STEP against AD

By Lev Osherovich, Senior Writer

Yale University researchers have made a case for blocking STEP, a brain-specific phosphatase involved in synaptic signaling and a key player in the early stages of Alzheimer's disease development.¹ Knocking down the enzyme forestalled AD symptoms in mice, but it remains unclear whether it can be targeted in humans.

STEP (protein tyrosine phosphatase non-receptor type 5 striatum-enriched; PTPN5) ordinarily dephosphorylates the NMDA receptor (NMDAR), a protein complex that strengthens synaptic connections during learning and memory formation. The dephosphorylation causes the receptor to be internalized and degraded.

In a previous study, a team led by Paul Lombroso, professor of neurobiology and psychiatry at Yale, found that STEP is misregulated in a range of neuropsychiatric conditions.²

"We found that STEP was elevated in three different disorders—AD, schizophrenia and fragile X syndrome," he said. "If you're trying to learn something, this enzyme isn't good for you."

Moreover, a different study by Lombroso showed that β -amyloid (A β) accumulation at the synapse leads to lower NMDAR levels.³

Thus, he hypothesized that knocking down STEP would increase NMDAR levels and counteract the effects of A β , a neurotoxic protein fragment that accumulates in patients with AD.

Now, Lombroso's group has shown that deleting the mouse gene encoding Step indeed slowed cognitive decline caused by A β .

The team's first step was crossing *Step* knockout mice with a strain that overproduces A β .

In an assay of spatial learning and memory, AD mice lacking Step performed better than AD mice with intact Step. Additionally, hippocampal slices from AD mice lacking Step had better synaptic function than slices from wild-type AD mice in a cell culture assay.

Lombroso's team next examined NMDAR at the synapse. Levels of an NMDAR subunit that is dephosphorylated by Step were lower in AD mice than in wild-type controls. Knocking out Step in AD mice restored synaptic levels of the subunit back to wild-type levels.

Although many AD therapies have focused on lowering A β , STEP deletion does not have that effect. Lombroso's team found that levels of A β were comparable in AD mice with and without Step. Likewise, *Step* deletion did not affect levels of hyperphosphorylated microtubule-associated protein- τ (MAPT; TAU; FTDP-17), another marker of neuronal dysfunction.

The findings were reported in the *Proceedings of the National Academy of Sciences*. Paul Greengard, professor of neuroscience at **The Rockefeller University**, coauthored the report.

STEP right this way

Lombroso thinks that tinkering with STEP can protect neurons from A β at a very early stage of disease. "Before there's any neurodegeneration, STEP activity is disrupting synaptic strength," he said.

Samuel Gandy, professor of neurology and psychiatry at **Mount Sinai School of Medicine**, said that STEP is shaping up to be a promising target for early intervention, provided that there are no deleterious effects of inhibiting the enzyme for an extended period of time.

Inhibiting STEP "antagonizes the effect of A β on NMDAR trafficking" said Gandy. Hitting STEP may thus be a more subtle way to modulate NMDAR activity than hitting the receptor itself, Gandy told *SciBX*.

He noted that timing will be important because later in AD excessive NMDAR activation can actually accelerate disease progression. **Forest Laboratories Inc.** markets an NMDAR antagonist, Namenda memantine, to treat AD in the U.S. **H. Lundbeck A/S** markets the drug as Ebixa in Europe, and **Merz GmbH & Co. KGaA** sells it elsewhere as Axura.

Excess NMDAR activity later in disease "is something you would certainly worry about, so you would want to study long-term effects" of blocking STEP, Gandy said.

Lombroso told *SciBX* that his team has been tracking AD mice with and without the *Step* gene into late adulthood and thus far has seen no neurological problems.

Out of STEP

Lombroso has not patented his discoveries but said that he is interested in working with companies to develop and test a STEP inhibitor in AD and other neurological indications. His group is running *in vitro* and cell culture screens to find small molecule antagonists of STEP.

However, finding a suitable small molecule may be challenging as STEP is part of a family of enzymes—the phosphatases—that has proven hard to selectively inhibit.

"This is a nice paper that validates STEP as a potential target to treat AD, but it's doubtful that pharmaceutical companies will try to develop drugs that inhibit STEP," said Sir Philip Cohen, professor of life sciences and director of the Medical Research Council Protein Phosphorylation Unit at the **University of Dundee**.

He said many pharmas believe that "protein tyrosine phosphatases are undruggable."

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"The fact that the *STEP* knockout is normal, viable and has perhaps even better-than-normal learning and memory is good for a drug approach, since inhibiting the enzyme should not have serious side effects. The difficulty is that it's a phosphatase."

—Steven Braithwaite, Signum Biosciences Inc.

approach, since inhibiting the enzyme should not have serious side effects,” said Steven Braithwaite, SVP of drug discovery at **Signum Biosciences Inc.** “The difficulty is that it’s a phosphatase.”

Braithwaite said the medicinal chemistry of phosphatases, which remove phosphates, is “about 10 years behind” that of kinases, which add them.

As a senior scientist at **AGY Therapeutics Inc.**, Braithwaite led a team that identified a STEP antagonist that was in preclinical testing for AD, stroke and schizophrenia.

“We were looking at fine-tuning neuronal function,” said Braithwaite. “We thought STEP was a viable target.”

AGY ceased operations in 2005.

Signum is targeting another phosphatase implicated in neurodegenerative diseases—protein phosphatase 2 (PPP2CA; PP2A). Braithwaite said PP2A removes phosphates from MAPT and α -synuclein (SNCA), the phosphorylation of which contributes to AD and Parkinson’s disease (PD), respectively. Signum has PP2A agonists in preclinical development.

Irach Taraporewala, CEO of **Ohr Pharmaceutical Inc.**, said that finding a sufficiently selective STEP inhibitor may be a challenge as there are many similar phosphatases involved in a range of physiological processes.

“Inhibitors would probably need to bind areas of STEP that are distal to the catalytic domain, which has substantial sequence homology with other tyrosine phosphatases,” said Taraporewala.

Ohr’s trodusquemine, a protein tyrosine phosphatase 1B (PTPN1; PTP-1B) inhibitor, is in Phase I testing for type 2 diabetes and obesity.

If STEP itself proves hard to hit, another possibility is to go upstream or downstream of the enzyme. However, this may prove equally challenging. STEP levels are regulated by ubiquitination, which is itself a hard process to target. On the other hand, it’s unclear how to directly tweak NMDAR to mimic the effect of inhibiting STEP.

Taraporewala also thinks that STEP’s brain-specific activity and

subcellular localization in the endoplasmic reticulum may pose a challenge.

“To be effective, an inhibitor would not only need to traverse the blood brain barrier but also be able to get into the endoplasmic reticulum where STEP is localized in nerve synapses,” he said.

Gandy noted that STEP antagonists would most likely be effective in patients who haven’t yet undergone the massive neuronal loss that characterizes clinical AD. But identifying early-stage patients using serum and cerebrospinal fluid biomarker profiles⁴ is a hard sell to regulators, said Gandy.

“We have to broaden our approach—targeting A β seems insufficient,” said Gandy. “But FDA is reluctant to allow us to go into an asymptomatic population based on a biomarker profile.”

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COMPANIES AND INSTITUTIONS MENTIONED

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