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PINK1-Based Screen Shines Light on Autophagy Enhancers for Parkinson's Disease

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Abstract

In this issue of *Cell Chemical Biology*, Zhang et al. (2017) report a zebrafish model of Parkinson's disease (PD), incorporating the PD-protein PINK1 and rotenone, a toxin linked to PD. Using it as a drug-screening platform, they identify trifluoperazine and other piperazine phenothiazines as protective compounds that enhance autophagy independent of PINK1.

The finding that mutations in the serine/ threonine-protein kinase PINK1 cause a familial form of Parkinson's disease (PD) (Valente et al., 2004) is perhaps the most compelling evidence that mitochondrial dysfunction causes PD. Moreover, although PINK1 mutations are a rare cause of PD, heterozygous loss of PINK1 is also a risk factor for sporadic PD (Puschmann et al., 2017), and PD is characterized by prominent mitochondrial changes. Understanding why nigrostriatal dopamine neurons are susceptible to PINK1 mutations may provide insights into how mitochondrial dysfunction contributes to sporadic PD. Thus, if the pathogenic processes that cause PINK1 PD also contribute to sporadic PD, then therapies that protect against PINK1 mutations may also protect against at least a subset of sporadic PD. No disease-modifying therapy for PD has been effective, and new therapies are urgently needed.

One promising strategy is to look for compounds that block the effects of PINK1 mutations on selective mitochondrial turnover (mitophagy). However, key questions remain. Is mitophagy truly impaired in PD? Does defective mitophagy underlie the preferential death of DA neurons in PD? Will restoring mitophagy protect DA neurons in PD? Thus, it is critical that screening efforts targeting PINK1 functions, including mitophagy, are complemented by assays based on neuronal survival. However, such assays are limited. Rodent models of PINK1 loss alone fail to show neurodegeneration and/or lack the throughput needed to screen for suppressors. The most established model organism for studying the toxicity of PINK1 mutations is *Drosophila*, which develops severe deficits in flight muscle and age-dependent loss of DA neurons when PINK1 is deficient (Park et al., 2006). In zebrafish, PINK1 knockout leads to complex I and III deficiency and modest loss of DA neurons in a subset of DA neurons in the ventral diencephalon. (Flinn et al., 2013)

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In this issue of *Cell Chemical Biology*, Zhang et al. (2017) add an important new resource for researchers. They report an elegant model for PINK1 deficiency in zebrafish based on the presence of a touch-evoked escape response. Notably, the model combines PINK1 deficiency with exposure to rotenone, a pesticide and mitochondrial complex I inhibitor whose exposure is associated with an increased risk of sporadic PD (Tanner et al., 2011). Combining PINK1 deficiency and rotenone disrupts the escape response and produces robust, preferential death of the 5,6,11 clusters of DA neurons in the ventral diencephalon. Interestingly, the toxicity of PINK1 loss depends on gene dose with heterozygotes showing intermediate susceptibility, perhaps modeling how heterozygous disruption of PINK1 may predispose to the toxicity of environmental insults in “sporadic” PD. Interestingly, an adjacent population of DA neurons is spared, which is a potentially important feature because PINK1 loss in particular produces relatively selective degeneration of nigrostriatal DA neurons. Whether the susceptible 5,6, 11 DA neurons in zebrafish share core intrinsic properties with human nigrostriatal DA that predispose them to degeneration remains to be determined.

Although the mechanism underlying the disruption of the touch-evoked escape response remains to be elucidated, the behavioral changes correlate with decreased mitochondrial bioenergetic function, and both PINK1 loss and rotenone disrupt respiration, strongly suggesting that mitochondrial dysfunction underlies the behavioral changes. Interestingly, the adverse effect of PINK1 deficiency on the touch response depends on concurrent complex I inhibition by either rotenone or piericidin and does not occur with complex (III) inhibition by antimycin. Does this preferential susceptibility to complex I inhibitors reflect the same susceptibility that predisposes nigrostriatal DA neurons in rats to the toxicity of rotenone (Cannon et al., 2009)? PINK1 deficiency does indeed inhibit complex I function (Flinn et al., 2013) (Morais et al., 2014); on the other hand, other studies have failed to find a selective vulnerability of DA neurons to complex I inhibition (Choi et al., 2011). These findings raise the possibility that the susceptibility depends on the context, the specific mechanism by which complex I is inhibited, or other undefined effects of these stressors.

