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Therapeutic Potential of PTB Inhibition Through Converting Glial Cells to Neurons in the Brain

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Keywords

Ptbp1, neuronal reprogramming, lineage tracing, pluripotent progenitors, neurodegeneration, behavioral benefits

Abstract

Cell replacement therapy represents a promising approach for treating neurodegenerative diseases. Contrary to the common addition strategy to generate new neurons from glia by overexpressing a lineage-specific transcription factor(s), a recent study introduced a subtraction strategy by depleting a single RNA-binding protein, Ptbp1, to convert astroglia to neurons not only in vitro but also in the brain. Given its simplicity, multiple groups have attempted to validate and extend this attractive approach but have met with difficulty in lineage tracing newly induced neurons from mature astrocytes, raising the possibility of neuronal leakage as an alternative explanation for apparent astrocyte-to-neuron conversion. This review focuses on the debate over this critical issue. Importantly, multiple lines of evidence suggest that Ptbp1 depletion can convert a selective subpopulation of glial cells into neurons and, via this and other mechanisms, reverse deficits in a Parkinson's disease model, emphasizing the importance of future efforts in exploring this therapeutic strategy.

Contents

1. INTRODUCTION

As age-related neurodegenerative diseases impose an increasing global burden, various strategies have been advanced to modify disease progression or replace dysfunctional or dying neurons; however, despite encouraging recent findings, none are yet proven safe and effective (Martier & Konstantinova 2020). For example, delivery of trophic factors, though promising, has not yet achieved desired results. Preclinical studies of cell replacement therapies employing stem cells are encouraging but come with concerns over which cells to transplant, the extent to which they replicate endogenous neuron structure and function, potential immune rejection, unregulated growth, and axonal mistargeting. Very exciting are recent data pointing to the possibility of converting endogenous nonneuronal cells to functional neurons to replace those lost in diseases (Barker et al. 2018). This in situ neuronal reprogramming approach is attracting the increasing attention of the research community (Bocchi et al. 2022, Qian & Fu 2021).

An important new era for regenerative medicine emerged when nonneuronal cells such as fibroblasts were converted to neurons by using various combinations of lineage-specific transcription factors (TFs), a demonstration abundantly accomplished in vitro (Vierbuchen & Wernig 2012). In contrast to the TF overexpression strategy, others explored neuronal reprogramming by repressing a master negative neurogenic regulator, polypyrimidine tract binding protein 1 (Ptbp1) (Hu et al. 2018, C.K. Vuong et al. 2016). *Ptbp1* is abundantly expressed in neural stem cells and is progressively downregulated during neurogenesis (**Figure 1*a***). During this process, while functioning as a splicing repressor (Boutz et al. 2007, Makeyev et al. 2007, Spellman et al. 2007), Ptbp1 is also involved in modulating the targeting of neuron-specific microRNAs, miR-124 and miR-9, to inactivate the negative *RE1*-silencing transcription factor (*REST*) (Xue et al. 2013). These actions are critical for neuronal induction, as coordinated downregulation of *Ptbp1* and *REST* leads to the induction of a large array of neuron-specific genes, including virtually all key TFs that have been shown to drive neurogenesis in nonneuronal cells, such as *Ascl1*, *Myt1l*, *NeuroD1*, and *Brn2* (Vierbuchen & Wernig 2012), all of which are REST targets (Xue et al. 2016). *Ptbp1* downregulation is coupled with the transient induction of its paralog, *Ptbp2* (also known as *nPTB*), which

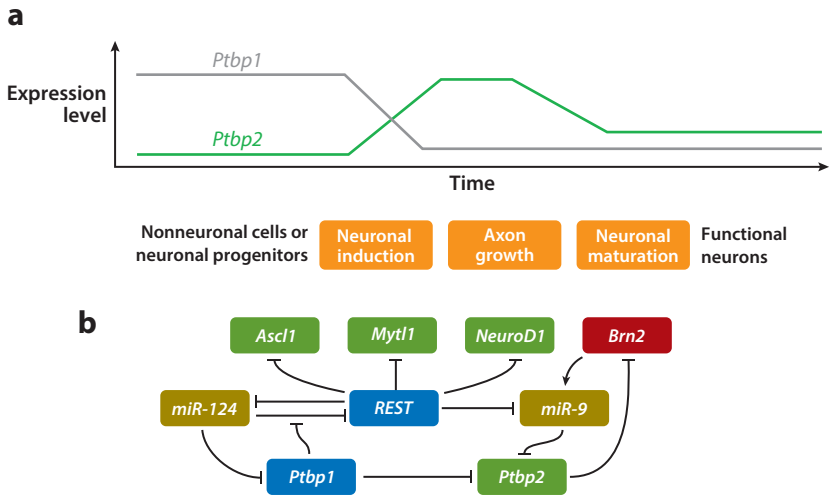

Figure 1

Dynamic regulation of *Ptbp1* and *Ptbp2* during neurogenesis. (*a*) In the initial phase of neurogenesis, *Ptbp1* is progressively downregulated, which is accompanied by the transient induction of *Ptbp2*. (*b*) Downregulation of *Ptbp1* enables initially induced miR-124 to become more effective in targeting the REST complex, and reduced REST further enhances miR-124 expression to suppress both *REST* and *Ptbp1*. This cascade of events induces a series of changes in gene expression at both the transcriptional and posttranscriptional levels, including the activation of multiple TFs, such as Ascl1, Mytl1, and NeuroD1. One of the consequences of *Ptbp1* downregulation is the induction of *Ptbp2*. After its initial induction, *Ptbp2* is then reduced by miR-9 to derepress *Brn2*, a key TF for neuronal maturation. *Brn2* helps maintain a high level of miR-9 to keep *Ptbp2* in check. This regulatory loop is critical for homeostatic gene expression in mature neurons. Abbreviations: miR, microRNA; PTBP, polypyrimidine tract binding protein; REST, RE1-silencing transcription factor; TF, transcription factor.

facilitates axonogenesis (Zhang et al. 2019). *Ptbp2* and miR-9 form another regulatory loop to activate *Brn2* (**Figure 1*b***), a TF that is critical to drive the maturation of induced neurons (Li et al. 2014, Xue et al. 2016). Interestingly, this stagewise neuronal reprogramming can be triggered by *Ptbp1* knockdown (KD) alone in mouse embryonic fibroblasts but requires separate cues to sequentially downregulate *Ptbp1* and *Ptbp2* in other cell types to generate functional neurons (Xue et al. 2016).

Mechanistic insights into the *Ptbp1/Ptbp2*-regulated loops have enabled neuronal reprogramming in vivo. Because different types of nonneuronal cells are likely to impose different epigenetic barriers to switch cell fate, a critical goal is to define which nonneuronal cell types are susceptible to neuronal reprogramming. We initially characterized astrocytes from both mice and humans, finding that, unlike fibroblasts, the *Ptbp2*-regulated loop is already activated (Qian et al. 2020). This observation suggested that *Ptbp1* KD alone may be sufficient to induce astrocyte-to-neuron (AtN) conversion. We showed that this was indeed the case using either a short hairpin RNA (shRNA) or an antisense oligonucleotide (ASO) against *Ptbp1* to generate new neurons in vitro and in the brain, and remarkably, we demonstrated that the newly induced neurons in the 6-hydroxydopamine (6-OHDA)-injured substantia nigra were able to reconstitute the damaged nigrostriatal pathway to restore dopamine biogenesis and reversed disease-associated phenotypes (Qian et al. 2020).

