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Commentary

GDNF Therapy: Can We Make It Work?

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Abstract. In two recent postmortem studies, Jeffrey Kordower and colleagues report new findings that open up for an interesting discussion on the status of GDNF/NRTN signaling in patients with Parkinson's disease (PD), adding an interesting perspective on the, admittedly very limited, signs of restorative effects previously seen in GDNF/NRTN-treated patients. Their new findings show that the level of the GDNF signaling receptor Ret is overall markedly reduced relative to the non-PD controls, and most severely, up to 80%, in nigral neurons containing α-synuclein inclusions, accompanied by impaired signaling downstream of the Ret receptor. Notably, however, the vast majority of the remaining nigral neurons retained a low level of Ret expression, and hence a threshold level of signaling. Further observations made in two patients who had received AAV-NRTN gene therapy 8–10 years earlier suggest the intriguing possibility that NRTN is able to restore Ret expression and upregulate its own signaling pathway. This "wind-up" mechanism, which is likely to depend on an interaction with dopaminergic transcription factor Nurr1, has therapeutic potential and should encourage renewed efforts to turn GDNF/NRTN therapy into success, once the recurring problem of under-dosing is resolved.

Keywords: Parkinson's disease, neurturin, Ret, Nurr1, phospho-S6

Efforts aimed to develop GDNF (or its close relative neurturin, NTRN) into a neuroprotective therapy for Parkinson's disease (PD) has faced numerous set-backs. But despite the disappointing outcome of several well-designed clinical trials, most recently the Bristol trial reported last year [1, 2], the interest in the therapeutic potential of this family of factors is still very much alive, as evidenced by the report from a recent workshop sponsored by Cure Parkinson's and The Michael J. Fox Foundation [3]. This is not only due to the intriguing neuroprotective and restorative properties of GDNF family of factors seen in PD model experiments, but also due to the signs of efficacy observed in some of the GDNF or NTRN treated patients. Although limited is size and magnitude, the signs of recovery in ^{18}F-DOPA PET imaging [1] and the increase in tyrosine hydroxylase (TH) immunostaining seen postmortem [4, 5] are particularly interesting and raise the question of the cellular mechanisms underlying these effects, their dependence on the underlying disease process and the ongoing α-synuclein pathology, in particular. To what extent is GDNF/NRTN signaling affected by the degenerative process? And is the ability of the affected nigral dopamine (DA) neurons to respond to the factor dependent on the severity of the degenerative changes?

The integrity of the GDNF/NTRN signaling pathway through their canonical receptor Ret is critical in this regard. The GDNF-induced protective and restorative effects are known to be mediated by Ret [6], and the expression of this receptor, in turn, is regulated by Nurr1, a transcription factor essential for DA neuron survival and maintenance of the DA phenotype [7–9]. Previous findings in brains from PD patients have shown that Nurr1 is reduced by more than 50% in nigral neurons containing α-synuclein inclusions [10], and studies in midbrain DA

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neurons in culture [11, 12] or rodent PD models [8, 13], have shown that overexpression of human wild-type or A53T mutant α-synuclein induces a marked, 30–60%, downregulation of Nurr1 and its downstream targets, including Ret, as observed on both the mRNA and protein level, and that this is accompanied by a blockade of the intracellular GDNF response. This α-synuclein-induced signaling blockade, as well as the reduced Ret expression, is effectively reversed by Nurr1 [8]. This effect has been observed in DA neurons expressing α-synuclein at a high level, 2–5-fold above normal [11–13], but not in a transgenic mouse with a more moderate, 0.5-fold, increase in α-synuclein expression [14], suggesting that the impact of α-synuclein on Ret expression is dose-dependent.

These findings raise the question whether downregulation of Ret, and hence disruption of GDNF signaling, may occur also in the affected midbrain DA neurons of PD patients. Although explored previously with divergent results [8, 14, 15], this issue has now, for the first time, been more systematically addressed in two recent reports by Jeffrey Kordower and colleagues [16, 17]. In their analysis of brains from diagnosed PD patients they show that the level of Ret in the still remaining nigral DA neurons was overall markedly reduced relative to the non-PD controls. Ret remained expressed at a low level in most of the cells, but below detection in about 15% in the remaining melanized neurons. The level of the phospho-S6 protein, which is commonly used as an indicator of downstream signaling through the Ret receptor (see Fig. 1), was similarly reduced and indeed below detection in many Ret positive neurons. In contrast to the non-PD controls where Ret and phospho-S6 were co-expressed at high levels, phospho-S6 was detectable only in 1/3 of remaining DA neurons in the PD subjects.

