Ramping up autophagy

By Lev Osherovich, Senior Writer

The most common therapeutic approach to treat Huntington's disease and Parkinson's disease is to reduce the amount of the misfolding-prone proteins huntingtin and α -synuclein, respectively, before disease sets in. A study published in *Nature Chemical Biology* now points to an alternative way of accomplishing this goal using generic calcium channel blockers already on the market.¹ But basic translational questions including model choices and drug dosage remain to be resolved.

A group at the **Cambridge Institute of Medical Research** screened a library of 253 well-characterized preclinical compounds and approved drugs to identify small molecules that promoted a cellular recycling program called autophagy. Autophagy, or 'self-eating', is a cellular response to starvation and stress that may slow the accumulation of toxic protein aggregates associated with HD and PD.

In autophagy, portions of the cytoplasm are sectioned off into a pre-autophagosome, a membrane-bound compartment that later

fuses with acidified lysosomes. This allows cells to scavenge captured proteins such as huntingtin and α -synuclein in order to return their amino acids back into circulation.²

The Cambridge team was led by David Rubinsztein, professor of molecular neurogenetics at the institute.

Previous work by Rubinsztein's team and others had shown that genetic and pharmacological tinkering with a metabolic sensor called mammalian target of rapamycin (mTOR)³ could stimulate autophagy and help rid cells of misfolded HD- and PD-associated proteins.⁴

The mTOR inhibitor Rapamune sirolimus is the only approved drug known to enhance autophagy in preclinical studies. Rapamune is marketed by **Wyeth** as an immunosuppressant to prevent organ-transplant rejection.

However, immunosuppression is not necessarily desirable in treating neurodegenerative diseases. In that setting, compounds that enhance autophagy independently of mTOR could potentially be more useful.

Thus, the surprise result from Rubinsztein's screen was that a group of L-type calcium channel antagonists boosted autophagy without affecting mTOR activity. These included verapamil, loperamide, nimodipine, nitrendipine and amiodarone, which all lowered intracellular levels of huntingtin and α -synuclein compared with levels of the proteins in controls. The five drugs are used to treat hypertension and angina.

Two other drugs also had strong, mTOR-independent effects on huntingtin and α -synuclein clearance in the cell culture screen: minoxidil, a potassium channel agonist used to treat hypertension and

Figure 1. Harnessing the central role of calpain in the recycling of Parkinson's disease- and Huntington's disease-associated proteins. Autophagy, a pathway for scavenging cytoplasmic proteins that is activated by cellular stress or starvation, offers potential targets for Huntington's disease and Parkinson's disease by calpain inhibition. Toxic misfolded proteins such as huntingtin and α -synuclein build up when autophagy is dormant [a]. Upon calpain inhibition [b], structures called autophagosomes envelop protein aggregates and degrade them, reducing their toxicity [c]. Upstream regulators of calpain can be hit with generic drugs approved for indications outside of HD and PD. These include: L-type Ca²⁺ channels (antagonized by verapamil, loperamide and other generic compounds); ATP-sensitive K⁺ receptors (agonized by minoxidil); and imidazole-1 receptors (agonized by clonidine). Alternatively, calpain itself can be directly inhibited with calpeptin and calpastatin.

alopecia, and clonidine, which is approved to treat hypertension and promotes the action of G_i , an inhibitory G protein. G proteins are signaling proteins that act downstream of GPCRs.

Together, these results pointed to a signaling pathway that turns up autophagic recycling of proteins when calcium levels fall. Using pharmacological probes, Rubinsztein's team elucidated the pathway, which involves G proteins, cyclic adenosine monophosphate levels and multiple other components (see **Figure 1, "Harnessing the central role of calpain in the recycling of Parkinson's disease- and Huntington's disease-associated proteins"**).

A key regulator of this autophagy-controlling pathway was calpain, a calcium-sensitive protease. The researchers showed that pharmacological and siRNA inhibition of calpain also produced the high levels of autophagy observed with calcium channel inhibitors.

"I'm excited by this because it gives us a pathway that is likely to be independent of mTOR that induces autophagy," Rubinsztein told *SciBX*. "There are a number of drugs that can get into the brain that are fairly safe."

Although autophagy is relevant to a variety of neurodegenerative diseases, Rubinsztein's group is focusing on HD, a fatal autosomal dominant disorder with no available therapies. The goal, he said, would be to treat genetically at-risk HD patients before neurodegeneration sets in.

"The dream is to delay HD from a mean age of 40 to a mean age of 100, which will be like curing the disease," Rubinsztein said.

Therapeutic long shot

Industry researchers told *SciBX* that because the mechanisms of HD and PD are still poorly understood, preventing these neurodegenerative diseases is a tall order. Instead, companies are focusing on therapeutics that diminish symptom severity.

"The good news is that they have used commercially available, marketed drugs that are used by many people," said Gerhard Koenig, CSO of **EnVivo Pharmaceuticals Inc.** "The not so good news is that these are all cellular models. The HD field is struggling with translation into humans."

EnVivo is developing EVP-0334 to treat HD and PD. The histone deacetylase inhibitor is thought to work by boosting the activity of CREB, a transcriptional regulator of cognition that is depleted in neurodegenerative diseases. EVP-0334 is slated to enter Phase I trials in late 2008.

Rubinsztein agreed that a next step is to assess broader neurophysiological effects in a mouse model of HD. He said his group has positive preliminary safety data with a brain-specific HD transgenic mouse. In the *Nature Chemical Biology* study, the team reported a modest decrease in huntingtin protein aggregation and pathology in flies and zebrafish models of HD compared with aggregation and pathology in controls.

