Introducing GSAP

By Tim Fulmer, Senior Writer

Following a lead provided by Gleevec, a team of U.S. researchers from **The Rockefeller University** and **Intra-Cellular Therapies Inc.** has identified a new regulatory protein, γ -secretase activating protein, that activates γ -secretase, a key Alzheimer's disease target.¹

Because γ -secretase activating protein (GSAP; PION) potentially regulates only the β -amyloid ($A\beta$)-related activity of γ -secretase, GSAP inhibitors might be safer than compounds that block all γ -secretase activity. Intra-Cellular is developing small molecule compounds that can enter the brain and target GSAP.

γ -Secretase is a proteolytic complex that converts amyloid- β (A4) precursor protein (APP) into $A\beta$, the protein fragment that forms pathogenic amyloid plaques thought to underlie Alzheimer's disease (AD). Thus, one therapeutic avenue in AD has been to inhibit γ -secretase to decrease $A\beta$ levels and prevent plaque-associated neuronal toxicity (see **Figure 1, "Targeting GSAP in AD"**).

Despite the rationale, two small molecule γ -secretase inhibitors have failed in their respective Phase III AD trials, at least in part because of poor safety.² The problem is that γ -secretase, a complex of at least four proteins, has multiple substrates other than APP, such as notch homolog 1 translocation-associated (NOTCH1), and inhibiting the complex can cause toxicity by disrupting multiple cell signaling pathways in healthy cells.

In their latest work, Paul Greengard and colleagues may have identified a solution to the problem: rather than target γ -secretase and shut it down completely, target only regulators or activators of the γ -secretase-APP interaction, leaving the other essential functions of γ -secretase intact.

Greengard, professor of molecular and cellular neuroscience at Rockefeller University and cofounder of Intra-Cellular, was initially looking for ways to treat AD by targeting the ATP requirement of $A\beta$ processing.

In work published in 1999, Greengard's team found that $A\beta$ production required ATP. This suggested that blocking the actions of ATP using kinase inhibitors could reduce $A\beta$ production and treat AD.³ In 2003, the group reported that the kinase inhibitor Gleevec imatinib, which targets multiple kinases including ABL, was able to block $A\beta$ production in cell culture and in rodents.

Importantly, the team found that Gleevec's $A\beta$ -lowering effects did not stem from the drug's action on ABL.⁴ Thus, the question was: What protein did Gleevec target to reduce $A\beta$?

In a new paper published in *Nature*, Greengard's team has now identified that protein as GSAP, a 16 kDa protein that forms a complex with γ -secretase and APP and helps promote cleavage of APP to $A\beta$.

Figure 1. Targeting GSAP in AD. A team of researchers led by Paul Greengard, professor of neuroscience at **The Rockefeller University**, identified γ -secretase activating enzyme (GSAP; PION), a protein the team thinks could be targeted to treat Alzheimer's disease without the toxicity associated with many γ -secretase inhibitors.

γ -Secretase, a multi-subunit protein complex, sits in the membrane, where it cleaves a variety of protein substrates in multiple signaling pathways [a]. Cleavage of one of those substrates, amyloid- β (A4) precursor protein (APP), generates the β -amyloid ($A\beta$) peptide [b], which forms the amyloid fibrils and plaques that are thought to be the primary drivers of AD-related cognitive and behavioral dysfunction [c]. Some evidence points to the potential of the second cleavage product of APP, the APP intracellular domain (AICD), to upregulate expression and/or activation of genes that promote AD [d].^{6,7}

In a paper published in *Nature*, Greengard's team identified GSAP as a selective regulator of the γ -secretase-APP interaction.

G protein-coupled receptor 3 (GPR3) also is known to interact with γ -secretase and help regulate its function.

By preventing GSAP from interacting with γ -secretase and APP, Gleevec could block downstream formation of amyloid plaques without binding γ -secretase. Indeed, small interfering RNA knockdown of GSAP was sufficient to reduce $A\beta$ levels by about 50% in cell culture compared with no treatment. Importantly, those reductions did not impair processing of NOTCH1 by γ -secretase.

The final question was whether the mechanistic difference between

targeting GSAP and targeting the γ -secretase complex would translate to improvements in an animal model.

In a transgenic mouse model of AD, small hairpin RNA-mediated knockdown of *Gsap* significantly reduced levels of toxic A β and amyloid plaque development compared with normal *Gsap* expression ($p < 0.01$ for both).

The A β -lowering effects of *Gsap* knockdown were similar to the effects of the γ -secretase inhibitor dibenzazepine, though without causing intestinal mucosal cell metaplasia, a side effect associated with the latter.

The researchers concluded in their paper that compounds that “accumulate in the brain and target GSAP represent a valid approach for development of potential therapies against Alzheimer’s disease.”

The identification of GSAP as a potential AD target “shows us that the goal is no longer to target γ -secretase itself but to target regulators of γ -secretase. By doing so, such a strategy could potentially lower toxic amyloid levels while minimizing the harmful side effects associated with direct γ -secretase blockade,” said Sanjay Pimplikar, associate professor of molecular medicine at **Cleveland Clinic**.

