Thinking outside the amyloid box

By Lev Osherovich, Senior Writer

Two European groups have proposed strategies for treating Alzheimer's disease that are focused on hitting intermediate protein fragments and enzymatic steps in the pathway that produces β -amyloid.^{1,2} A team at **Institut National de la Santé et de la Recherche Médicale** and the **University of Nice Sophia Antipolis** thinks the goal should be reducing levels of C99, a protein fragment that is a precursor of β -amyloid. A team at **Catholic University Leuven** expects that hitting a newly identified cofactor of γ -secretase can yield the same effect as eliminating C99 and β -amyloid while potentially acting more selectively on the β -amyloid pathway than γ -secretase inhibitors.

AD is thought to be driven by the accumulation of β -amyloid ($A\beta$), a toxic fragment of amyloid precursor protein (APP). Thus, blocking $A\beta$ production has been a prime approach to treating AD.

Toward this goal, academic and industry researchers have spent decades working out the enzymatic steps that lead to the conversion of APP into $A\beta$ ³ (see Figure 1, "APP processing in AD"). In the two most critical steps of the pathway, full-length APP is first cut by β -site APP-cleaving enzyme 1 (BACE1) to yield the C99 fragment, which is then cut a second time by γ -secretase, a multisubunit proteolytic complex, to yield $A\beta$.

Initially, researchers deemed γ -secretase the most therapeutically tractable target in this pathway. However, safety problems cratered multiple clinical trials of first-generation γ -secretase compounds. mAbs that directly hit $A\beta$, such as **Johnson & Johnson** and **Pfizer Inc.**'s bapineuzumab and **Eli Lilly and Co.**'s solanezumab, avoided the safety issues associated with blocking γ -secretase but nonetheless failed in Phase III trials for lack of efficacy.

Now, a team at the University of Nice Sophia Antipolis branch of Institut National de la Santé et de la Recherche Médicale (INSERM) has mouse data showing that accumulation of C99, which previous work has suggested could play a role in AD disease progression, is actually made worse by γ -secretase inhibitors.

The INSERM team's findings help build a case for treating AD by preventing C99 accumulation rather than focusing exclusively on $A\beta$.

Meanwhile, a group at Catholic University Leuven thinks hitting a specific subunit of γ -secretase could reduce levels of both APP fragments. The team found that γ -secretase converts C99 into $A\beta$ through an intracellular cofactor, arrestin β 2 (ARRB2), and a cell surface protein called G protein-coupled receptor 3 (GPR3), and they found that blocking the GPR3-ARRB2 pathway alters the activity of γ -secretase in a way that lowers levels of both C99 and $A\beta$.

C99 problems

Previous work by other researchers showed that C99 overexpression was highly toxic to cultured neurons, suggesting the APP cleavage intermediate could contribute to AD pathology independently of $A\beta$.⁴

The INSERM team, led by Research Director Frédéric Checler, used an antibody that detects C99 to show that the protein fragment accumulated inside the hippocampal neurons of aging mice. The buildup occurred prior to the accumulation of extracellular $A\beta$ deposits, which are a hallmark of AD pathology.

C99 accumulation also occurred in a different mouse model of AD in which there was relatively little extracellular $A\beta$. Thus, Checler thinks the accumulation of intracellular C99 is an early marker of AD pathology.

"We have found a new phenotype in these mice that is linked with what is seen in AD patients. C99 is present in older mice and correlates with the onset of symptoms" more closely than $A\beta$ deposits, said Checler.

Figure 1. APP processing in AD. In early Alzheimer's disease pathogenesis, amyloid precursor protein (APP) is processed by a series of proteases to yield several neurotoxic protein fragments. First, APP is cut by β -site APP-cleaving enzyme 1 (BACE1) [a] to yield a fragment called C99 [b], which is subsequently cut by the γ -secretase complex [c] to yield β -amyloid ($A\beta$).

Lauritzen *et al.* have provided *in vivo* evidence in a mouse model of AD that suggests intracellular C99 accumulation contributes to early AD pathology. Thathiah *et al.* have shown that arrestin β 2 (ARRB2) acts as a γ -secretase cofactor that specifically regulates the processing of C99.

The team then showed that ELND006, a discontinued γ -secretase inhibitor from **Elan Corp. plc**, increased levels of C99 compared with vehicle. Checler thinks this finding could help explain why two other discontinued γ -secretase inhibitors, Lilly's semagacestat and **Bristol-Myers Squibb Co.**'s avagacestat, appeared to worsen rather than relieve AD symptoms in respective Phase III and Phase II trials.⁵

The findings were published in *The Journal of Neuroscience*.

Bart De Strooper, professor of human genetics at Catholic University Leuven, said Checler's study "does a very careful analysis of the transgenic mice, showing that C99 is accumulating at a very early stage."

De Strooper, who discovered γ -secretase, said the findings provide potential explanations for "the problems with broad-spectrum γ -secretase inhibitors, since one of the consequences of these compounds is the accumulation of C99."

Human touch

The big question is whether C99 plays a role in human disease, but answering this is technically challenging.

