C** Astrocyte-to-neuron reprogramming in a one-step strategy by downregulating PTB.

downregulated in astrocytes. This one-step trans-differentiation procedure has been demonstrated to efficiently reconstruct damaged neural circuits, restore striatum dopamine levels, and reverse dyskinesia in a mouse model of PD [7].

At the beginning of the exploration of PTB effects in astrocytes, the PTB-regulated loop was found to be similar in fibroblasts and astrocytes while the nPTB-regulated loop was similar in astrocytes and neurons. This suggests that PTB knockdown alone can convert astrocytes into neurons in both mice and humans. Transfection with a lentivirus vector expressing shRNA against *Ptbp1* (shPTB) converts both isolated mouse and human astrocytes into functionally mature $TUJ1^+$/ $MAP2^+$ neurons with normal electrophysiological properties. This process differs from that of HAFs-to-functional neurons conversion, which requires sequential downregulation of PTB and nPTB [6].

Is it possible to directly reprogram astrocytes into neurons by targeting *Ptbp1* in the mouse brain? *GFAP-cre* transgenic mice expressing Cre recombinase under the astrocyte-specific *GFAP* promoter was used in the study. By designing an AAV vector driving shPTB expression (AAV- shPTB) with RFP tracing which is activated in cells expressing GFAP-Cre recombinase and injecting it into the transgenic mouse midbrain, researchers found that neurons converted from astrocytes progressively increased, and these newly-generated dopamine (DA) neurons were close to the injection site, where they gradually matured and formed synaptic connections. This indicates a time-dependent incorporation into the nigrostriatal pathway. The propensity and efficiency of midbrain astrocyte-to-DA neuron conversion following PTB downregulation maybe attributed to the higher expression of DA neuron-specific transcription factors in midbrain astrocytes and the influence of the local midbrain microenvironment.

How can such an *in vivo* reprogramming strategy be exploited to reconstitute an injured nigrostriatal pathway? Researchers first used a mouse model of PD induced by 6-hydroxydopamine (6-OHDA), a dopamine analogue that is toxic to DA neurons [8]. They found that about 90% of tyrosine hydroxylase positive ($TH^+$) DA neuronal cell bodies in the substantia nigra and about 90% of $TH^+$ fibers in uninjured brain were reduced after 6-OHDA lesion. By injecting of AAV-shPTB into the lesioned side, increased $TH^+$ DA neurons and $TH^+$ fibers were induced, which restored $TH^+$ DA neurons to one third of the uninjured brain and $TH^+$ fiber density to about 30% of the initial level, indicating that AAV-shPTB partially replenishes lost DA neurons through endogenous midbrain astrocyte conversion in the PD model. This also shows that axons of the new DA neurons are gradually incorporated into the nigrostriatal dopamine pathway. Furthermore, AAV-shPTB restored the level of striatal dopamine to nearly 65% of the uninjured level after it was reduced to about 25% by 6-OHDA injury. The DA level was measured using HPLC and the restored function of DA neurons was confirmed by using an activity-induced DA release assay in AAV-shPTB-reprogramming PD mice. The release of DA

in the striatum is thought to be important for the regulation of movement [9]. In addition, AAV-shPTB efficiently reversed the PD-like motor phenotype after 6-OHDA-induced lesioning, during which contralateral rotation, ipsilateral rotation, and spontaneous motor activity tests were performed. Given that antisense oligonucleotide (ASO) technology has shown great potential for the treatment of neurodegenerative diseases [10], the authors also demonstrated that astrocyte-to-DA neuron conversion was achieved by ASO-mediated PTB mRNA degradation. This strategy also efficiently rescued the 6-OHDA lesion-induced PD phenotype, and laid a solid foundation for the discovery of antisense medicines related to PD.

In summary, Qian and colleagues reported a one-step strategy to convert brain astrocytes to functional neurons. Similarly, using the newly-developed, highly-specific, and efficient RNA-targeted CRISPR system CasRx, and injecting an AAV-GFAP-CasRx-*Ptbp1* (gRNA targeting *Ptbp1* driven by an astrocyte-specific GFAP promoter) vector into the striatum of the PD mouse model, similar results of converting DA neurons from astrocytes and rescuing PD-like motor dysfunction were achieved [11]. In addition, AAV-GFAP-CasRx-*Ptbp1* also converted M *ü* ller glia into functional retinal ganglion cells (RGCs) in the mature retina, thereby alleviating symptoms associated with RGC loss [11]. Thus, this one-step procedure that converts glia into functional neurons by targeting PTB shows great potential to be exploited to treat PD or other neurodegenerative diseases, or injuries caused by irreversible nerve damage such as trauma, tumor, or stroke.

