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● PERSPECTIVE

Targeting transcriptional regulators to regenerate midbrain dopaminergic axons in Parkinson's disease

Introduction: Parkinson's disease (PD) is a chronic, age-related neurodegenerative disorder that affects 1–2% of the population over the age of 65. PD is characterised by the progressive degeneration of nigrostriatal dopaminergic (DA) neurons. This leads to disabling motor symptoms, due to the striatal DA denervation. Despite decades of research, there is still no therapy that can slow, stop or regenerate the dying midbrain DA neurons in PD. Current drug treatment regimes typically involve dopamine-replacement strategies. While these are effective in controlling the symptoms for several years, they do not attenuate the progressive neurodegeneration. There is now robust evidence that retrograde degeneration of nigrostriatal axons occurs early in PD pathogenesis, and precedes neuronal loss (Kordower et al., 2013). Therefore, a promising approach for restoring motor function in PD patients may be to develop strategies which regenerate nigrostriatal DA axons, so that they can re-establish their lost connections (**Figure 1**). To address this, investigation of the potential for reactivation of the intrinsic axon growth capacity of midbrain DA neurons is needed. Information on the molecular mechanisms regulating nigrostriatal DA axonal growth and target innervation during their normal development will provide novel targets for axon regenerative therapy in PD.

Axon regeneration in the central nervous system (CNS): Regeneration of CNS axons remains a major challenge for cotemporary neuroscience. While their peripheral nervous system (PNS) counterparts can typically regenerate after damage in adulthood, the spontaneous regeneration of injured CNS axons is inhibited by a number of extrinsic factors, such as glial barriers and myelin-associated inhibitors. Moreover, it is now well established that intrinsic growth ability is a critical determinant of CNS axon regeneration (He and Jin, 2016). In the case of the degenerating nigrostriatal DA axons in PD, reactivating their intrinsic axon growth ability is a major goal for promoting their regeneration.

Using the extensively employed optic nerve injury model for CNS axon regeneration, several methods have been identified to activate growth-promoting pathways and regeneration in mouse retinal ganglion cells (RGCs). These include knockdown of phosphatase and tensin homolog (PTEN), or overexpression of osteopontin with insulin-like growth factor 1 (IGF1) or brain-derived neurotrophic factor (BDNF), which are thought to activate mammalian target of rapamycin (mTOR) and/or growth factor signalling pathways (Park et al., 2008; Duan et al., 2015). Interestingly, a number of these manipulations, such as PTEN deletion, also promote axon regeneration in the mouse spinal cord injury model (Du et al., 2015; He and Jin, 2016), suggesting conserved regenerative mechanisms for mammalian CNS axons.

In addition to the manipulation of growth-promoting pathways in CNS neurons, another effective strategy for inducing axon regeneration following disease or injury involves the identification of cell-autonomous, master regulators of axon growth in CNS neuronal subtypes during their development. These factors can be subsequently targeted to reprogram neurons towards an axon growth-competent state. Indeed, a screen of developmentally-regulated genes in rodent RGCs identified a zinc finger transcription factor, Krüppel-like factor 4 (KLF4), as a transcriptional repressor of axonal growth. Knockdown of KLF4 was then shown to promote RGC regeneration post optic nerve injury *in vivo* (Moore et al., 2009). More recently, overexpression of Sox11, a transcription factor important for RGC differentiation, was also shown to promote RGC regeneration in this model (Norsworthy et al., 2017). Similarly, identification of developmentally-relevant, cell-autonomous regulators of midbrain DA axonal growth may reveal strategies to regenerate these axons in PD.

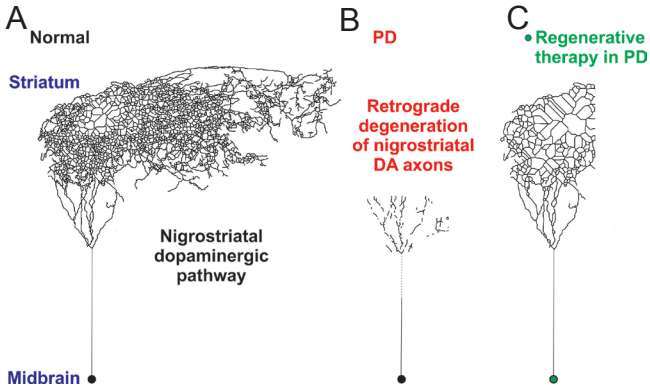

Figure 1 The potential of regenerative therapy in Parkinson's disease (PD). (A) Drawing of the intact nigrostriatal pathway showing ascending axons of dopaminergic (DA) neurons that project from the midbrain and innervate the striatum. (B) In PD, the progressive retrograde degeneration of these axons leads to a loss of DA innervation in the striatum, which causes motor dysfunction. (C) Regenerative therapy in PD (green), such as targeting transcriptional regulators of axon growth, may regenerate midbrain DA axons so that they can reinnervate the striatum.