These findings suggested a promising therapeutic strategy by replenishing neurons lost in Parkinson's and perhaps other neurodegenerative diseases (Bocchi et al. 2022, Contardo et al. 2022, Qian et al. 2021, Wei & Shetty 2021). Maimon et al. (2021) first independently supported

this approach by enhancing neurogenesis in the dentate gyrus (DG) of the hippocampus of aged mice with an anti-Ptbp1 ASO. However, another group examining the striatum failed to link newly induced neurons to astrocytes through lineage tracing by using tamoxifen-induced, *Aldh1l1* promoter–regulated expression of Cre (Wang et al. 2021). In this study, evidence was also provided for extensive leakage of exogenous *NeuroD1* in endogenous neurons, demonstrating the need for caution in interpreting neuronal conversion results. A series of follow-up experiments by multiple labs produced conflicting observations (**Table 1**). Notably, however, none of the published studies to date has directly examined potential neuronal leakage caused by *Ptbp1* KD. This review thus focuses on *Ptbp1* KD–induced neuronal reprogramming and, when relevant, discusses some key *NeuroD1*-related observations. Readers are encouraged to consider opposing opinions in this debate (Wang & Zhang 2022). We believe that carefully examining evidence for and against AtN conversion will serve to drive research in this important research field.

2. PTBP1 SUPPRESSION IN VIVO AND IN DISEASE MODELS

Short hairpin against Ptbp1 (shPtbp1) suppression to effect in situ neuronal reprogramming was first tested in rat striatum (Weinberg et al. 2017) using a newly derived adeno-associated virus (AAV) vector, Olig001, that exhibits a greater than 95% tropism for oligodendrocytes (Powell et al. 2016). Six weeks postinjection, apparently oligodendrocyte-derived neurons appeared capable of incorporating into endogenous neural circuitries, as indicated by spontaneous postsynaptic currents and action potentials and the presence of their axons in globus pallidus and substantia nigra. Fluorescent beads injected into these regions were endocytosed and retrogradely transported to the cell bodies of newly converted neurons. This study provided initial evidence for *Ptbp1* KD–induced conversion of nonneuronal cells to neurons.

Targeting astrocytes in mice, Qian et al. (2020) tested *Ptbp1* depletion in the substantia nigra of transgenic mice expressing the Cre recombinase from the mouse glial fibrillary acidic protein (GFAP) promoter (mGFAP-Cre) and detected new dopaminergic (DA) neurons with an AAV vector that expresses red fluorescent protein (RFP) and shPtpb1 (AAV-LoxP-Stop-LoxP-RFP-shPtbp1) but not with the empty vector that expresses RFP alone (AAV-LoxP-Stop-LoxP-RFP) as control. A parallel study reported *Ptbp1* KD mediated by CRISPR-associated protein from *Ruminococcus flavefaciens* XPD30002 (CasRx) (Zhou et al. 2020), but multiple follow-up studies failed to show that the reagent downregulated *Ptbp1* (Wang et al. 2021, Xie et al. 2022), a finding acknowledged by some of the same original authors (Yang et al. 2022). Thus, the work by Zhou et al. (2020) could not be considered as evidence for *Ptbp1* KD–induced AtN conversion.

Another independent study tested AAV-GFAP-shPtbp1 in a spinal cord injury model, showing the induction of new neurons and improvement in motor function (Yang et al. 2023). This study employed a vector containing a minimal *GFAP* promoter to target astrocytes, but the presumed AtN conversion was not established by lineage tracing. Notably, Leib et al. (2022) also tested GFAP-shPtbp1 in tamoxifen-treated *Aldh1L1*^{CreERT2}*:Rosa26-LSL-tdTomato* transgenic mice and detected no AtN conversion. Importantly, however, *Ptbp1* KD in vivo was not confirmed in this study.

Guo et al. (2022) tested AAV-GFAP-shPtbp1 in the hippocampus, showing efficient *Ptbp1* KD but no new neurons and no apparent phenotypic benefit in two Alzheimer's disease mouse models (5xFAD and PS19). The same strategy also failed to find AtN conversion in striatum or substantia nigra. Of note, Guo et al. detected little or no leakage of AAV2/9-delivered GFAP-shPtbp1 in endogenous neurons, a somewhat unexpected finding because engineered GFAP promoters are all known for significant leakage in neurons, and most AAV capsids show neurotropism (Borodinova et al. 2021). The lack of enhanced neurogenesis in the hippocampus is contradictory to the

Table 1 Induced (or lack of) neuronal reprogramming in the brain by Ptbp1 KD or KO

Research group (reference)	Targeting vector(s)	Animal model(s)	Brain region(s)	Ptbp1 KD in brain?	Appearance of new neurons?	Lineage traced?	Behavioral benefits?
T.J. McCown (Weinberg et al. 2017)	Olig001-CAG-shPtbp1	Rat WT	Striatum	Not done	Yes	Not done	Not done
X.-D. Fu (Qian et al. 2020)	AAV2-CMV-LSL-RFP-shPtbp1	Mouse WT 6-OHDA lesioned	Cortex, striatum Substantia nigra	Not done	Yes	Not done	Yes
D.W. Cleveland (Maimon et al. 2021)	Anti-Ptbp1 ASO	Aged mouse WT	Hippocampus	Yes	Yes	Not done	Yes
K. Zhang (Fu et al. 2020)	AAV2-CMV-LSL-RFP-shPtbp1	Mouse WT	Retina	Not done	Yes	Not done	Yes
C.L. Zhang (Wang et al. 2021)	AAV2/5-GFAP-CasRx-Ptbp1 AAV5-GFAP-mCherry-shPtbp1 AAV2/5-CMV-LSL-RFP-shPtbp1 AAV5-CAG-LSL-mCherry-shPtbp1	Mouse WT	Striatum	No Yes Yes	Yes	No	Not done
M. Li (Chen et al. 2022)	Anti-Ptbp1 ASO AAV2/5-GFAP-EGFP-shPtbp1	Mouse WT 6-OHDA lesioned	Striatum Substantia nigra	Not done Not done	Yes	Not done	Not detected
G. Chen (Yang et al. 2023)	Anti-Ptbp1 ASO AAV2/GFAP-EGFP-shPtbp1	Mouse WT or injured spinal cord	Spinal cord	Not done Not done	Yes	Not done	Yes
Y. Zhao (Guo et al. 2022)	Anti-Ptbp1 ASO AAV2/9-GFAP-mCherry-shPtbp1	Mouse WT, 5xFAD, PS95	Hippocampus	Yes Yes	No	Not done	Not detected
B. Chen (Xie et al. 2022)	PHP.eB-GFAP-CasRx-Ptbp1 PHP.eB-CMV-LSL-RFP-shPtbp1	Mouse WT NMDA lesioned	Retina	No Yes	No	No	Not detected
S. Blackshaw (Hoang et al. 2022)	Ptbp1 lox/lox	Mouse Ptbp1 KO	Retina	Yes	No	No	Not detected

Abbreviations: 6-OHDA, 6-hydroxydopamine; AAV, adeno-associated virus; ASO, antisense oligonucleotide; CAG, chicken beta-actin; CMV, cytomegalovirus; CasRx, CRISPR-associated protein from *Ruminococcus flavefaciens* XPD30002; EGFP, enhanced green fluorescent protein; GFAP, glial fibrillary acidic protein; KD, knockdown; KO, knockout; lox, locus of crossing over; LSL, LoxP-Stop-LoxP; NMDA, *N*-methyl-D-aspartate; Ptbp1, polypyrimidine tract binding protein 1; RFP, red fluorescent protein; shPtbp1, short hairpin against Ptbp1; WT, wild type.

findings of Maimon et al. (2021), although they almost exclusively detected converted cells in the subgranular zone and dentate granule cell layer, while Guo et al. focused on the hilus of the dentate.

