Turning up the heat on HD

By Lev Osherovich, Senior Writer

Researchers at **Duke University** have found small molecules that help cells untangle aggregates of the misfolded protein thought to cause Huntington's disease.¹ The molecules are now the lead compounds for **Chaperone Therapeutics Inc.**, a neurology startup focused on therapeutics that stimulate protein folding.

Many neurodegenerative disorders including HD are characterized by intracellular aggregates that are thought to compromise neuronal function and survival. HD pathogenesis involves polyglutamine expansion mutations in huntingtin (HTT) that cause the protein to misfold and aggregate, thus triggering neuron death.

Strategies for treating HD therefore have focused on either blocking the expression of mutant HTT or boosting neuronal survival in the presence of the misfolded protein (*see Table 1, "Selected compounds in development for Huntington's disease"*).

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Chaperone Therapeutics Inc.

The new study, published in *PLoS Biology*, proposes a different approach—helping the cell put the mutant protein into working order. A team led by Dennis Thiele, professor of pharmacology and cancer biology at Duke, focused on activating heat shock transcription factor 1 (HSF1), the key transcriptional regulator of the network of chaperone proteins that aids protein folding.²

HSF1 normally is turned on by heat and other forms of cellular stress that impede protein folding. Thus, the Duke researchers hypothesized that activation of the transcription factor could protect HTT-sensitive neurons by raising the cell's capacity for dealing with the misfolding problem.

The Duke team designed a yeast strain to screen for activators of HSF1, with the screening approach relying on the fact that HSF1 is essential for survival in yeast. The yeast strain conditionally expressed human HSF1; when human HSF1 expression was turned off, the yeast could only survive in the presence of HSF1 activators.

The screen yielded two benzyl pyrazole compounds that promoted yeast survival and activated HSF1 compared with mock treatment.

In cultured rat neurons expressing polyglutamine-expanded HTT, the lead compound, as compared with mock treatment, promoted cell survival, suppressed HTT aggregation and promoted heat shock 70kDa protein 4 (HSPA4; Hsp70) expression, which indicated activation of Hsf1.

In flies engineered to express another polyglutamine-expanded protein that underlies a rare neurological disorder called Machado-Joseph disease, the lead compound increased chaperone levels and reduced the severity of eye degeneration compared with those in mock-treated controls.

Table 1. Selected compounds in development for Huntington's disease.

Source: BCIQ: BioCentury Online Intelligence

Company	Product	Description	Status
Medivation Inc. (NASDAQ:MDVN)/ Pfizer Inc. (NYSE:PFE)	Dimebon latrepirdine	Oral small molecule that increases mitochondrial function	Phase III
NeuroSearch A/S (CSE:NEUR)	Huntexil pridopidine	Dopamine stabilizer	Phase III
Prana Biotechnology Ltd. (ASX:PBT)	PBT2	Metal-protein-attenuating compound (MPAC)	Phase I
Raptor Pharmaceutical Corp. (NASDAQ:RPTP)	DR Cysteamine	Delayed-release formulation of a degradation product of the amino acid cystine	Phase I
Siena Biotech S.p.A./Elixir Pharmaceuticals Inc.	SEN0014196 (EX-527)	Oral sirtuin 1 (SIRT1) inhibitor	Phase I
Accera Inc.	AC-0523	Not available	Preclinical
Alnylam Pharmaceuticals Inc. (NASDAQ:ALNY)/Medtronic Inc. (NYSE:MDT)	ALN-HTT	Small interfering RNA against the <i>huntingtin</i> (HTT) gene delivered to the CNS using Medtronic's implantable pump infusion system	Preclinical
Ceregene Inc.	CERE-120	Adeno-associated viral (AAV) type 2 vector encoding <i>neurturin</i> (NTN) gene	Preclinical
Chaperone Therapeutics Inc.	Heat shock transcription factor 1 (HSF1) agonist	Small molecule activators of HSF1	Preclinical
Medesis Pharma	NP03	Lithium citrate tetrahydrate delivered using Aonys buccal reverse-micelle formulation technology	Preclinical
Neurologix Inc. (OTCBB:NRGX)	dXIAP	Mutated form of the gene <i>X-linked inhibitor of apoptosis</i> (XIAP) delivered by AAV vector	Preclinical

“For many neurodegenerative diseases, enhanced expression of protein chaperones can confer stress resistance” in the face of misfolded proteins, said Thiele, who was senior author on the *PLoS Biology* paper.

“HSF1 is an especially good target because it’s the master regulator of the protein folding and chaperone system,” said William Janzen, president and CEO of Chaperone. “The ability to upregulate all of the chaperones” at once by targeting HSF1 is more appealing than trying to tinker with levels of individual chaperones, he added.

Staying on target

The Duke findings suggest that small molecule HSF1 agonists protect neurons from polyglutamine protein aggregation and toxicity. But whereas pretreatment of model cells with the compounds prevented *de novo* aggregation and toxicity, it’s still unclear whether the agonists can trigger the clearance of pre-existing aggregates or reverse the course of HD *in vivo*.

Walter Newman, interim CSO of **Proteostasis Therapeutics Inc.**, thinks the Duke team’s results in simple model systems have a good chance of bearing out in advanced organisms.

“We’re dealing with some very conserved features in the protein folding network, and this suggests that even yeast-based screens can yield interesting molecules,” Newman said.

Proteostasis is developing small molecule modulators of protein folding to treat neurodegenerative diseases.

In addition to uncertainty about whether the yeast findings will translate to more advanced organisms, the precise mechanism of the agonists identified by the Duke screen remains unclear. Thiele’s team doesn’t yet have evidence that the compounds bind to and activate HSF1 directly. He believes the compounds could bind to a chaperone complex involved in general protein folding, but it’s not yet known whether this interaction leads to HSF1 activation.

“There are so many ways that cells maintain protein folding balance,” said Robert Kalb, associate professor of neurology at the **University of Pennsylvania**. “The proteasome system controls degradation; autophagy is involved. What cellular compartment is this compound acting in?”

Last year, Kalb’s team identified a small molecule activator of fork-

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head box O3 (FOXO3; FOXO3A), another transcription factor that works together with HSF1 to aid clearance of misfolded proteins.³

Identifying the targets of the two Duke compounds will make it easier to generate

more drug-like derivatives of the compounds, which were effective only at micromolar concentrations.

“Having a defined molecular target allows you to understand what properties of the compound are really critical for activity,” said Newman.

Thiele has ruled out at least one potential mechanism—inhibition of heat shock protein 90 (HSP90AA1; Hsp90), a chaperone that ordinarily blocks HSF1 activity by trapping the protein in a partially unfolded state. Hsp90 antagonists are in clinical testing for cancer but are considered unsuitable for neurodegenerative diseases because of toxicity concerns, said Thiele.

Thiele is planning further mechanistic studies to work out the compounds’ mode of action.

Chaperone was founded by Thiele and has licensed the compounds and the screening platform from Duke. The biotech plans to test the molecules in preclinical models of Parkinson’s disease (PD) and HD.

Janzen said the company has a grant from **The Michael J. Fox Foundation for Parkinson’s Research** to screen for additional HSF1 activators that could be useful for treating PD, which is also thought to be caused by intracellular protein aggregates.

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COMPANIES AND INSTITUTIONS MENTIONED

Chaperone Therapeutics Inc., Durham, N.C.
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