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A Repulsive Environment Induces Neurodegeneration of Midbrain Dopaminergic Neurons

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Review of Korecka et al.

The motor impairments in Parkinson's disease (PD) are caused mainly by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc). The SNpc extends axons to the dorsal striatum, forming the nigrostriatal pathway, which is involved in motor function. In PD, degeneration of these axons and the consequent loss of dopaminergic input to the striatum alter activity patterns in basal ganglia circuits. This loss precedes motor symptoms (Cheng et al., 2010; Dijkstra et al., 2015; Tagliaferro and Burke, 2016). Although pharmacological dopamine replacement therapy remains the standard treatment for PD, it does not prevent progression of the disease. Efforts to halt this progression have instead focused on targets, such as protein misfolding and aggregation, oxidative stress-induced apoptosis, and neuroinflammation.

Recently, axon guidance molecules have emerged as potential contributors to neurodegeneration in several conditions, including PD (Van Battum et al., 2015). One such molecule is repulsive guidance molecule a (RGMa), a cell-membrane-associated glycosylphosphatidylinositol-anchored glycoprotein that interacts with its receptor neogenin to mediate repulsive axonal guidance and regulate neuron survival (Matsunaga et al., 2004; Itokazu et al., 2012). In previous work, transcriptional profiling revealed that RGMa levels were twofold higher in the SNpc of PD patients than in controls (Bossers et al., 2009). In a recent study published in *The Journal of Neuroscience*, *in situ* hybridization analysis of SN tissue from PD patients and age-matched controls revealed that RGMa expression was restricted to dopaminergic neurons (Korecka et al., 2017). These results motivated the authors to further investigate the role of RGMa with respect to dopaminergic neurons in the nigrostriatal pathway.

To elucidate whether overexpression of RGMa in the SN alone could induce PD-like neurodegeneration, Korecka et al. (2017) injected adeno-associated viral vectors (AAVs) encoding mouse RGMa under the control of the human synapsin-1 promoter (AAV-RGMa) into the SN of mice. Intrastriatal administration of AAV-RGMa resulted in RGMa expression in both the SN

and the striatum, which is consistent with an anterograde transport of RGMa along nigrostriatal projections. AAV-RGMa overexpression reduced the number of tyrosine hydroxylase-expressing (TH⁺), presumably dopaminergic, neurons in the SNpc by 38%–40% and induced significant motor impairments over the subsequent ~19 weeks. Importantly, numbers of non-TH⁺ neurons remained unaltered after AAV-RGMa administration, suggesting that the effect was specific to dopaminergic neurons. Moreover, the effects were comparable with those produced in rodent models of PD involving injection of the dopamine-neuron-specific neurotoxin 6-hydroxydopamine or viral delivery of PD-linked mutant α -synuclein (Alvarez-fischer et al., 2008; Ip et al., 2017).

Interestingly, when AAV-RGMa was injected bilaterally, the decrease in the number of TH⁺ neurons was accompanied by an increase in TH expression in the remaining neurons. While an increase in TH expression following an insult might be indicative of a compensatory mechanism, it does not necessarily translate into the restoration of dopamine signaling along the nigrostriatal pathway. Indeed, the authors detected no such compensatory increase in TH expression in the striatum after high-dose AAV-RGMa administration (Korecka et al., 2017, their Fig. 6B–E), supporting the hy-

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pothesis that loss of striatal dopamine underlies the observed motor deficits. In face of these results, one can hypothesize that overexpression of RGMA leads to a “die-back” or retrograde axonal degeneration, which prevents any compensatory mechanism from countering disease progression. This would be consistent with the observed increase in levels of RGMA in the striatum after the administration of both high- and low-titer dosages of AAV-RGMA because this RGMA could act locally to induce axon retraction.