3. FAILED ATN CONVERSION BASED ON LINEAGE TRACING OF ASTROCYTES

Many labs examining AtN conversion used lineage tracing by employing tamoxifen-inducible Cre recombinase in astrocytes, wherein the activated Cre-estrogen receptor (CreER) fusion protein translocates from the cytoplasm to the nucleus to induce reporter expression from the *Rosa26* locus. Contrary to AAV-delivered reagents, recombinase expressed from the endogenous promoter appears to be more specific, although some neuronal leakage may persist. In one such study, Wang et al. (2021) examined the effect of *Ptbp1* KD in the striatum of *Aldh1L1-CreER^{T2}:R26R-YFP* mice. In one series, AAV5 encoding CAG-LSL-mCherry-shPtbp1 or the shLuc control was injected into striatum of *Aldh1L1-CreER^{T2}:R26R-YFP* mice, and 60 days after tamoxifen treatment and viral injection, a 3.5-fold larger number of mCherry+NeuN+ neurons were detected in *Ptbp1*-suppressed mice relative to controls. Injecting the same viruses into the striatum of *mGfap-Cre:R26R-YFP* mice produced the same result. Importantly, in neither study were mCherry+NeuN+ cells traced with YFP. In another series, AAV5 or AAV2 viruses encoding CMV-LSL-RFP-shPtbp1 or the RFP-alone control were injected into the striatum of *mGfap-Cre:R26R-YFP* mice. Again, while *Ptbp1* suppression caused an approximate 3.5-fold increase in RFP+NeuN+ neurons 60 days after viral injection, none were YFP marked. The authors concluded that *Ptbp1* KD in striatal astrocytes did not affect AtN conversion. To explain the increase in labeled neurons with *Ptbp1* suppression, they speculated that mCherry+NeuN+ or RFP+NeuN+ cells might result from leaked expression of mCherry or RFP in endogenous neurons but provided no evidence for this. How shPtbp1 in the 3′UTR of CAG-LSL-mCherry or CMV-RFP would differ from controls in driving mCherry or RFP expression remains unclear.

Similarly, in another independent study, AAV-delivered GFAP promoter–driven green fluorescent protein (GFP) and shPtbp1 (GFAP-GFP-shPtbp1), as compared to a scrambled shRNA, was found to induce NeuN+ neurons in both substantia nigra and striatum (Chen et al. 2022), suggesting apparent AtN conversion. Indeed, in the substantia nigra at 1 month postinjection, ∼13% of cells were NeuN+/GFP+ with the shPtbp1 vector versus ∼2% in the control, and by 3 months, ∼46% of GFP+ cells were NeuN+ in shPtbp1-expressing cells. At 3 months, ∼38% of GFP+ cells were tyrosine hydroxylase positive (TH+). In striatum, ∼10% of shPtbp1-expressing cells were GFP+NeuN+, but none were TH+. This regional specificity, as originally reported (Qian et al. 2020), is consistent with the shared gene signatures between astrocytes and neurons in different brain regions (Herrero-Navarro et al. 2021). To confirm neuronal conversion from astrocytes, Chen et al. (2022) further performed lineage tracing in *Aldh1l1-CreER^{T2}:Rpl22^{lsl-HA}* mice in which a large ribosome subunit protein (Rpl22) was hemagglutinin (HA) tagged. Following tamoxifen treatment, AAV-shPtbp1 or AAV-shscramble was injected into substantia nigra or striatum and HA+ cells were counted. No HA+ cells could be identified among GFP+NeuN+ neurons, and the same negative results were also obtained in the striatum and substantia nigra of mice treated with 6-OHDA; correspondingly, no motor improvement was registered following *Ptbp1* suppression (Chen et al. 2022). Thus, these authors concluded that shPtbp1 did not induce AtN conversion but left open the cellular origin of neurons induced by GFAP-GFP-shPtbp1.

The anti-Ptbp1 strategy has also been independently pursued in the retinal system by Xie et al. (2022) using shPtbp1 and by Hoang et al. (2022) employing genetic *Ptbp1* knockout (KO) mice. These studies provided extensive evidence against the conversion of Müller glia to retinal ganglion cells. Interestingly, however, the published single-cell RNA-sequencing (RNA-seq) data on *Ptbp1* KO retina appear to show elevated rod photoreceptor cells, similar to the original report by Zhang

and colleagues (Fu et al. 2020), but those rod cells could not be traced to Müller glia (Hoang et al. 2022). The authors claimed to have detected little changes in gene expression in homozygous *Ptbp1* KO Müller glia (Hoang et al. 2022), which is surprising because *Ptbp1* KD has been shown to induce widespread changes in gene expression at both transcriptional and posttranscriptional levels (Han et al. 2022; Xue et al. 2009, 2013, 2016). One should consider overcompensation by *Ptbp2* in *Ptbp1* KO cells as a possibility (see below).

In summary, independent studies have resulted in conflicting claims with respect to *Ptbp1* KD–induced AtN conversion (**Table 1**). Some observed both AtN conversion and phenotypic benefit, whereas others detected new neurons but could not trace their origin to astrocytes. Still others provided evidence against AtN conversion. Discrepant findings could arise from several experimental variables, as discussed below. Of note, none of the negative lineage-tracing studies reported the use of a positive control, that is, with TFs, such as *Sox2* (Niu et al. 2013) or *Ascl1+Dlx2* (Lentini et al. 2021), that are bench-marked as being effective for AtN conversion.

4. INSIGHTS INTO THE DISCREPANCIES IN FINDINGS

The astrocyte-specific human *GFAP* promoter (hGFAP) was used in several studies to drive the expression of mCherry-shPtbp1 (Guo et al. 2022, Wang et al. 2021) or enhanced green fluorescent protein–shPtbp1 (EGFP-shPtbp1) (Chen et al. 2022, Yang et al. 2023). shLuc and scrambled shRNA served as controls in these experiments. Curiously, as compared to controls, two studies employing hGFAP-mCherry-shPtbp1 failed to detect any AtN conversation, while the other two studies using hGFAP-EGFP-shPtbp1 observed apparent AtN conversion, although EGFP-labeled neurons could not be traced to astrocytes. The reason for this difference is unclear, as all studies confirmed efficient *Ptbp1* KD. Considering variability in neuronal leakage as a possible source for differences, it is unclear as to why EGFP-shPtbp1 should differ from mCherry-shPtbp1.