In addition to the lack of mouse data, another concern of industry researchers is the high concentration of calcium channel blockers that was needed to elicit effects on autophagy. Whereas most of the drugs in the study are effective at nanomolar concentrations for their approved indications, Rubinsztein's team used approximately 100-fold higher levels.

"Even though all these drugs work, it's at one micromolar, so you could have a lot of problems," said David Hung, CEO of **Medivation Inc.** Hung told *SciBX* that the clinical effects of such high doses might be very different than what was observed in cell culture.

Moreover, Hung suspected the misfolded protein clearance observed by Rubinsztein's team may occur through some other mechanism than the calcium-modulating effects of these drugs.

"When you talk about micromolar concentrations, you have to wonder what the true mechanism is," he said. As a result, Hung thinks the calcium channel blocker findings in the *Nature Chemical Biology* paper are "a significant way off from prime time."

EnVivo's Koenig also doubted whether the observed effects really had to do with intracellular calcium levels. "If you are somehow saturating the channels by 100-fold, this becomes multipharmacy and is less interesting."

To resolve questions about the mechanism of action of these drugs, Koenig suggested the researchers directly confirm that L-type calcium channels are functioning in the cell lines used for this study and can indeed be inhibited by these drugs at high doses. For example, if the calcium channels become completely blocked by doses of antagonists that are much lower than needed to see improved autophagy, the autophagy effect would be likely due to a different mechanism.

Moreover, boosting autophagy could be a double-edged sword. According to Hung, high levels of autophagy could have side effects such as stimulating apoptosis.

Indeed, Medivation is targeting the neurotoxic effects of intracellular calcium with Dimebon, a small molecule inhibitor of L-type calcium channels and mitochondrial

pores. The mitochondria of HD patients are especially susceptible to the toxic effects of high intracellular calcium, leading to apoptosis.

According to Hung, Dimebon diminishes calcium toxicity by blocking mitochondrial pores. However, Dimebon has additional effects on mitochondrial activity that are independent of calcium.

"The autophagy pathway is unrelated to what we're doing," said Hung. "We don't think that inhibition of calcium influx is sufficient for the effects that we're seeing."

Dimebon is in Phase II trials to treat HD.

Rubinsztein dismissed concerns about off-target effects, citing multiple lines of evidence that calcium signaling converges on calpain to regulate autophagy. "I think we have very robust data suggesting that the effects are specific for calcium levels," he told *SciBX*.

Calcium shock

Rubinsztein's findings come on the heels of a paper in *Public Library of Science Biology* by researchers at **The Scripps Research Institute** that suggests calcium channel blockers could also be used to treat lysosomal storage disorders.⁵ That study focused on a protein called glucocerebrosidase. In Gaucher's disease, glucocerebrosidase accumulates in a misfolded form in the endoplasmic reticulum (ER).⁶

"I'm really impressed with the paper" published by Rubinsztein's team, said Jeffery Kelly, professor of chemistry at Scripps and an author on the *PLoS Biology* paper. "It's extremely well executed experimentally."

"The dream is to delay HD from a mean age of 40 to a mean age of 100, which will be like curing the disease."

**—David Rubinsztein,
Cambridge Institute of
Medical Research**

It's very difficult to prove the mechanism of a drug, but they've done as good a job as you can possibly do."

In Kelly's *PLoS* paper, blocking intracellular calcium channels led to an increase in chaperone activity in the ER and stimulated the folding of several lysosomal proteins.

"We need to figure out whether this is fortuitous or a signaling pathway that ramps up the protein-folding pathway for the cell," said Kelly. "Maybe this is pointing toward regulators that are calcium sensitive."

Kelly suspects that calcium-responsive changes in protein-folding homeostasis could be "part of a general phenomenon" that affects a number of disease-associated misfolded proteins. However, he thinks targeting calcium levels directly would have too many side effects.

Kelly suggested that calcium-sensitive factors such as calpain and the inhibitory G protein subunit identified in the Rubinsztein study might be better, more specific targets.

Rubinsztein told *SciBX* that the discoveries reported in the *Nature Chemical Biology* paper have been patented and are available for licens-

ing through **Cambridge Enterprise Ltd.**, a subsidiary of Cambridge University.

REFERENCES

1. Williams, A. *et al. Nat. Chem. Biol.*; published online March 23, 2008; doi:10.1038/nchembio.79
Contact: David C. Rubinsztein, Department of Medical Genetics, University of Cambridge, U.K.
e-mail: dcr1000@hermes.cam.ac.uk
2. Rubinsztein, D.C. *et al. Nat. Rev. Drug Discov.* **6**, 304–312 (2007)
3. Haas, M. *SciBX* **1**(6), 9–10; March 6, 2008
4. Floto, R.A., *et al. Autophagy* **3**, 620–622 (2007)
5. Mu, T.W. *et al. PLoS Biol.* **6**, e26; published online Feb. 5, 2008; doi:10.1371/journal.pbio.0060026
6. Fulmer, T. *SciBX* **1**(5), 4–5; Feb. 28, 2008

COMPANIES AND INSTITUTIONS MENTIONED

Cambridge Enterprise Ltd., Cambridge, U.K.
Cambridge Institute of Medical Research, Cambridge, U.K.
EnVivo Pharmaceuticals Inc., Watertown, Mass.
Medivation Inc. (NASDAQ:MDVN), San Francisco, Calif.
The Scripps Research Institute, La Jolla, Calif.
Wyeth (NYSE:WYE), Madison, N.J.