One biotech already is targeting a regulator of γ -secretase to treat AD. Last year, **Galapagos N.V.** reported in *Science* that knockout of G protein-coupled receptor 3 (GPR3), a membrane protein that helps regulate γ -secretase, led to lower A β levels than those in wild-type controls without impairing NOTCH processing.⁵ The company is now developing GPR3 antagonists.

Moving forward

Corresponding author Greengard told *SciBX* that GSAP “may be the tip of an iceberg” that represents a new pathway that controls γ -secretase activity. He now wants to identify other proteins in that pathway that potentially interact with GSAP to increase or decrease its activity.

Greengard is not actively developing compounds that target GSAP. Gleevec itself is not a likely option because the drug, which **Novartis AG** markets to treat chronic myelogenous leukemia (CML) and gastrointestinal stromal tumors (GIST), does not readily accumulate in the brain. But Gleevec derivatives capable of penetrating the blood brain barrier (BBB) and remaining in the brain could be an option for treating AD.

One way would be “to modify Gleevec to ensure it can cross the blood brain barrier and stay in the brain long enough to have a therapeutic effect,” said Greengard.

Bart De Strooper, professor of human genetics at the **Catholic University Leuven**, suggested a high throughput approach to generate compounds that dissociate the binding of GSAP with either the γ -secretase complex or APP. De Strooper is collaborating with Galapagos on the GPR3 studies.

“Additional structural data on how Gleevec binds GSAP and how that binding alters the interaction of GSAP with both APP and γ -secretase will be important for developing Gleevec derivatives that can effectively block the interaction *in vivo*,” noted Pimplikar. “It will also be important to confirm that reductions in A β levels actually

“The goal is no longer to target γ -secretase itself but to target regulators of γ -secretase.”

**—Sanjay Pimplikar,
Cleveland Clinic**

correlate with behavioral benefits when GSAP is inhibited.”

Lawrence Wennogle, VP of drug discovery at Intra-Cellular, told *SciBX* the company is already developing “an undisclosed class of small molecule compounds that can accumulate in the brain and selectively target GSAP with a higher affinity than Gleevec.”

Wennogle said the company has ongoing studies to characterize those compounds in rodent models of AD and other neurodegenerative diseases. The company is also using spectroscopic techniques to further elucidate the folding and secondary structure of purified GSAP.

Intra-Cellular’s lead compound, ITI-007, a dual serotonin (5-HT_{2A}) receptor antagonist and dopamine receptor phosphoprotein modulator (DPPM), completed a Phase Ib/II trial to treat schizophrenia in July.

Regardless of how GSAP is inhibited, one researcher said it will be essential to show that antagonizing GSAP does not disrupt other potential functions of the protein besides its role in activating APP processing by γ -secretase.

“It needs to be determined whether targeting GSAP is safe; that is, whether GSAP has essential functions other than its involvement in APP processing,” Jie Shen, a professor of neurology at **Harvard Medical School** and **Brigham and Women’s Hospital**, told *SciBX*.

Those studies should help shed light on “whether GSAP is involved in γ -secretase-mediated cleavage of other substrates that carry out essential functions of the γ -secretase complex,” said Shen.

According to Sharon Mates, chairman, CEO and cofounder of Intra-Cellular, patents covering the *Nature* findings are held by Rockefeller University and/or Intra-Cellular. The company has exclusively licensed all the IP, Mates told *SciBX*.

Fulmer, T. *SciBX* 3(36); doi:10.1038/scibx.2010.1082
Published online Sept. 16, 2010

REFERENCES

1. He, G. *et al. Nature*; published online Sept. 2, 2010; doi:10.1038/nature09325
Contact: Paul Greengard, The Rockefeller University, New York, N.Y.
e-mail: greengard@rockefeller.edu
2. Osherovich, L. *BioCentury* 18(37), A9–A11; Aug. 23, 2010
3. Greenfield, J.P. *et al. Proc. Natl. Acad. Sci. USA* 96, 724–747 (1999)
4. Netzer, W.J. *et al. Proc. Natl. Acad. Sci. USA* 100, 12444–12449 (2003)
5. Thathiah, A. *et al. Science* 323, 946–951 (2009)
6. Ghosal, K. *et al. Proc. Natl. Acad. Sci. USA* 106, 18367–18372 (2009)
7. Ghosal, K. *et al. PLoS ONE* 5, e11866; published online July 30, 2010; doi:10.1371/journal.pone.0011866

COMPANIES AND INSTITUTIONS MENTIONED

Brigham and Women’s Hospital, Boston, Mass.
Catholic University Leuven, Leuven, Belgium
Cleveland Clinic, Cleveland, Ohio
Galapagos N.V., Mechelen, Belgium
Harvard Medical School, Boston, Mass.
Intra-Cellular Therapies Inc., New York, N.Y.
Novartis AG (NYSE:NVS; SIX:NOVN), Basel, Switzerland
The Rockefeller University, New York, N.Y.