Zhang et al. (2017) use their model system to screen 727 small-molecule compounds, identifying three structurally related piperazine phenothiazines (i.e., trifluoperazine [TFP], fluphenazine [FLU], and prochlorperazine [PRO]) as the only hits that normalized their behavioral screen, improved mitochondrial function and blocked DA neuron death. Furthermore, they found these compounds increase autophagy, consistent with prior reports (Tsvetkov et al., 2010), while inhibiting autophagy abrogates the protective effects.

These specific compounds are unlikely to be useful therapies in PD: they are D_2 dopamine receptor antagonists. This property underlies their use as antipsychotic medications, but can worsen parkinsonism. As such, drug development will be required to determine if the dopamine antagonist properties can be dissociated from the effects on autophagy. Nonetheless, these findings highlight the therapeutic potential of boosting autophagy in PD and the need to identify more specific and robust approaches to boost autophagy in neurons. Interestingly, the mTor inhibitor rapamycin is less effective in inducing autophagy in neurons than non-neuronal cells (Tsvetkov et al., 2010), and it disrupts other pathways and causes adverse effects. Indeed, boosting autophagy over many years, as may be required for

neurodegenerative disease, could have a range of adverse effects including tumorigenesis and disruption of neuronal functions.

One approach to increase the specificity of autophagy-based therapies is to enhance a subset of autophagy, for example, by promoting PINK1/Parkin mitophagy by boosting the kinase activity of PINK1 (Hertz et al., 2013) or by inhibiting deubiquitinase enzymes that oppose mitophagy (Bingol et al., 2014). In this case, however, Zhang et al. (2017) show that TFP induces autophagy by activating transcription factor EB (TFEB) and its target gene SQSTM1 independent of PINK1 and Parkin, presumably indicating that the pathway acts in parallel or downstream of PINK1 and Parkin. Interestingly, increased autophagy through this PINK1-independent mechanism also appears to underlie the improved mitochondrial function, as the improvement in a range of bioenergetic parameters is blocked by bafilomycin. As such, it will be important to understand how the activation of TFEB improves mitochondrial function independent of PINK1- and Parkin-based mitophagy. Are the mitochondria turned over through other mechanisms? These studies also raise questions about the mechanism by which TFP activates TFEB. Phosphorylation of TFEB inhibits nuclear translocation, raising the question of if TFP prevents TFEB phosphorylation, either directly or indirectly by decreasing the phosphorylation of AKT and mTOR (Wu et al., 2016)?

Overall, Zhang et al. (2017) establish a robust model system that complements existing approaches and may be a valuable platform for screening therapeutic compounds. In particular, combining genetic and environmental risk factors is an important step and likely necessary to accurately model neurodegenerative diseases, such as PD, that are heterogeneous and primarily sporadic. However, it will be challenging to know which stressors to use and how to best combine them. It will also be important to determine if such genetic-environmental systems better identify promising therapies than model systems that consider only individual genetic or environmental insults. Ultimately, the capacity of a given screening platform to identify effective therapies may depend on matching the platform to the appropriate sub-population of PD patients, but achieving this will require both better defining the subtypes of sporadic PD and developing appropriate model systems for each subtype. Whether this level of mechanistic resolve will actually be required to develop disease-modifying therapies remains unknown. PD is defined by the preferential loss of nigrostriatal DA neurons, which presumably have core properties that predispose them to degeneration across the range of stressors that cause PD. Therefore, there may also be core therapeutic strategies, such as boosting autophagy, that will benefit many or even all forms of PD.

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