One potential explanation may be related to the dynamic activity of the *GFAP* promoter coupled with differential influence of downstream protein-coding sequences. For instance, the promoter likely has the highest activity in initial AAV-infected astrocytes, but once the astrocyte fate is switched off, the promoter activity may progressively decline. This mechanism has been suggested to enable the transient overexpression of *Sox2* to drive AtN conversion, as constitutively expressed *Sox2* from the CMV promoter failed to do so (Niu et al. 2013). Given such transient *GFAP* promoter activity during the AtN conversion process, it is possible that a sustainable level of shPtbp1 would be needed to induce cell fate switching after the promoter becomes attenuated. Conceivably, initial *Ptbp1* KD may be achievable with a wide range of AAV doses; a much higher dose may be required to sustain the effect of shPtbp1 to fully execute the entire cell fate conversion process. This may explain different efficiencies with different constructs or even the same construct in different experimental settings (e.g., see different outcomes with the same construct shown in Wang et al. 2021, figure 7*j* versus 7*m*). While a sufficiently high initial dose may be required, the dilemma is that high-titer AAV may also cause significant neuronal leakage (Chen 2021, Xiang et al. 2021). A cell type–specific promoter coupled with an effective enhancer may help solve this problem, as illustrated by a recent study (Xu et al. 2022).

A more common strategy to ensure a sustainable level of shPtbp1 is to induce its expression from a strong CMV or CAG promoter via tamoxifen-inducible CreER recombinase, and once induced in a chosen cell type, it would allow for constitutive, high-level expression of shPtbp1 regardless of cell fate. One potential problem with this strategy is a higher barrier to cell fate switch in tamoxifen-treated cells, as was recently suggested (Xu et al. 2022). In a typical lineage-tracing setting, tamoxifen is first used to induce Cre expression from a cell type–specific promoter (e.g., *Aldh1l1* for astrocytes), thus turning on a reporter (e.g., LSL-tdTomato or LSL-YFP) integrated in the *Rosa26* locus. In the untreated mouse brain, initiation of neuronal reprogramming typically

takes at least one month to become detectable (Qian et al. 2020). In response to tamoxifen treatment, activated Cre is known to transiently induce a DNA damage checkpoint response (de Alboran et al. 2001, Loonstra et al. 2001, Pepin et al. 2016), and even in the absence of any LoxP site, Cre may attack related genomic sequences (Janbandhu et al. 2014). Support for this possibility is tamoxifen-induced impairment of hematopoietic (Higashi et al. 2009) and intestinal stem cells (Bohin et al. 2018). A more recent study directly demonstrated that tamoxifen treatment induces deficits in mouse neural stem cells (NSCs) in developing cerebral cortex and adult hippocampus (Lee et al. 2020). Thus, tamoxifen-induced toxicity may contribute to the elevated barrier to neuronal reprogramming, thus compromising lineage tracing of converted neurons.

Potential tamoxifen-induced toxicity suggests that cells inefficiently traced by tamoxifen-induced CreER might be less impacted by such toxic effects, thus preserving their potential for neuronal reprogramming. In support of this possibility, multiple successful lineage-tracing experiments on TF-converted neurons waited for at least one or two weeks to allow tamoxifen-treated mice to recover before delivery of a reprogramming agent (Tai et al. 2021, Zhang et al. 2022). As the optimal period for recovery has yet to be systematically determined, future studies should define the presence of tamoxifen-induced DNA damage and the period required for affected cells to recover. An alternative strategy would be to monitor fate switching for a sufficiently long period of time, as shown in a recent neuronal reprogramming induced by *NeuroD1* overexpression (Xiang et al. 2021, Xu et al. 2022). Of note, shPtbp1-treated cells have also been traced in *mGfap-Cre:R26R-YFP* mice in the absence of tamoxifen treatment (Wang et al. 2021). Critical insights from this set of experiments are separately discussed in Section 7 below.

5. DISTINCT PHENOTYPES GENERATED BY KNOCKDOWN VERSUS KNOCKOUT

Hoang et al. (2022) appear to have provided compelling genetic evidence against neuronal conversion in the mouse retina and in cortex, striatum, and substantia nigra (Hoang et al. 2021), as genetic approaches would circumvent many shortcomings of shRNA/ASO-based strategies, including variable efficiency in reducing the expression of targeted genes and potential off-target effects. These authors detected little AtN conversion, which is reminiscent of early genetic studies showing that *Ptbp1* KO prevents not only proliferation but also differentiation of embryonic stem cells (ESCs) (Shibayama et al. 2009) and NSCs (Shibasaki et al. 2013). This may result from distinct phenotypes generated by KO versus KD, as is the case for another master negative regulator of neurogenesis, REST. *REST* KD derepresses a large set of neuronal-specific genes to induce the neurogenesis program in nonneuronal cells (Aoki et al. 2012, Ballas et al. 2005, Johnson et al. 2007), but *REST* KO impairs self-renewal of NSCs in the brain, leading to early embryonic lethality (Gao et al. 2011). Interestingly, a systematic comparison between genetic ablation versus morpholino-mediated KD revealed that about half of the genes in the zebrafish genome showed distinct phenotypes (Kok et al. 2015).

There is an ongoing debate on experimental artifacts versus genetic compensations induced by KO versus KD in diverse biological systems (El-Brolosy & Stainier 2017). For example, while *Tet1* KD skewed ESC differentiation (Koh et al. 2011), *Tet1* KO showed no phenotype (Dawlaty et al. 2011), apparently because ESCs express a major (*Tet1*) and a minor (*Tet2*) member of the Tet family of DNA demethylases; thus, forced induction of *Tet2* likely compensated for total loss of *Tet1*. In another example, *egfl7* encodes for endothelial extracellular matrix gene in zebrafish, and *egfl7* KD induced severe vascular defects but *edfl7* KO exhibited no obvious phenotype (Rossi et al. 2015). The difference was attributed to the genetic compensation by another induced extracellular matrix protein in *edfl7* KO animals. These observations illustrate genetic compensation by other proteins with related functions acting in the same biological pathways.

What would be the mechanism for genetic compensation to *Ptbp1* KO? *Ptbp2* induction in response to *Ptbp1* KO has been extensively documented in diverse cell types (Boutz et al. 2007, Makeyev et al. 2007, Polydorides et al. 2000, Xue et al. 2016, Zheng et al. 2012), including Müller glia (Hoang et al. 2022). Importantly, simultaneous inactivation of *Ptbp1* and *Ptbp2* causes cell death (Xue et al. 2016), suggesting that cell viability requires at least one of the gene products. Indeed, *Ptbp1* and *Ptbp2* have been found to have both distinct and redundant functions: When ablated, *Ptbp2* was complemented by constitutively expressed *Ptbp1*, and the function of NSCs was still greatly compromised, even though viability and function of mature excitatory forebrain neurons were largely preserved (J.K. Vuong et al. 2016). These findings could help explain the differences between *Ptbp1* KO versus KD: In *Ptbp1* KO cells, it may be essential to maintain a high level of induced *Ptbp2* to fulfill essential cellular functions, thus inducing precocious neurogenesis but preventing the complete switch of cell fate to the neuronal lineage; in *Ptbp1* KD cells, residual Ptbp1 protein may be sufficient to ensure cell viability, thus permitting transient *Ptbp2* induction to promote axonogenesis (Zhang et al. 2019), with subsequent downregulation of *Ptbp2* to facilitate synaptogenesis (Zheng et al. 2012) and longevity (Lin et al. 2020). These findings demonstrate the functional interplay between *Ptbp1* and *Ptbp2* in both immature and mature neurons to regulate axonal structure and synaptic plasticity.

