GEN Genetic Engineering & Biotechnology News

Special Reports

Examining Both Sides of the Sirtuin Coin

Jury is still out on whether inhibition and/or activation can treat neurodegenerative disorders.

By Patricia F. Dimond, Ph.D.
2/22/2010

Huntington disease is caused by the accumulation of a mutated form of the neuronal protein huntingtin, or Htt. *(V. Yakobchuk/Fotolia.com)*

On January 8, Elixir Pharmaceuticals reported that its partner, Siena Biotech, had commenced Phase I testing of Elixir's SIRT1 (sirtuin-1) inhibitor, a potentially first-in-class treatment for Huntington disease (HD). Unlike Sirtris Pharmaceuticals and GlaxoSmithKline's (GSK) drug candidates that increase SIRT1 activity to treat age-associated disorders, Elixir's EX-527 for HD is a small molecule aimed at decreasing SIRT1 activity.

Elixir was formed in 1999 based on discoveries from founders Cynthia Kenyon, Ph.D., currently at the University of California San Francisco, and Leonard Guarente, Ph.D., Novartis professor of biology at Massachusetts Institute of Technology. Dr. Guarente decamped from Elixir in 2007 to become co-chair of Sirtris' scientific advisory board. Sirtris was bought in 2008 by GSK for $720 million.

While Sirtris seems to be focusing on SIRT1 activators like analogues of resveratrol, a compound found in some red wines, Elixir's pipeline includes both activators and inhibitors, with Elixir's candidate entering the clinic first. But mechanisms of how sirtuins work to influence the pathological changes associated with these diseases remain incompletely characterized.

The SIRT1 seven-member enzyme family, which works by removing chemical and acetyl groups from its target proteins, has over two dozen known substrates. These enzymes affect a variety of cellular processes ranging from metabolism, growth and differentiation, senescence, apoptosis, stress response, and aging. As knowledge accumulates regarding this enzyme family's mechanisms of action, the true scope and complexity of their effects is emerging.

"None of us should be naïve enough to think resveratrol won't have multiple effects, including some you don't want," commented Patrick Vallance, M.D., Ph.D., head of drug discovery at GlaxoSmithKline.

Both Activation and Inhibition Show Promise

One desirable outcome of research on SIRT1 modulation would be the discovery of a new treatment for currently untreatable neurodegenerative diseases like HD. These diseases are generally related to the

accumulation of an abnormal form of a protein in specific brain cells, which causes their destruction. Other neurogenerative diseases related to abnormal protein accumulation include Alzheimer disease (AD) and so-called tauopathies characterized by the presence of filamentous protein aggregates in neurons. In the case of HD, the disease is caused by accumulation of a mutated form of the neuronal protein huntingtin, or Htt.

In 2005, Sirtris co-founder David Sinclair, Ph.D., of Harvard Medical School, reported that SIRT1 is upregulated in mouse models for AD and amyotrophic lateral sclerosis (ALS) as well as in primary neurons in cell culture challenged with neurotoxic insults. In cell-based models for AD and ALS, SIRT1 and resveratrol both promoted neuronal survival. In the inducible p25 transgenic mouse, a model of AD and tauopathies, resveratrol reduced neurodegeneration in the hippocampus, prevented learning impairment, and decreased the acetylation of the known SIRT1 substrates PGC-1alpha and p53. Direct injection of SIRT1 lentivirus into the hippocampus of p25 transgenic mice conferred significant protection against neurodegeneration.

Dr. Sinclair told *GEN* that neuroprotection via upregulation of SIRT1 may have several mechanisms: It may prevent apoptosis and may also control the way proteins aggregate. And in 2008, Dr. Guarante presented data showing that in a preclinical model of HD, mice lived longer and had less disease pathology in their brains with increased SIRT1. At the time, Sirtris CEO, Christoph Westphal, said, “These neuroprotective data expand the promise for small molecule sirtuin activators as potential therapeutics for a broad range of diseases of aging.”

