Heat shock and awe

By Tim Fulmer, Senior Writer

U.S. researchers have identified several small molecules that decrease toxic protein aggregation *in vitro*.[1] The first-generation compounds have been licensed by **Proteostasis Therapeutics Inc.** and are proof of concept for the biotech's approach to treating protein misfolding diseases like Huntington's and cystic fibrosis.

Cells have evolved a complex network of quality control enzymes to prevent the formation and accumulation of misfolded proteins during normal cellular function. However, this protein homeostasis (or proteostasis) network often is overwhelmed in protein misfolding diseases, which generate high levels of protein aggregates that are impossible to clear and lead to cell death.

The challenge has been figuring out how to boost the activity of the network, which consists of hundreds of proteins involved in the folding, trafficking and degradation of misfolded proteins.[2]

Rather than tackling single components of the network, a research team led by Proteostasis Therapeutics founder and **Northwestern University** professor of biology Richard Morimoto hypothesized that activating a global regulator of many components simultaneously would improve the likelihood of a therapeutic effect.

The group turned to a known transcriptional factor of the network—heat shock transcription factor 1 (HSF1)[3,4]—and set out to identify compounds that activated that target. Morimoto and colleagues first devised a high throughput cell-based screen that allowed them to measure activation of HSF1. The assay worked by monitoring the activity of the promoter of one of HSF1's many target genes, *heat shock protein 70* (*Hsp70*).

A screen of about 900,000 small molecules resulted in 233 compounds that increased *Hsp70* promoter activity. The compounds encompassed a variety of chemical scaffolds and were grouped into seven classes based on structural similarity.

Additional *in vitro* studies confirmed that the five best compounds upregulated multiple proteins in the protein homeostasis network, including Hsp40 and Hsp27, and did so by acting through HSF1.

The next priority was determining whether any of the five HSF1 activators had a disease-modifying effect *in vitro*.

The researchers looked at cell culture models of Huntington's disease (HD), which is characterized by toxic huntingtin (HTT) protein aggregates, and of cystic fibrosis (CF), which is characterized by a misfolded and defective cystic fibrosis transmembrane conductance regulator (CFTR) protein.

In the HD model, compounds A1, D1 and F1 decreased the number of HTT aggregates compared with vehicle control. In the CF model, compounds A3, C1 and F1 increased CFTR ion conductance compared with vehicle.

Finally, in a *Caenorhabditis elegans* model of HD, compounds A1, D1 and F1 lowered toxic aggregation compared with vehicle.

The findings were published in *Nature Chemical Biology*.

Prioritize and optimize

"A basic part of our initial optimization process is the generation of a proteostasis network signature for each compound. The signature reflects how each compound affects pathways within the network and helps prioritize our choice of disease models for further testing," said Peter Reinhart, president and CSO of Proteostasis Therapeutics. "By focusing on several pathways at once, our approach differs from more traditional optimization strategies that focus on a single target from the outset."

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Proteostasis Therapeutics founder Jeffrey Kelly said the strength of the approach and the screen used in the paper is that "distinct compounds with distinct mechanisms of action will likely be discovered. A more reductionist biochemical screen can then be added subsequently to identify actual targets and demonstrate mechanistic details."

Kelly is a professor of chemistry at **The Scripps Research Institute**.

Reinhart said the company plans to study some of the compounds in animal models of CF, Parkinson's disease (PD), HD and Alzheimer's disease (AD).

AD and PD are characterized by toxic aggregates of β-amyloid (Aβ) and α-synuclein (SNCA) proteins, respectively.

Screening the screens

Experiments reported in the paper confirmed that some of the most active compounds in the *in vitro* disease models did not have off-target effects on the proteasome or Hsp90 that might lead to toxicity.

Nevertheless, Matt Kaeberlein, associate professor of pathology at the **University of Washington**, said that beyond the screens and assays it will be important "to see if any of the compounds are effective in mouse models of human proteotoxic diseases without too many negative side effects."

"There are a variety of mechanisms of action that promote the heat shock response, and some of those MoAs are undesirable because they result from acute cellular stress, not from activation of HSF1," noted William Janzen, cofounder, president and CEO of **Chaperone Therapeutics Inc.** "For that reason, we designed our yeast-based screen to identify only compounds that have an HSF1-dependent MoA."

The screen described in *Nature Chemical Biology* cannot by itself distinguish heat shock–promoting compounds that have an HSF1-dependent mechanism of action from those that have an HSF1-

independent one. For that reason, the researchers had to use additional *in vitro* experiments to confirm that their activators had an HSF1-dependent mechanism of action.

In contrast, Chaperone Therapeutics' screen is, by itself, sufficient to identify only compounds that have an HSF1-dependent mechanism of action.

Thus, while the two companies are seeking to identify the same types of compounds, they differ in the screening platforms they are using. It is not yet clear whether one approach will yield better therapeutic candidates than the other.

Last year, Chaperone Therapeutics cofounder Dennis Thiele published in *Public Library of Science Biology* that the company's screen identified multiple compounds that activated HSF1.[5] This month, **The Michael J. Fox Foundation for Parkinson's Research** announced it was funding research to test some of those HSF1 activators in preclinical models of PD. Thiele is professor of pharmacology and cancer biology at the **Duke University School of Medicine**.

The *Nature Chemical Biology* findings are covered by patents from Northwestern University and licensed to Proteostasis Therapeutics.

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COMPANIES AND INSTITUTIONS MENTIONED

Chaperone Therapeutics Inc., Research Triangle Park, N.C.
Duke University School of Medicine, Durham, N.C.
Northwestern University, Evanston, Ill.
The Michael J. Fox Foundation for Parkinson's Research, New York, N.Y.
Proteostasis Therapeutics Inc., Cambridge, Mass.
The Scripps Research Institute, La Jolla, Calif.
University of Washington, Seattle, Wash.