These data suggest that Ret expression and signaling is depressed overall, but to a variable extent among the remaining DA neurons. In their previous study, Chu et al. [10] showed that the downregulation of TH and Nurr1 is most pronounced in neurons containing α-synuclein inclusions. In the new study [16] they now report that Ret is reduced in both types of cells, with or without α-synuclein inclusions, but most severely, by about 80%, in neurons with inclusions. Interestingly, in a group of subjects with presumed prodromal PD, Ret was suppressed in nigral neurons with α-synuclein inclusions but maintained at a near-normal level in neurons without inclusions.

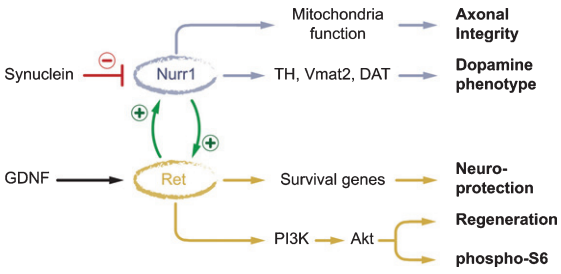

Fig. 1. New data obtained from two PD patients who had received NRTN gene therapy 8-10 years earlier [17], point to an interesting interplay between Nurr1 and the GDNF/NRTN signaling receptor Ret, providing a feed-forward mechanism that allows GDNF (or NTRN) to potentiate its own signaling via Ret-induced activation of Nurr1. In the proposed model, downregulation of Nurr1 is caused by the appearance of toxic levels of α-synuclein and/or α-synuclein inclusions [8, 10], or by α-synuclein entering the nucleus [11, 12], resulting in suppression of Ret signaling that is most pronounced in aggregate-containing DA neurons lacking TH expression [16]. Upregulation of Ret, as seen in a sub-portion of the affected nigral DA neurons in the NRTN-treated patients, activates in turn Nurr1 and its downstream targets, including TH [11]. Relations indicated by the arrows are based on references [7, 11, 18–20].

Taken together, these findings provide support for a progressive model of Ret-dependent signaling impairment that reflects not only the overall progression of the disease, but also the heterogeneity of disease progression among the individual remaining nigral neurons at any given stage. At an early symptomatic stage, it seems likely that the majority of the nigral DA neurons are GDNF responsive, but as the disease progresses and the DA neurons become more affected the GDNF responsiveness it retained in only a sub-fraction of neurons that become fewer in number as the disease progresses.

The second of the two new papers [17], which reports postmortem data from two patients who had received AAV-NRTN gene therapy 8–10 years earlier, adds interesting further observations in support of this model. The patient receiving the vector in substantia nigra (in addition to the putamen) is particularly interesting. In this patient the fraction of melanized neurons expressing TH was increased about 3-fold compared to the non-operated control, and by about 5-fold compared to the second patient receiving the vector in putamen only, indicating a marked upregulation of both Ret and TH in response to NTRN. Double-immunostaining for Ret and TH showed, moreover, that melanin-containing nigral neurons with high Ret expression were TH-positive, while Ret-negative neurons were TH-negative. These observations are interesting in that they suggest that

NRTN is able to enhance Ret expression in nigral neurons where this receptor is suppressed, implying that NRTN is able to upregulate its own signaling pathway, provided that it is acting at the cell body level.

What is the mechanism underlying this intriguing effect? Chu et al. [10] have previously shown that the TH downregulation seen in PD patients is closely correlated with the reduction in Nurr1, and they show now that the reduction in TH is closely correlated with the downregulation of Ret, suggesting an interesting interplay between these three factors. In DA neuron cultures, increased expression of α-synuclein at a level 3-fold above normal induces a 50–80% downregulation of all three factors, Nurr1, TH and Ret, leading to blockade of GDNF signaling as determined by the induction of phospho-S6 [11]. The α-synuclein induced suppression of TH and Ret is reversed by Nurr1, which is consistent with the fact that both TH and Ret are transcriptionally regulated by Nurr1 [7, 9, 18]. Interestingly, however, this interaction also works in the opposite direction, such that forced expression of Ret reverses the α-synuclein induced suppression of Nurr1 and TH.

As illustrated in Fig. 1, this reciprocal interaction can serve as a feed-forward mechanism that allows GDNF (or NTRN) to regulate its own signaling pathway via Ret-induced activation of Nurr1, as suggested by the restoration of Ret and TH in NTRN-responding nigral neurons in the Chu et al. study [17]. This wind-up mechanism is proposed to lead to a gradual potentiation of GDNF signaling in those DA neurons that retain a low level of Ret expression, leading to maintenance of DA neuron survival, recovery of DA neuron phenotype, axonal regrowth, and restored function. Since the vast majority of the remaining nigral DA neurons appear to retain a low level of Ret expression, and hence a threshold level of signaling, even in advanced stages of the disease, this should encourage renewed efforts to turn GDNF/NRTN therapy into success, once the recurring problem of under-dosing is resolved.

CONFLICT OF INTEREST

The author has no conflict of interest to report.

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