Unnerving atrophy

By Chris Cain, Staff Writer

A team at **The University of Texas Southwestern Medical Center at Dallas** has shown that deletion of class IIa histone deacetylases prevents muscle atrophy after nerve damage.¹ The result suggests that inhibitors of these molecules could help treat neuromuscular diseases, a hypothesis the researchers are testing by deleting class IIa HDACs in a mouse model of amyotrophic lateral sclerosis.

ALS and other neuromuscular diseases damage motor neurons, causing denervation that leads to muscle atrophy. Previous studies by UT Southwestern's Eric Olson, professor and chairman of molecular biology, identified a compensatory regeneration pathway that acts to recover the neuromuscular junction after nerve injury.² However the signaling pathway leading to muscle atrophy remained poorly understood.

Now, Olson's lab has identified the specific factors involved in denervation-mediated muscle atrophy. The team's first clue came from previous experiments demonstrating that many genes, including the class IIa histone deacetylases *HDAC4* and *HDAC5*, are upregulated upon denervation.³

HDAC4 and *HDAC5* are chemically and evolutionarily distinct from the class I HDACs that have been extensively targeted in cancer indications.

Systemic deletion of *HDAC4* in mice is lethal, so Olson's team used a conditional knockout to assess its role in muscle atrophy. In wild-type animals, severing the sciatic nerve led to about 50% leg muscle weight loss compared with 0% in unsevered control leg muscle.

Mice lacking *HDAC4* in skeletal muscle, or *HDAC5* systemically, did not lose as much muscle weight—only about 30%. The most striking result came when both HDACs were deleted, in which case denervation led to only 10% muscle weight loss. That result showed the two HDACs are required for neurogenic muscle atrophy.

"We knew HDACs 4 and 5 were upregulated during denervation, but we were very surprised to see that neurogenic atrophy was almost completely abated in these knockout mice," said Rhonda Bassel-Duby, a professor of molecular biology at UT Southwestern. "The mouse experiments helped bring many previous results together into a pathway for neurogenic muscular atrophy."

The researchers went on to detail the regulatory pathway downstream of *HDAC4* and *HDAC5* that leads to muscle atrophy (see **Figure 1, "A pathway for neurogenic muscular atrophy"**). They discovered the key downstream gene was a muscle-specific transcriptional regulator called *myogenin*.

"Myogenin is absolutely required for muscle development, so it initially seems crazy that it would also be required for muscle atrophy. But

Figure 1. A pathway for neurogenic muscular atrophy.

Researchers at **The University of Texas Southwestern Medical Center at Dallas** have elucidated a pathway leading to muscle atrophy following neuronal damage. Modulating the pathway could help treat neuromuscular diseases like amyotrophic lateral sclerosis (ALS). In response to skeletal muscle denervation that can occur during neuronal injury [a], histone deacetylase 4 (*HDAC4*) and *HDAC5* are upregulated. *HDAC4* and *HDAC5* repress the transcriptional regulator dachshund homolog 2 (*DACH2*), leading to upregulation of myogenin, a muscle-specific transcriptional regulator. Myogenin then upregulates the muscle-specific E3 ubiquitin ligases tripartite motif-containing 63 (*TRIM63*; *MURF1*) and f-box protein 32 (*FBXO32*; *atrogin-1*). *MURF1* and *atrogin-1* ubiquitinate muscle proteins and trigger muscle atrophy [b].

if you think about it, it makes sense to use a muscle-specific transcription factor to turn on a muscle-specific pathway such as atrophy," said Viviana Moresi, a postdoctoral fellow in Olson's lab and lead author of the paper in *Cell* that described the findings.

Indeed, conditional *myogenin* knockout mice were resistant to atrophy and did not have upregulation of tripartite motif-containing 63 (*TRIM63*; *MURF1*) and f-box protein 32 (*FBXO32*; *atrogin-1*), two E3 ubiquitin ligases known to play central roles in the proteolytic pathway that causes muscle atrophy.

The UT Southwestern group went on to show that myogenin binds the promoters of MURF1 and atrogen-1 and directly turns on their expression. Importantly, muscle atrophy caused by starvation did not require myogenin, suggesting this pathway is specific to denervation.

Daniel Goldman, professor of biological chemistry at the **University of Michigan Medical School**, whose lab first described the HDAC-myogenin signaling cascade, wrote in an e-mail to *SciBX* that “the main advance this paper makes is connecting the HDAC4/5-DACH2 [dachshund homolog 2]-myogenin signaling system to the regulation of E3 ubiquitin ligases that control muscle atrophy. The potential for using HDAC inhibitors to treat ALS and other neuromuscular diseases is of great interest.”^{4,5}

Hot for HDACs

There are no disclosed class IIa-specific HDAC inhibitors in the clinic. Indeed, companies contacted by *SciBX* suggested the reason is not because they are hard to develop but rather that they have been a target class in search of an indication.

“Class II HDACs are druggable; however, this is one case where the chemistry was ahead of the biology, because it was unclear how these drugs could best be used in a therapeutic setting,” **MethylGene Inc.** CSO and EVP of R&D Jeffrey Besterman told *SciBX*. “We have discovered many class II-specific inhibitors with the belief that someone would find an indication which they would be useful for, and we have demonstrated they are not overtly toxic in mouse models.”

Besterman said that in addition to ALS, a host of rare motor neuron disorders, including spinal muscular atrophy, could benefit from inhibition of the HDAC-myogenin pathway.

Three compounds from MethylGene's HDAC chemistry platform have entered clinical development. These include one antifungal HDAC inhibitor, one class I HDAC inhibitor in the clinic for oncology and one HDAC inhibitor licensed to **EnVivo Pharmaceuticals Inc.** for neurodegenerative disorders. The most advanced of these is in Phase II testing for multiple forms of lymphoma in collaboration with **Taiho Pharmaceutical Co. Ltd.**, a subsidiary of **Otsuka Pharmaceutical Co. Ltd.** In addition, MethylGene is exploring the role of its class II HDAC inhibitors in preclinical models of inflammation and diabetes.

Acetylon Pharmaceuticals Inc. also is developing inhibitors of class II HDACs. In February, the company licensed a discovery platform for HDAC inhibitors from **Harvard University** and the **Dana-Farber Cancer Institute**.⁶

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**—Walter Ogier,
Acetylon Pharmaceuticals Inc.**

its potential,” Acetylon president and CEO Walter Ogier told *SciBX*.

Acetylon has small molecule HDAC6 inhibitors in preclinical development for multiple myeloma (MM) and inflammation. HDAC6 is a class IIb HDAC.

Olson's lab intends to follow up the results in the *Cell* paper on two fronts. First, the lab is in the process of generating an HDAC4 knockout in a mouse model of ALS. The group will ana-

lyze the effects of HDAC deletion on lifespan and muscle functionality. Second, the researchers have started an academic collaboration with Timothy McKinsey, associate professor in the Division of Cardiology at the **University of Colorado Denver**, to test the effect of class IIa-specific HDAC inhibitors on neurogenic muscular atrophy.

Tso-Pang Yao, associate professor of pharmacology and cancer biology at the **Duke University School of Medicine**, whose lab first determined that HDAC4 linked neural activity to muscle,³ wrote in an e-mail to *SciBX* that he believes targeting HDAC4 is an exciting therapeutic opportunity for treating ALS-related disease. His lab has a patent on regulating HDAC4 activity in relation to muscle atrophy.

Olson's team did not disclose the patent and licensing status of its work.

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COMPANIES AND INSTITUTIONS MENTIONED

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