Korecka et al. (2017) also sought to address the role of RGMA in neuroinflammation, a strong component of the pathophysiology of PD. Reactive gliosis, as identified by levels of the ionizing calcium-binding adaptor molecule 1 (expressed in reactive microglia) and GFAP (expressed in reactive astrocytes), was significantly increased in the SNpc after both high- and low-titer AAV-RGMA injections compared with injection of control vectors. The glial response elicited by RGMA overexpression is notable because it suggests that RGMA might induce neurodegeneration through glial activation in addition to retraction of striatal terminals.

Whether neuroinflammation is a causative factor in PD or only aggravates neurodegeneration in response to cell-autonomous factors (e.g., protein homeostasis dysregulation, mitochondrial dysfunction) is an unresolved question. In the work of Korecka et al. (2017), it is unlikely that the observed neuroinflammatory response is the main mechanism driving dopaminergic neurodegeneration because high- and low-titer AAV-RGMA elicited comparable increases in ionizing calcium-binding adaptor molecule 1 and GFAP expression but produced different degrees of motor impairment. Therefore, the results suggest that the glial response is secondary to neuronal dysfunction caused by RGMA overexpression. Future work should address whether exposure to proinflammatory cytokines (e.g., interleukin-1) increases the susceptibility of dopaminergic neurons to RGMA-induced neurodegeneration, as is observed in an LPS-primed 6-hydroxydopamine rat model of PD (Koprich et al., 2008).

The molecular mechanisms by which RGMA promotes neurodegeneration remain unclear. Previous work points to a mechanism in which the prosurvival protein kinase Akt is dephosphorylated in response to RGMA-neogenin binding (Tanabe and Yamashita, 2014). Moreover, evidence from postmortem analysis

of brain tissue from PD patients supports the notion that dephosphorylation of Akt is present in PD-like neurodegeneration (Malagelada et al., 2008). Korecka et al. (2017) evaluated the levels of phosphorylated Akt but did not identify significant differences between AAV-RGMA and control-injected animals. Other pathways, however, might be involved in RGMA-induced neurodegeneration. For instance, other known inhibitory factors in the CNS, such as ephrins, Nogo-A, and chondroitin sulfate proteoglycans, converge on the RhoA/ROCK signaling pathway to induce axonal degeneration. RGMA might therefore be acting through this pathway to induce neuropathological changes. Indeed, there is evidence that RGMA induces growth cone collapse and neurite retraction via RhoA GTPase activation (Conrad et al., 2007). The precise mechanism of RGMA-induced RhoA activation involves the recruitment of the RGMA coreceptor Unc5, which associates with the leukemia-associated Rho guanine nucleotide exchange factor to induce RhoA activation and growth cone collapse (Hata et al., 2009). Moreover, RhoA activation has been linked to neuronal apoptosis (Dubreuil et al., 2003; Semenova et al., 2007), and its inhibition has been shown to improve neuron survival and regeneration *in vivo* (Koch et al., 2014). Thus, it is possible that RGMA overexpression in SN triggers a signaling cascade orchestrated by active RhoA that affects both dopaminergic neuron survival and the integrity of striatal axon terminals.

The identification of potential mechanisms by which RGMA drives dopaminergic cell loss could translate into effective therapeutic interventions in two ways. First, it could lead to enhanced cell-replacement therapies if modulation of RGMA-neogenin signaling facilitates the survival and growth of neural grafts in the PD brain. Second, biotherapeutics (e.g., gene therapy, antibody-directed therapy) modulating RGMA expression could provide neuroprotection in early stages of PD. Consistent with this therapeutic strategy, antibody-mediated inhibition of RGMA has been shown to be neuroprotective in models of stroke (Tassew et al., 2014) and spinal cord injury (Mothe et al., 2017). It will also be valuable to consider the interplay between repulsive axonal guidance molecules, such as RGMA, and other major neuropathological factors contributing to the inhibitory milieu of the PD brain, such as intracellular inclusions containing α -synuclein, to further understand the neuropathology of PD

and thus identify novel therapeutic targets.

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