All in the family

By Amy Donner, Senior Editor

PTEN induced putative kinase 1 is a tantalizing player in familial forms of Parkinson's disease, in which it is inactivated by mutation, but thus far the kinase has been a no-fly zone for drug development because of challenges in activating kinases. **Mitokinin LLC** thinks it finally has an angle to modulate the kinase after the company's cofounders identified an ATP analog that selectively activates the target.[1]

The company plans to develop the analog or optimized variants to treat PD.

Mitochondria are particularly important for cells such as neurons that have a high demand for energy. Defects in mitochondrial quality control have been implicated in neurodegenerative conditions such as PD largely based on genetic mutations associated with familial, early onset forms of the disease.[2,3]

In particular, there have been multiple associations between disease and mutations that disrupt a process called mitophagy, or the ability of damaged mitochondria to self-destruct. Mitophagy is a cellular quality control mechanism that selectively eliminates the damaged organelle and thereby protects the cell from death.

Despite a solid genetic connection between disrupted mitophagy and PD, "we are only at the beginning of understanding the whole mitophagy pathway and, more generally, mitochondrial dysfunction associated with PD," said Helene Plun-Favreau, a senior lecturer in the Department of Molecular Neuroscience at the **UCL Institute of Neurology**.

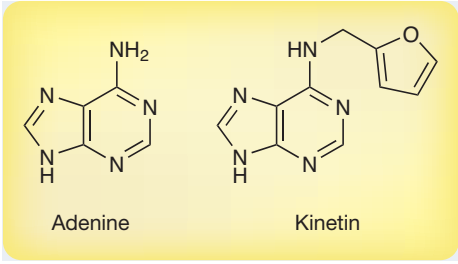

Figure 1. Adenine and kinetin. Similar to adenine, the precursor to ATP, kinetin can be taken up by cells, modified with a ribose sugar and converted to N6 furfuryl ATP (KTP). KTP is a selective substrate for and activator of PTEN induced putative kinase 1 (PINK1) because the kinase contains an unusual insertion in its kinase domain. This is the first example of a wild-type kinase that can use an N^6-modified ATP analog as a substrate.

To elucidate the molecular relationships between mitochondrial dysfunction and PD, a group led by Kevan Shokat focused on PTEN induced putative kinase 1 (PINK1). Shokat is a professor and chair of cellular and molecular pharmacology at the **University of California, San Francisco**.

PINK1 is a mitochondrial kinase that, in response to damage, accumulates in the outer mitochondrial membrane and recruits other proteins essential for mitophagy to the damaged organelle[4] (*see* **Box 1, "Targeting mitophagy in Parkinson's disease"**). The gene is mutated in early onset, familial forms of PD.

To study PINK1, Shokat's group turned to its chemical genetics platform for studying kinases. The technology combines engineered variants of a kinase with chemical inhibitors targeting the ATP-binding site that are selective for a particular variant.

Box 1. Targeting mitophagy in Parkinson's disease.

Mitophagy is the selective and programmed destruction of damaged mitochondria in a cell. The process can protect cells, including neurons, from death. Several genes encoding the proteins that orchestrate mitophagy, including *PTEN induced putative kinase 1*, *Parkin* and *F-box protein 7*, are mutated in familial forms of Parkinson's disease.

Parkin (PARK2) and F-box protein 7 (FBXO7) are both E3 ubiquitin ligases that are recruited to damaged mitochondria in a PTEN induced putative kinase 1 (PINK1)-dependent fashion and are activated in response to mitochondrial stress. Mitophagy depends on the enzymatic activation of PARK2 and FBXO7.

Although the Shokat group used kinetin to activate PINK1 and mitophagy, separate research groups from the U.K. and Canada are exploring alternative therapeutic targets.

A team of British researchers led in part by Helene Plun-Favreau, a senior lecturer in the Department of Molecular Neuroscience at the **UCL Institute of Neurology**, recently reported that, similar to PARK2, FBXO7 plays a role in mitophagy downstream of PINK1.[3]

"We are planning to do some compound screening in the near future to try and correct or improve mitophagy. One possibility would be to try to improve PARK2 and/or FBXO7 recruitment to damaged mitochondria," she said.

Similarly, a Canadian team led in part by Edward Fon, an associate professor of neurology and neurosurgery at **McGill University** and director of the McGill Parkinson Program, recently reported the first structure of Park2, demonstrating with multiple structural methods that the protein adopts an autoinhibited conformation.[6]

Mutation of the protein to disrupt formation of this conformation increased the activity of the ligase and its recruitment to mitochondria compared with no mutation. Fon told *SciBX*, "We are now looking for small molecules that disrupt this autoinhibitory interaction. We are setting up screens to find small molecules that will mimic the mutations." —*AD*

The approach lets scientists selectively inactivate a given kinase using small molecules, which in turn allows for the biological function of the kinase to be elucidated.

