Fixing mitophagy in Parkinson's disease

By Kai-Jye Lou, Senior Writer

Genentech Inc. has identified ubiquitin specific peptidase 30 as a potentially disease-modifying target involved in the clearance of damaged mitochondria in Parkinson's disease.¹ The **Roche** unit now needs to determine whether the deubiquitinase is indeed druggable.

Mitophagy is a form of autophagy that involves ridding cells of damaged mitochondria and is one of the key pathways for maintaining mitochondrial quality control.^{2,3} Defects in the process can result in the accumulation of damaged mitochondria within cells, leading to increased oxidative stress and cellular dysfunction.

Genetic studies have identified loss-of-function mutations in two regulators of mitophagy that are associated with PD.^{4,5} One regulator is PTEN induced putative kinase 1 (PINK1), a mitochondria-targeted serine/threonine kinase, and the other is the E3 ubiquitin ligase parkin (PARK2).^{6,7}

PINK1 recruits parkin to damaged mitochondria, which leads to parkin-mediated ubiquitination of mitochondrial proteins and mitophagy.

Mitokinin LLC has a PINK1 activator in preclinical development to treat PD. The rationale is that increasing PINK1 activity could rescue the impairments in mitophagy observed in PD.

Now, Genentech researchers have published a paper in *Nature* that identifies another way to target this pathway. The group linked ubiquitin specific peptidase 30 (USP30) to parkin-mediated mitophagy.

"Our study grew out of Parkinson's disease genetics that indicate defects in the mitophagy pathway—specifically the genes *PINK1* and *parkin*—cause familial Parkinson's disease in humans," said Baris Bingol, the co-lead author on the study and a scientist at Genentech. "Given that parkin is an E3 ubiquitin ligase, we wondered if there is a deubiquitinating enzyme that counteracts parkin and inhibits mitophagy. USP30 hit the mark in a screen designed for identifying deubiquitinating enzymes that block mitophagy."

After picking out USP30 from the screen, the team carried out a series of *in vitro* studies that showed the peptidase blocks mitophagy in dopaminergic neurons and deubiquitinates mitochondrial proteins that parkin ubiquitinates. The group also showed that parkin ubiquitinates USP30 and induces its degradation and that two different PD-associated parkin mutants lacked that activity. This result suggests that wild-

type parkin removes a brake on mitophagy set by USP30 and that PD-associated parkin mutants fail to do this.

In neuronal cell lines expressing a PD-associated parkin mutant, siRNA against USP30 rescued mitophagy defects, whereas control siRNA did not. In fly models of PD in which the disease phenotype is caused by loss-of-function mutations in *pink1* or *parkin*, knockdown of *usp30* rescued the defects in mitophagy and improved motor function.

In flies treated with a PD-linked mitochondrial toxin, *usp30* knockdown improved motor function and survival.

"We believe USP30 inhibition offers a novel strategy by activating clearance of damaged, unhealthy mitochondria to boost mitochondrial quality control," said Bingol. He noted that USP30 inhibition could potentially offer a disease-modifying therapy that slows down or halts the progression of PD.

"If you could prevent the death of dopaminergic neurons with a USP30 inhibitor in patients with early Parkinson's disease, you could potentially extend the period where L-dopa remains effective," said Mitokinin cofounder and CSO Nicholas Hertz.

"I think this approach could be a good idea to explore in Parkinson's

if they can find a molecule that is able to selectively inhibit USP30 and cross the blood brain barrier," said Richard Youle, chief of the biochemistry section at the Surgical Neurology Branch of the NIH's **National Institute of Neurological Disorders and Stroke**. He cautioned that the extent to which the PINK1-parkin pathway drives PD in humans is still unknown.

Moreover, the majority of human PD cases are idiopathic. PD cases with genetic origins account for about 5%–10% of total cases.⁸

Mulling over mammals

Before considering a possible drug discovery effort, Bingol said that the function of USP30 and safety of USP30 inhibition in mammals will need to be investigated.

Youle noted that studies in some mammalian models could be challenging. He said that mice with mutations in *Pink1* and *parkin* do not have a PD phenotype. Instead, he said, dogs with mitochondrial mutations might represent a better model system for evaluating USP30 inhibition.

Another option could be rats, added Kevan Shokat, chair of the Department of Cellular and Molecular Pharmacology at the **University of California, San Francisco** and a **Howard Hughes Medical Institute** investigator. Shokat also is a Mitokinin cofounder and a member of Genentech's scientific resource board. He noted that *Pink1* knockout rats recapitulate a PD phenotype,⁹ although *parkin* knockouts do not.

Hertz agreed that knockout rat studies could be a good option for studying USP30 inhibition. "If you saw that knocking out *Usp30* in rats does not result in a developmental phenotype, that result suggests that USP30 inhibition could be achieved without deleterious effects," he said. "One would then want to know if knocking out *Usp30* could rescue the

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Stroke**

for USP1, USP7 [HAUSP] and USP14 [TGT] have already been described. However, biophysical characterization, pharmacokinetic experiments and medicinal chemistry optimization will be required for these published inhibitors to be considered viable leads for drug discovery. These types of studies will help determine whether deubiquitinating enzymes, including USP30, are druggable as a class,” Bingol told *SciBX*.

Beyond PD, Youle noted that there also could also be opportunities for USP30 inhibitors in other diseases caused by mitochondrial dysfunction such as Leber hereditary optic neuropathy.

Parkinson’s disease phenotype in the rat model.”

Hertz said that another important next step will be to screen human populations for mutations in *USP30* and then determine whether those individuals are at lower risk of PD.

Although the druggability of USP30 remains to be determined, Bingol said that there is some precedent to suggest that deubiquitinating enzymes could be targeted with small molecules.

“For example, inhibitors

Genentech declined to provide details of its programs in PD or disclose the patent and licensing status related to the data described in *Nature*.

Lou, K.-J. *SciBX* 7(27); doi:10.1038/scibx.2014.782

Published online July 17, 2014

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