Because Checler's anti-C99 mAbs are immunohistochemical probes that cannot be used *in vivo*, it is not possible to use them to scan the brains of early stage AD patients for C99 accumulation.

An alternative approach is to pharmacologically block production of C99 with BACE1 inhibitors. But such compounds would also prevent A β production, making it difficult to unravel which aspects of AD arise from intracellular C99 vs. extracellular A β .

"The best way to investigate this in patients is to try to detect C99 postmortem, but at best this would be correlative," said De Strooper.

Virginia Lee, director of the Center for Neurodegenerative Disease Research and professor of pathology and laboratory medicine at the **Perelman School of Medicine at the University of Pennsylvania**, wanted to see more evidence of C99 accumulation in human tissue. She cautioned that Checler's studies were done in mice that overexpress APP. At more physiological levels of APP expression, she said the conversion of APP into C99 and then A β may work differently.

"We don't yet know the biological significance of C99 accumulation," said Lee. "In models that overexpress APP, C99 may be important, but you don't know what it's like in wild-type animals and humans."

To resolve this question, Lee recommended developing selective anti-C99 mAbs and using them in additional immunohistochemistry studies in a variety of mouse models of AD and in human tissue.

Checler said he is collaborating with an undisclosed company to identify and characterize next-generation antibodies against C99.

Gamma world

Meanwhile, De Strooper's team is identifying proteins that interact with γ -secretase and could be targeted with safer compounds than the failed broad-spectrum inhibitors.

In 2009, De Strooper and researchers at **Galapagos N.V.** reported that an orphan GPCR called GPR3 plays an essential role in γ -secretase activity.⁶

Now, De Strooper has found that ARRB2 is required for GPR3's effects on γ -secretase.

In cell culture, mutations in GPR3 disrupted the receptor's ability to bind ARRB2 and also prevented γ -secretase from converting C99 into A β . The finding suggests ARRB2 transmits a signal from GPR3 to γ -secretase that governs the enzyme's activity.

Indeed, overexpression of ARRB2 increased production of A β and small interfering RNA knockdown of ARRB2 decreased A β levels compared with what was seen using vector controls.

Arrb2 knockout mice with AD had lower γ -secretase activity and A β levels than wild-type controls. Finally, the brains of patients with AD had higher levels of ARRB2 than brains from age-matched healthy controls.

Altogether, the findings suggest ARRB2 facilitates γ -secretase activity on C99 by coupling the enzyme to GPR3 at the cell surface.

De Strooper's team also found that knocking down ARRB2 in cell culture lowered both C99 and A β levels, suggesting that blocking the GPR3-ARRB2 pathway works differently than inhibiting γ -secretase outright, which Checler's team found to prevent A β production at the cost of increasing C99 levels. Although the exact mechanistic details are not known, decreasing ARRB2 levels led to increased proteasomal degradation of C99.

Moreover, knocking down ARRB2 did not interfere with γ -secretase's ability to cleave other substrates besides C99, suggesting that ARRB2 is a fairly AD-specific target.

Results were published in *Nature Medicine*.

De Strooper thinks the findings will help focus screening efforts for GPR3-binding compounds that specifically antagonize ARRB2 activity. De Strooper said a prior collaboration with Galapagos to identify such compounds has ended, and he is now working with a different, undisclosed company. A spokesperson for Galapagos said that the company retains patents and a discovery-stage screening program targeting GPR3 in AD.

De Strooper said that although γ -secretase inhibitors have failed in the clinic, his findings are proof of principle that modulating γ -secretase activity can reduce both C99 and A β levels without compromising the enzyme's other functions.

"There is a way to affect γ -secretase activity without causing C99 accumulation," said De Strooper. "The message is that we shouldn't give up on γ -secretase."

The results from Checler's team are not patented. De Strooper has filed a patent on modulating ARRB2 to treat AD. That patent is not available for licensing.

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REFERENCES

1. Lauritzen, I. *et al. J. Neurosci.*; published online Nov. 14, 2012; doi:10.1523/JNEUROSCI.2775-12.2012
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2. Thathiah, A. *et al. Nat. Med.*; published online Dec. 2, 2012; doi:10.1038/

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3. Citron, M. *Nat. Rev. Drug Discov.* **9**, 387–398 (2010)
4. Jin, L.-W. *et al. J. Mol. Neurosci.* **19**, 57–61 (2002)
5. Osherovich, L. *BioCentury* **19**(44), A8; Oct. 24, 2011
6. Thathiah, A. *et al. Science* **323**, 946–951 (2009)

COMPANIES AND INSTITUTIONS MENTIONED

Bristol-Myers Squibb Co. (NYSE:BMJ), New York, N.Y.

Catholic University Leuven, Leuven, Belgium

Elan Corp. plc (NYSE:ELN), Dublin, Ireland

Eli Lilly and Co. (NYSE:LLY), Indianapolis, Ind.

Galapagos N.V. (Euronext:GLPG; Pink:GLPYY), Mechelen, Belgium

Institut National de la Santé et de la Recherche Médicale, Sophia-Antipolis, France

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