Surprisingly, the group's search for a PINK1 inhibitor turned up the exact opposite—an ATP analog called N^6 furfuryl ATP (KTP) that could activate the target and restore mitochondrial quality control pathways.

KTP activated both wild-type PINK1 and a PD-associated mutant version of PINK1 that contained a G309D substitution.

For cell culture experiments, the researchers used a membrane-permeable precursor of KTP, called kinetin, which is a natural cytokinin derived from adenine (*see* **Figure 1, "Adenine and kinetin"**).

In a human cell culture model of chemically induced mitochondrial damage, kinetin accelerated both wild-type and G309D PINK1–dependent Parkin (PARK2) recruitment to mitochondria and inhibited stress-induced cell death. PARK2 is an E3 ubiquitin ligase important for mitophagy (*see* **Box 1, "Targeting mitophagy in Parkinson's disease"**).

In primary rat hippocampal neurons, kinetin decreased mitochondrial motility compared with vehicle. Thus, kinetin promoted PINK1 function and multiple features of mitophagy in cells with damaged mitochondria.

Results were published in *Cell*. The research team also included scientists from the **Gladstone Institute of Neurological Disease**.

"For a long time, I thought these results were experimental error. This outcome was a big surprise," said Nicholas Hertz, the study's lead author and a cofounder and CSO of Mitokinin.

"Our data suggest the effect of kinetin is significant, and the rescue of mitochondrial homeostasis in the face of mitochondrial damage is a highly attractive mechanism to delay PD," said Shokat.

Taking kinetin outside the family

Mitokinin is launching a medicinal chemistry effort to generate kinetin analogs optimized for activating PINK1, but a primary challenge will be selecting animal models.

"All models for PD are problematic," said Dan de Roulet, a managing member of Mitokinin. "There are a number of examples where models have proved to be nonpredictive in the clinic."

Thus, Mitokinin plans to look beyond traditional models to firm up the mechanism of action for kinetin. "We will make our best case by using a combination of models where we can show improvement in mitochondrial quality control," said de Roulet.

"For PD, there are no great animal models. Genetically, the most robust model is the fly because *Parkin* and *Pink1* knockout mice do not get PD," noted Edward Fon, an associate professor of neurology and neurosurgery at **McGill University** and director of the McGill Parkinson Program.

Fon suggested that simply extending results obtained in cultured HeLa cells to primary neurons or induced pluripotent stem cell–derived neurons could be valuable.

Mitokinin hopes to take kinetin into the clinic for some familial as well as idiopathic forms of PD.

"There is increasing evidence that mitochondrial defects are central to PD pathology. If we can improve mitochondrial quality control—targeting and clearing damaged mitochondria—we have the potential to slow the progression of the disease," said de Roulet.

"A lot of what we know about the molecular pathways associated with PD comes from the study of the rare genetic forms of PD as opposed to the most frequent sporadic forms. Mitochondrial dysfunction is also associated with idiopathic PD, so therapies for the former will very likely be beneficial to sporadic PD patients as well," noted Plun-Favreau.

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—Dan de Roulet, Mitokinin LLC

Because there are no disease-modifying therapies in PD, "even a small effect could be a huge breakthrough in this disease," said Hertz.

"In the U.S. alone there are roughly one million cases of PD, and 60,000 new cases are diagnosed per year. Right now, therapies can only manage symptoms," added de Roulet.

Mitokinin and **Dysautonomia Foundation Inc.** anticipate having a data-sharing deal to help move kinetin toward Phase I trials in PD and familial dysautonomia, respectively.

Familial dysautonomia is a neuropathy caused by mutations that disrupt the splicing of *IKBKAP* (*inhibitor of κ-light polypeptide gene enhancer in B cells kinase complex–associated protein*). Kinetin has been reported to improve expression of IKBKAP in cells from patients with the disease. The foundation previously helped fund a study of kinetin in humans[5] that showed the molecule was safe, achieved high serum concentrations and crossed the blood brain barrier.

UCSF has filed for a method-of-use patent and patents for kinetin analogs. The IP is licensed to Mitokinin, which was cofounded by Shokat, Hertz and de Roulet.

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COMPANIES AND INSTITUTIONS MENTIONED

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