Molecular regulation of midbrain DA axonal growth: To identify such regulators of midbrain DA axon growth, we recently performed a polymerase chain reaction (PCR) screen to profile gene expression in the mouse midbrain during nigrostriatal pathway development (Hegarty et al., 2017). We found that expression of zinc finger E-box-binding homeobox 2 (Zeb2) was significantly decreased during midbrain DA axon growth and target innervation (from embryonic day (E)12 to E18). Zeb2 was first identified as a transcriptional repressor that interacts with Smad transcription factors, and has since been shown by many studies to be a multifunctional regulator of nervous system development (Hegarty et al., 2015). In our study, we discovered that Zeb2 acts as a repressor of midbrain DA axon growth. Genetic silencing of Zeb2 enhanced, while Zeb2 overexpression repressed, midbrain DA axonal growth *in vitro*, in a Smad signalling-dependent manner (Hegarty et al., 2017). Moreover, we demonstrated that conditional (Nestin-Cre based) Zeb2 knockout mice exhibit DA hyperinnervation of the striatum, indicating that nigrostriatal DA axons grow excessively in the absence of Zeb2 *in vivo*.

Zeb2: a novel target for midbrain DA axon regeneration: Another zinc finger transcription factor, KLF4, is expressed during optic nerve development, is a repressor of RGC axonal growth, and its deletion promoted RGC regeneration after optic nerve injury *in vivo* (Moore et al., 2009). If the

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mechanistic findings obtained from the widely-used optic nerve crush model are comparable or predicative for other CNS axonal projections, then Zeb2 may be a promising target for the regeneration of nigrostriatal DA axons, since we have found that it represses midbrain DA axonal growth during development (Hegarty et al., 2017). In support of this suggestion, axon regeneration strategies developed from the optic nerve model have been used to regenerate corticospinal axons (Du et al., 2015; He and Jin, 2016). To explore the potential of Zeb2 as a therapeutic target in PD, the regenerative effects of Zeb2 knockdown on nigrostriatal DA axons should be investigated in animal models of PD. Furthermore, those regeneration-enhancing manipulations that relate to both RGC and corticospinal axons, and thus may be universally applicable throughout the CNS, *e.g.*, PTEN deletion, should also be investigated for their ability to regenerate midbrain DA axons.

Potential limitations for regenerative therapy in PD: To date, experimental regeneration strategies have achieved successful regrowth of only a proportion of the population of injured or degenerating CNS axons (Park et al., 2008; Moore et al., 2009; Duan et al., 2015; He and Jin, 2016; Norsworthy et al., 2017). Further research is therefore required to overcome this limitation, such as the investigation of subtype-specific growth mechanisms in CNS neuronal populations. However, since nigrostriatal DA axons extensively innervate their target areas, axon regeneration of the total population of dying midbrain DA neurons may not be necessary to achieve clinical benefits in PD. For example, a neuronal labelling study showed that a single midbrain DA axon can innervate up to 5.7% of the total volume of the striatum (Matsuda et al., 2009). Therefore, it is theoretically possible to restore a therapeutically efficacious amount of striatal innervation in PD by inducing the regeneration of only a proportion of midbrain DA axons.

Another important consideration before regenerative therapy becomes a clinical possibility for PD is whether the nigrostriatal DA axons, which are subject to pathological, degenerative signals, will require both survival- and regeneration-promoting signals to effectively reinnervate the striatum. KLF4 deletion increases the regeneration of adult RGCs without affecting their survival (Moore et al., 2009), while Sox11 overexpression promotes regeneration of some RGC subtypes but kills others (Norsworthy et al., 2017). Because survival and regenerative signalling can be mutually exclusive, it will be important to test whether manipulations aimed at regenerating nigrostriatal DA axons, such as the proposed deletion of Zeb2, also have effects on neuronal survival in animal models of PD. For the effective, long-term restoration of nigrostriatal DA innervation in PD, regeneration therapeutics may need to be co-administered with survival-promoting treatments, *e.g.*, neurotrophic factors. Other avenues that should be explored before translation from animal models to the clinic include: the quantification of the number of functional synapses established by regenerating axons, determination of any adverse effects of the genetic manipulation(s) on other key cellular functions, evaluation of the potential of task-specific training to enhance functional recovery after regenerative therapy, and optimisation of non-invasive methods for targeting regeneration enhancers in the CNS.

Despite these potential limitations, targeting transcriptional regulators, such as Zeb2, to regenerate midbrain DA axons is a promising disease-modifying therapeutic strategy for PD, and may be capable of achieving functional recovery for PD patients.

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