6. INSIGHTS INTO LEAKED EXPRESSION IN NEURONS

Leaky expression of reprogramming agents in endogenous neurons is a major concern even when attempting vigorous control. Qian et al. (2020) presented five lines of evidence to argue against leakage in endogenous neurons: (*a*) a time-dependent appearance of both TH+ cell bodies in substantia nigra and fibers in striatum over the course of 3 months (if RFP-shPtbp1 were leaked in endogenous neurons, one would expect a much faster labeling of RFP+NeuN+ neurons); (*b*) abnormal targeting of RFP+ axons to the septal nucleus, which is not typically targeted by endogenous nigral neurons, suggesting a level of aberrant axonal growth from newly converted neurons; (*c*) progressive acquisition of the electrophysiological properties of mature DA neurons; (*d*) restoration of dopamine biogenesis and activity-induced dopamine release following 6-OHDA lesioning of endogenous TH+ neurons; and (*e*) chemogenetic evidence from using the DREADD (designer receptors exclusively activated by designer drugs) strategy to control the function of newly reprogrammed neurons.

Similarly, Maimon et al. (2021) reported five lines of evidence for new neurons generated through inhibition of *Ptbp1* in the hippocampus: (*a*) Ptbp1-ASO-dependent enhancement of new neurons, (*b*) gradual maturation of neuronal morphology, (*c*) Ptbp1-ASO-mediated conversion in the organoid model of neurodevelopment, (*d*) the induction of doublecortin (DCX)+ immature neurons as intermediates during cell fate switching, and (*e*) improved memory in aged mice. Qian et al. (2020) did not detect the induction of DCX+ neurons in the shPtbp1-treated substantia nigra, possibly due to distinct cellular sources for new neurons in substantia nigra versus hippocampus.

Despite the evidence thus referenced, the possibility remains that leakage of a reprogramming reagent, but not its control, occurs in endogenous neurons. This has been demonstrated with *NeuroD1*-mediated AtN conversion where a large number of endogenous neurons were found to express mCherry-NeuroD1 but not mCherry alone (Wang et al. 2021). However, an independent study in which *NeuroD1* was overexpressed clearly detected a degree of AtN conversion in both hippocampus and cerebellar cortex (Leib et al. 2022). Furthermore, Chen and colleagues (Xiang et al. 2021) provided their own lineage-tracing data to demonstrate *NeuroD1*-mediated AtN conversion more than 4 months after tamoxifen treatment. In a more recent study, these

authors suggested a potential mechanism for selective damage of neurons that, when infected with high-titer AAV, in some way alters the specificity of the *GFAP* promoter (Xu et al. 2022), but the mechanism remains elusive. Alternatively, the *GFAP* promoter may be quite weak but not completely inactive in neurons, and as a result, in neurons infected by AAV at a high multiplicity of infection, the resulting messenger RNAs (mRNAs) may accumulate to a level sufficient to allow for the protein to reach a detectable level. This may account for leaked expression of the control GFP vector in endogenous neurons with high-titer AAV (Xu et al. 2022). The problem may be exacerbated by enhanced mRNA stability with certain protein-coding sequences, such as those in the *NeuroD1* complementary DNA (cDNA), thus contributing to the accumulation of translated protein in endogenous neurons. This appears to provide a more plausible explanation than what was offered earlier for the altered specificity of the *GFAP* promoter by the *NeuroD1* cDNA to cause enhanced *NeuroD1* leakage, even with DNA binding–deficient *NeuroD1* (Wang et al. 2021).

A key question is whether any of these potential mechanisms would account for selective leakage of RFP-shPtbp1 in endogenous neurons. As a general principle of molecular biology, a control with point mutations would be considered a rigorous control. For example, in the case with shLuc or scrambled shRNA as control for shPtbp1, it is quite difficult to envision how a few differences in nucleotide sequences in the shRNA hairpin would significantly alter the specificity of the CMV promoter or the stability of the RFP-shPtbp1 transcript. One may also consider selective RFP-shPtbp1 leakage compared to RFP-shLuc control via shPtbp1-mediated off-target effects. However, multiple different shPtbp1 constructs that target different *Ptbp1* regions have been tested and shown to induce similar AtN conversion via AAV or ASO (Maimon et al. 2021, Qian et al. 2020). Wang et al. (2021) offered additional potential mechanisms, such as Cre-independent recombination (Fischer et al. 2019) or AAV concatemerization (Yang et al. 1999), resulting in aberrant activation of the targeting vector in existing neurons (Wang et al. 2021). These mechanisms do not seem to apply to shPtbp1 because the brain of wild-type mice showed no RFP signal when injected with AAV-LSL-RFP-shPtbp1 or control AAV-LSL-RFP (Qian et al. 2020).

An interesting alternative to these considerations is that apparent neuronal leakage might result from selective killing of initially infected astrocytes by an overexpressed reprogramming reagent, while sparing infected neurons because of the limited expression of such reagent. Such damaged cells may be difficult to detect. This potential mechanism might account for time-dependent appearance of converted neurons even in rigorously lineage-traced systems because the density of leaked neurons would appear progressively increased if initially infected nonneuronal cells are progressively reduced. In any case, neuronal leakage remains a formal possibility, requiring additional studies to determine the underlying mechanism(s).

7. EVIDENCE FOR NEURONAL CONVERSION FROM IMMATURE GLIA

To address the concern of tamoxifen-induced toxicity, Wang et al. (2021) further tested *Ptbp1* KD in constitutive, nontamoxifen-treated *mGfap-Cre* mice, where astrocytes were premarked with YFP expressed from the Cre-dependent *Rosa26* locus (**Figure 2*a***). Injection of AAV-CMV-LSL-RFP-shPtbp1 is expected to activate RFP-shPtbp1 in these YFP-traced mice (**Figure 2*b***). Cells that are YFP+ but RFP− likely correspond to YFP-traced cells that were not infected by the injected AAV (**Figure 2*c***); cells positive for both labels would be those infected by the AAV vector expressing RFP (**Figure 2*c***). Surprisingly, numerous RFP+ but YFP− cells are also evident (**Figure 2*c***). As discussed in Section 6, there is no evidence for shPtbp1-mediated, Cre-independent recombination that would account for the selective induction of RFP expression in AAV-CMV-LSL-RFP-shPtbp1-infected cells. In fact, there were about an equal number of RFP+YFP− cells in shPtbp1 and control AAV-infected brain samples, arguing against a selective