Elixir reported that in its preclinical, unpublished studies, its SIRT1 inhibitor drug candidate EX-527 also reduced neuronal death caused by Htt in cell-based assays. In addition, according to the company, the molecule showed efficacy in a widely used transgenic model of HD, demonstrating favorable safety profiles and pharmaceutical properties.

Elixir entered into a research collaboration with Siena in 2007 and licensed EX-527 to the firm in 2009, giving Siena exclusive, worldwide rights to the small molecule for certain indications. The compound was granted orphan designation by the EMEA on October 28, 2009, and by the FDA on December 7, 2009.

Besides EX-527, Elixir’s pipeline also features two preclinical SIRT1 activators and two SIRT1 inhibitors, one of each for type 2 diabetes and obesity. The company licensed its technology through an agreement with Boston University for intellectual property covering discoveries related to SIRT1 modulators.

The Science behind Inhibition

So what is the putative mechanism of SIRTI activation and/or inhibition and their potential as treatments for HD? Initial interest in modifying the expression of SIRT1 as a treatment for HD focused on enhancing rather than inhibiting it, since the onset of neurodegenerative diseases appears to be associated with the aging process as cells’ defense mechanisms become less efficient.

SIRT1 as a deacetylase inhibitor appears to modify acetylation of the HD protein, causing enhanced clearance of the abnormal but not the normal version. It is this function that Elixir credits for producing the positive effects in cell and animal Huntington’s models.

Acetylation as a target mechanism for getting rid of abnormal, neurotoxic proteins may have some support from preclinical studies at the Mass General Institute for Neurodegenerative Diseases (MIND). New research shows that post-translational modifications, specifically acetylation, of Htt promote its clearance and may reveal new therapeutic targets for this disorder.

The MIND research team reported in the April 2009 issue of *Cell* that clearance of Htt protein can be achieved by post-translational modification of the mutant protein Htt by acetylation at lysine residue 444 (K444). Increased acetylation at this specific site on the abnormal protein facilitates its trafficking into

autophagosomes. This improves htt clearance by macroautophagy and reverses the toxic effects of the mutant huntingtin in primary cell cultures of neurons as well as in a transgenic *C. elegans* model of HD. Conversely, according to the authors, mutant Htt that is rendered resistant to acetylation dramatically accumulates and leads to neurodegeneration in cultured neurons and in mouse brain.

Current compounds used to increase acetylation lack the required specificity to create a safe and efficient medication, according to Dimitri Krainc, M.D., Ph.D., of MIND and associate professor of neurology at Harvard Medical School. “Among several candidate HD drugs currently in development are some that increase acetylation. But we need to identify more specific versions of these drugs that target only the mutant protein and don’t affect other cellular pathways. In addition to huntingtin, we are examining whether acetylation of other proteins affects their degradation in Parkinson’s and Alzheimer’s diseases.”

Reinforcing the complexities of working with activation or inhibition of sirtuins, researchers working in cell-based models of neuronal damage have reported opposing effects of sirtuins on neuronal survival. In particular, studies have revealed that SIRT1-mediated neuroprotection is independent of its deacetylase activity. In vitro studies of neuronal cell cerebellar granule neurons examined the effect of expressing each of the seven SIRT proteins in healthy neurons or in neurons induced to die by low potassium (LK) treatment.

The team found that SIRT1 protected neurons from LK-induced apoptosis, while SIRT2, SIRT3, and SIRT6 induced apoptosis in otherwise healthy neurons. They also discovered that SIRT5, which generally exerts a protective effect, localized to the mitochondria in a specific subset of the neurons and promoted neuronal death.

Given the sirtuin family of enzymes’ broad range of fundamental biological effects across multiple species, much more basic research is needed to identify specific drug targets for various diseases. A broader functional understanding of the physiological contexts in which sirtuins exert their regulatory influences will also be necessary.

♦♦♦

Patricia F. Dimond, Ph.D., is a principal at BioInsight Consulting. Email: drpdimond@comcast.net.