This breakthrough discovery by Fu's laboratory took several years, starting from the initial observation of the biological phenomenon, subsequent exploration of the neuron trans-differentiation mechanisms by a PTB-regulated loop, and then now to the in-depth theoretical exploration for the purpose for clinical transformation for disease treatment [5–7]. However, there still exist several hurdles to be overcome before clinical practice in patients. Since neurodegenerative disorders are more common in older people, how can the age-related limits of reprogramming be solved as discussed by the authors? In addition, downregulation of PTB is a reinforcing process for neurons converted from astrocytes. Astrocytes are the most abundant type of glial cells with vital roles in brain development and functions, including blood brain barrier formation and maintenance, synaptogenesis, neurotransmission, and metabolic regulation [12]. Therefore, does the reduction of astrocytes have any potential negative effect? This should be confirmed in further studies. Besides, a safe plasmid delivery system targeting PTB in the human still needs to be confirmed, making sure it is safe without potential risk. In the mouse midbrain, both astrocytes and neurons differentiate from radial-glial progenitor cells [13] with PTB expression [14]; and differentiated astrocytes still have PTB expression but neurons do not. Since PTB serves as a key gatekeeper for astrocyte-to-neuron trans-differentiation, whether PTB plays a role during the astrocyte differentiation and neuron differentiation process or not, a decider for cell fate or regulated by other molecular? Further studies are needed to explore the specific mechanism. In the long run, the findings of this study show good clinical translation potential for therapy of PD and other neuron-loss diseases.

## References


1. Poewe W, Seppi K, Tanner CM, Halliday GM, Brundin P, Volkmann J. Parkinson disease. Nat Rev Dis Primers 2017, 3: 17013.
2. Li S, Le W. Milestones of Parkinson's disease research: 200 years of history and beyond. Neurosci Bull 2017, 33: 598–602.
3. Barker RA, Gotz M, Parmar M. New approaches for brain repair-from rescue to reprogramming. Nature 2018, 557: 329–334.
4. Gil A, Sharp PA, Jamison SF, Garcia-Blanco MA. Characterization of cDNAs encoding the polypyrimidine tract-binding protein. Genes Dev 1991, 5: 1224–1236.
5. Xue Y, Ouyang K, Huang J, Zhou Y, Ouyang H, Li H, *et al.* Direct conversion of fibroblasts to neurons by reprogramming PTB-regulated microRNA circuits. Cell 2013, 152: 82–96.
6. Xue Y, Qian H, Hu J, Zhou B, Zhou Y, Hu X, *et al.* Sequential regulatory loops as key gatekeepers for neuronal reprogramming in human cells. Nat Neurosci 2016, 19: 807–815.
7. Qian H, Kang X, Hu J, Zhang D, Liang Z, Meng F, *et al.* Reversing a model of Parkinson's disease with in situ converted nigral neurons. Nature 2020, 582: 550–556.
8. Thiele SL, Warre R, Nash JE. Development of a unilaterally-lesioned 6-OHDA mouse model of Parkinson's disease. J Vis Exp 2012, 60: 3234.
9. Chen L, Xie J. Dopamine in Parkinson's disease: Precise supplementation with motor planning. Neurosci Bull 2018, 34: 873–874.
10. Bennett CF, Krainer AR, Cleveland DW. Antisense oligonucleotide therapies for neurodegenerative diseases. Annu Rev Neurosci 2019, 42: 385–406.
11. Zhou H, Su J, Hu X, Zhou C, Li H, Chen Z,*et al.* Glia-to-neuron conversion by CRISPR-CasRx alleviates symptoms of neurological disease in mice. *Cell* 2020, 181: 590–603.e16.
12. Molofsky AV, Deneen B. Astrocyte development: A guide for the perplexed. Glia 2015, 63: 1320–1329.
13. Kriegstein A, Alvarez-Buylla A. The glial nature of embryonic and adult neural stem cells. Annu Rev Neurosci 2009, 32: 149–184.
14. La Manno G, Gyllborg D, Codeluppi S, Nishimura K, Salto C, Zeisel A, *et al.* Molecular diversity of midbrain development in mouse, human, and stem cells. Cell 2016, 167: 566–580.e19.