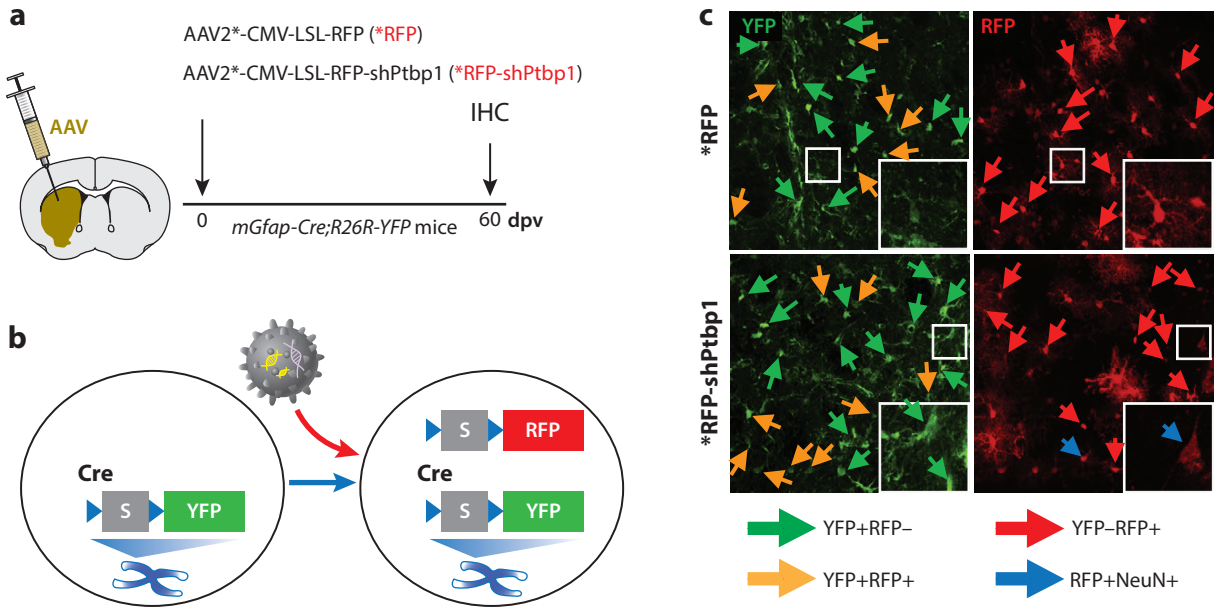

Figure 2

RFP+ cells that failed to be traced with YFP may correspond to a population of immature glial progenitors in the striatum of adult mouse brain. (*a*) The experimental scheme (derived from Wang et al. 2021, figure 7*k*) for injecting AAV into the striatum of *mGfap-Cre* transgenic mice, which are traced with YFP expressed from the *Rosa26* locus. (*b*) Illustration of reporter genes before and after AAV infection. After infection, any cell expressing RFP would be indicative of the presence of active Cre, which would also be expected to turn on YFP. (*c*) Actual data derived from Wang et al. (2021, figure 7*l*). Green arrows show cells labeled with YFP but not infected by AAV, thus lacking RFP. Orange arrows show cells traced with YFP and infected with AAV, thus also expressing RFP. Red arrows show cells expressing RFP but not traced with YFP. Blue arrows show cells expressing RFP, which are also marked by NeuN+, a marker for mature neurons. Abbreviations: AAV, adeno-associated virus; CMV, cytomegalovirus; dpv, days post viral infection; IHC, immunohistochemistry; LSL, LoxP-Stop-LoxP; NeuN, neuronal nuclei; RFP, red fluorescent protein; shPtbp1, small hairpin against Ptbp1; YFP, yellow fluorescent protein.

effect of shPtbp1 in mediating Cre-independent recombination. These observations raise an intriguing possibility that RFP+YFP− cells may represent a distinct population that is distinct from RFP+YFP+ astrocytes. As such, they could be a source of newly generated RFP+ neurons in shPtbp1-treated brains.

In fact, astrocytes are now well known to be heterogeneous in both the normal and diseased brain (Ben Haim & Rowitch 2017, Escartin et al. 2021), as further evidenced by the most recent spatial transcriptomic analysis of both mouse and human brains (Fang et al. 2022). Contrary to some claims, most astrocytes can be marked in a given specific transgenic mouse strain, and it has been argued that "there is no uniform and unequivocal definition of astrocytes" and, indeed, "astrocytes are actually the cell population in the brain, which is left over after one would remove neurons, oligodendrocytes, and microglia" (Kettenmann & Verkhratsky 2016, p. 552). Earlier lineage-tracing studies demonstrated that radial glia are the common ancestor of both neurons and glial cells during early development. In adult brain, neuronal progenitors are largely restricted to specific brain regions such as the subventricular zone (SVZ) and DG of hippocampus, but radial glia–derived progenitors are present in many brain regions, wherein they may maintain homeostasis of astrocytes and oligodendrocytes (Anthony et al. 2004, Kriegstein & Alvarez-Buylla 2009) (**Figure 3**).

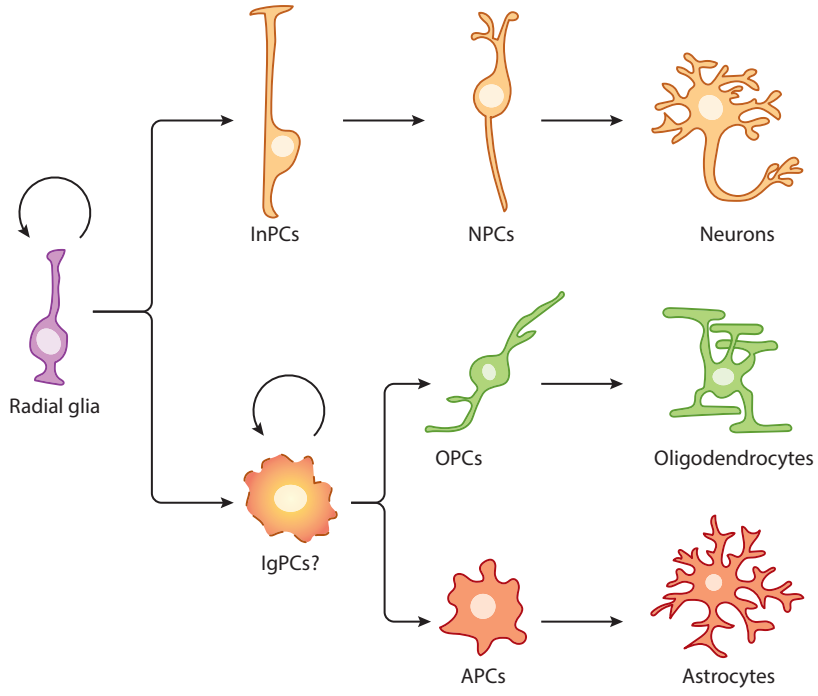

Figure 3

A population of immature glia progenitor cells in adult mouse brain. During development, radial glia function as neural stem cells (NSCs). After birth, these NSCs are specified into immature neuronal progenitor cells (InPCs), which give rise to the neural progenitor cells (NPCs) that are largely preserved in the subventricular zone and dentate gyrus of the hippocampus. Besides these specialized brain regions, it is possible that later radial glia cells are first specified into a population of immature glial progenitor cells (IgPCs), which are further differentiated into the oligodendrocytic and astrocytic lineages to produce oligodendrocyte progenitor cells (OPCs) and astrocytic progenitor cells (APCs, commonly known as immature astrocytes). These progenitors are responsible for maintaining homeostasis of mature oligodendrocytes and astrocytes in adult mouse brain.

It is currently unclear how many intermediate cell types exist. Could one or more subsets of glial progenitors be directed to the neuronal lineage? Based on this line of thought, we envision a scenario that could account for the discrepant findings for neuronal reprogramming with shPtbp1. During the maturation of specific progenitors, the expression of lineage-specific markers is gradually induced. For example, *GFAP* is known to be modestly expressed in radial glia (Kriegstein & Alvarez-Buylla 2009) and is strongly induced in mature astrocytes (Rowitch & Kriegstein 2010), likely resulting from differential activities of the *mGfap* promoter during gliogenesis (Robel et al. 2011). In the brain of *mGfap-Cre* mice, this would result in differential levels of Cre expression in different subclasses of immature glia and astrocytes. When Cre levels are relatively modest, the episomal viral DNA in the AAV-LSL-RFP-shPtbp1 expression unit could combine more efficiently than the chromosomal DNA in the R26-CAG-LSL-YFP locus. In support of this suggestion, different reporters engineered in the same *Rosa26* locus show distinct sensitivities to Cre (Álvarez-Aznar et al. 2020). Therefore, as suggested above, RFP+ but YFP− cells may mark a population of immature glial cells expressing a relatively low level of Cre. Like radial glia, this population of cells may preserve the ability to divide and thus would be more prone than mature astrocytes to switch cell fate to the neuronal lineage. Future efforts will be needed to test this idea

and to identify glial progenitors with these characteristics. While challenging, such studies could open new opportunities for generating new neurons in neurodegenerative diseases.

8. IS CELL CYCLING A PREREQUISITE FOR SWITCHING CELL FATE?

What would be the best approach for identifying the most reprogrammable subpopulation of astrocytes? One suggestion is there would be value in characterizing the population of RFP+YFP− cells from *mGfap-Cre:R26R-YFP* mice injected with CMV-LSL-RFP-shPtbp1 by sorting these cells for single-cell RNA-seq; their transcriptome could then be compared to those of other cell types, including RFP+YFP+ cells. Longitudinal studies may demonstrate the steps through which RFP+YFP− cells transition to NeuN+ neurons in response to *Ptbp1* KD. These data may enable one to deduce the pathway(s) for neuronal reprogramming, a strategy that has been widely applied in multiple biological systems (Van den Berge et al. 2020).

A more general question pertinent to the current discussion is why certain cell populations show a higher propensity than others to neuronal reprogramming. In many neuronal reprogramming studies, a retrovirus has been used to deliver lineage-specific TFs to induce a cell fate switch (Gascon et al. 2016, Guo et al. 2014, Lentini et al. 2021). As retroviruses only infect proliferating cells, this raises the intriguing question as to whether dividing cells are particularly prone to neuronal reprogramming. If so, this would suggest that a more dynamic epigenetic landscape favors cell fate switching. This suggestion appears applicable to instances of not only cellular dedifferentiation but also redifferentiation, and even transdifferentiation, as recently reviewed (Qian & Fu 2021). Compared to TF-mediated neuronal reprogramming, *Ptbp1* KD–induced neurogenesis in nonneuronal cells appears to reflect a true transdifferentiation process, because shPtbp1 treatment potentially pulls cells out of the cell cycle to promote neuronal differentiation (Cheung et al. 2009, Wang et al. 2022, Xue et al. 2016). However, even in this case, it is still possible that the cycling state is critical for epigenetic remodeling for acquiring a new cell fate. If cell cycling state is a key determinant for neuronal reprogramming, this population of cells could be labeled with BrdU to support their evaluation by single-cell RNA-seq.

A prerequisite for cycling cells to change their fate may underlie the induction of new neurons from reactive astrocytes by lineage-specific TFs in various injury models (Bocchi et al. 2022, Heinrich et al. 2015, Wells & Watt 2018). BrdU-labeled glial progenitors are detected beyond the SVZ and subgranular zone in the uninjured adult mouse brain (Laywell et al. 2000). In substantia nigra, for example, an early study showed that half of BrdU-labeled glia are NG2 cells, which correspond to oligodendrocyte progenitors (OPCs); the cellular identity of the other half is yet unknown (Lie et al. 2002). Notably, NG2+ OPCs, which could not be efficiently traced with mature astrocytic markers, were recently shown to be the major cell population for *Sox2*-induced neuronal reprogramming in injured mouse spinal cord (Tai et al. 2021). Interestingly, BrdU+ cells isolated from the substantia nigra were able to differentiate under defined conditions in vitro into different populations of glia as well as neurons, and impressively, when grafted in different adult mouse brain regions, these progenitors largely matured into glia but a significant fraction became neurons in the hippocampus, evidence for a role of environmental cues in cell fate induction (Lie et al. 2002). Modern functional genomics can now be applied to fully characterize these cells and to explore their therapeutic potential.

9. CONTRIUBTION OF MULTIPLE MECHANISMS TO BEHAVIORAL BENEFITS?

Despite the ongoing debate on the cellular origin of *Ptbp1* KD–induced neurons, independent studies have linked these new neurons in disease models to recordable behavioral benefits,

including reversal of a 6-OHDA-induced Parkinsonian-related phenotype (Qian et al. 2020), correction of age-dependent decline in cognitive activity (Maimon et al. 2021), restoration of mechanical and thermal sensitivity of injured limbs (Alber et al. 2020), and improvement of motor activity and reduction of scar formation after spinal cord injury (Yang et al. 2022). That new neurons contributed to phenotypic rescue was supported in one of these studies by using a chemogenetic approach, showing that motor recovery in the 6-OHDA model of Parkinson's disease was reversed when the electrophysiological activity of reprogrammed neurons was suppressed via an engineered G protein–coupled receptor (Qian et al. 2020). An additional effect of anti-Ptbp1 treatment in the same model system was the restoration of dopamine biogenesis above the gain in DA neuron number. For example, following 6-OHDA lesion, endogenous RFP−TH+ DA neuron cell bodies in substantia nigra and axons in striatum were both reduced to ∼10% of the wild-type level. Following shPtbp1 treatment, while RFP+TH+ cell bodies and their striatal axons were both increased ∼20%, there was also a ∼10% increase in RFP−TH+ striatal axons, resulting in total TH+ axons of ∼40–45% of those of the unlesioned mouse in striatum. Furthermore, dopamine biogenesis and activity-induced dopamine release returned to ∼60% of wild type. Therefore, the fiber density and the level of dopamine biogenesis were both increased beyond the increase of DA neuron cell bodies, suggesting that events in addition to neuronal reprogramming contributed to the outcome.

Besides *Ptbp1* KD–induced neuronal reprogramming, we envision three potential additional mechanisms that may collectively contribute to the rescue of phenotypic deficits (**Figure 4**). First, *Ptbp1* may be induced in injured neurons to negatively impact their structure and function. In fact, *PTBP1* has been found to be induced in patients with Alzheimer's disease (Roussarie et al. 2020) and in response to peripheral nerve injury (Alber et al. 2020). This may be due to reactivation of developmental patterns of gene expression program in the aged and/or diseased brain (Caldwell et al. 2020, Tollervey et al. 2011). Remarkably, in the peripheral nerve injury model, induced Ptbp1 protein was found in the cytoplasm of injured neurons, reminiscent of cytoplasmic Ptbp1 in response to signaling (Ma et al. 2007, Xie et al. 2003) and stress (Galban et al. 2008) or during cell spreading (Babic et al. 2009). Importantly, suppression of such injury-induced *Ptbp1* with AAV9-shPtbp1 or an anti-Ptbp1 ASO was found to partially ameliorate reduced mechanical and thermal sensitivity, indicative of a therapeutic benefit (Alber et al. 2020). Early studies showed that *Ptbp1* is directly or indirectly involved in suppressing genes important for synaptogenesis, one of which corresponds to *PSD-95* (Zheng et al. 2012). Therefore, injury or disease-induced reexpression of *Ptbp1* may repress this and other genes to impair the function of mature neurons. Moreover, *Ptbp1* has also been shown to directly regulate the expression of a key apoptosis regulator, *Bak1* (Lin et al. 2020). During neurogenesis, downregulated *Ptbp1* causes de-repression of a mini-exon in the Bak1 pre-mRNA to trigger its nonsense-mediated decay, thereby diminishing Bak1 protein and leading to the increased longevity of induced neurons. Based on these mechanistic insights, it is conceivable that under certain disease conditions reinduced *Ptbp1* may impair various neuron structures and functions; if so, *Ptbp1* suppression in these neurons may provide therapeutic benefit.

Second, *Ptbp1* may support neurons by enhancing the local trophic environment. *Ptbp1* KD has been shown to form a feedforward loop to cause *REST* downregulation in a coordinated fashion in nonneuronal cells (see **Figure 1**). Interestingly, REST is responsible for direct repression of the gene encoding for brain-derived neurotrophic factor (BDNF), as evidenced by the strong REST chromatin immunoprecipitation sequencing (ChIP-seq) signal on the *BDNF* promoter in brain tumor–derived cell lines (Arnold et al. 2013, Gertz et al. 2013) as well as in human hippocampus (McGann et al. 2021). Thus, *Ptbp1* KD in nonneuronal cells, such as mature astrocytes, may induce BDNF, thereby supporting the structure and function of nearby endogenous neurons.

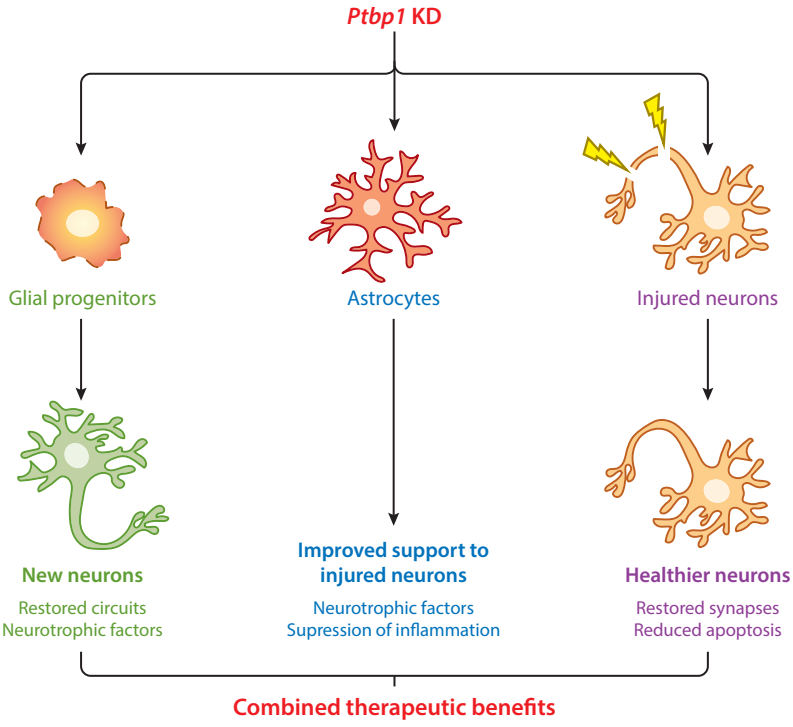

Figure 4

Potential contribution of polypyrimidine tract binding protein 1 (*Ptbp1*) knockdown (KD) to behavioral benefits via more than one mechanism. Besides generating new neurons (*left*), *Ptbp1* knockout in mature astrocytes may alter the gene expression program that somewhat resembles that of neurons (*middle*). As a result, even without a switch in cell fate, those cells may secrete various neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), to support recovery of injured neurons. Small hairpin RNA against *Ptbp1* may also help confine harmful inflammatory responses in diseased brain. When the reprogramming agent is leaked into endogenous neurons, it may make injured neurons healthier by improving synaptogenesis and repressing apoptosis (*right*). These mechanisms may collectively contribute to the observed phenotypic recovery.

Last, but not least, *Ptbp1* inhibition may help reduce inflammation. Neural injury and certain disease conditions trigger inflammatory responses in the brain (Kinney et al. 2018, Tansey et al. 2022). Recent studies have linked such responses to neural senescence, inducing the senescence-associated secretory phenotype (SASP), which may cause further neural damage (Baker & Petersen 2018, Si et al. 2021). SASP has been intensively investigated in the context of tumorigenesis, representing a promising avenue to develop therapeutic approaches against cancer (Birch & Gil 2020). A recent genome-wide screen was designed to search for SASP suppressors without inducing cells to reenter the cell cycle, and interestingly, *Ptbp1* emerged as a top hit (Georgilis et al. 2018). More recently, a targeted CRISPR screen also identified *Ptpb1* as a key gene responsible for the activation of the nuclear factor κB (NF-κB) pathway in endothelial cells from a mouse model of atherosclerosis (Hensel et al. 2022). In this study, *Ptbp1* KD was found to blunt the inflammatory response of endothelial cells to a variety of cytokines, including tumor necrosis factor-α (TNF-α). Because TNF-α is one of the key cytokines secreted by lipopolysaccharide (LPS)-activated microglia to cause neurotoxicity in the central nervous system (Liddelow & Barres 2017, Liddelow

et al. 2017), *Ptbp1* KD may help repress such an inflammatory response as part of SASP in injured brain or during neurodegeneration—an exciting possibility to be explored in future studies.

10. CONCLUDING REMARKS

Ptbp1 has emerged as a promising target for treating neurodegenerative diseases. Its disease relevance does not arise from studies showing that it is a disease-causing gene; rather, interest stems from basic science research advances in which *Ptbp1* was first identified as a splicing regulator and later found to be dynamically regulated during neurogenesis. Indeed, *Ptbp1* and its paralog, *Ptbp2*, are now known for their key roles in neurodevelopment. The initial serendipitous discovery that *Ptbp1* KD converts diverse cell types into neurons in vitro (Xue et al. 2013), followed by the extension of the discovery to generate new neurons in both mouse and human cells (Xue et al. 2016), and more recently in diseased brain (Qian et al. 2020), established the foundation for motivating studies to explore its therapeutic value.

Our analysis of conflicting data and observations, as discussed herein, demonstrates that more evidence must be produced to determine whether new neurons come from mature astrocytes, as initially proposed based on the use of promoters active in astrocytes. The important alternatives to this conclusion include (*a*) reprogramming of a yet unidentified glial cell population and (*b*) mistaking existing neurons labeled by promoter leakage as new neurons. If we accept neuronal leakage as a possible explanation for earlier findings, then additional studies are needed to carefully define the extent of leakage and its significance. Important lessons have also been learned about distinct phenotypes resulting from gene KD versus KO due to genetic compensation and from accumulating evidence suggesting that REST and Ptbp1 are Goldilocks regulators in both neuronal development and homeostasis.

The evidence points to the exciting possibility that as yet poorly defined populations of immature glial progenitors derived from radial glia play important roles in the mature brain for both glial homeostasis and neurogenesis. After birth, a fraction of radial glia–derived pluripotent cells may be largely committed to the glial cell lineage, pending possible specification into OPCs or immature astrocytes. This population may serve as a reservoir to maintain homeostasis of mature oligodendrocytes and astrocytes in normal brain, thus preserving the ability to respond to neural injury. Because of their close developmental relationship with neuronal progenitors, we speculate that this population of immature glial progenitors may be particularly susceptible to induced cell fate switch to the neuronal lineage in response to downregulation of *Ptbp1* or overexpression of lineage-specific TFs. Studies to test this hypothesis, and if true, to exploit it, are essential for exploring their therapeutic potential for brain repair.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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