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RESEARCH HIGHLIGHT

Cutting in on a secretase *pas de deux*

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Two proteolytic enzymes, β- and γ-secretases, work together to produce the amyloid β-peptide of Alzheimer's disease. New evidence suggests that these proteases directly interact and compounds that disrupt this interaction reduce amyloid β-peptide levels without directly blocking either enzyme's solo activity.

Alzheimer's disease (AD) is a devastating neurodegenerative disorder and the most common form of dementia [1]. Pathological hallmarks include plaques composed of the amyloid β-protein (Aβ) and neurofibrillary tangles composed of the otherwise microtubule-associated protein tau. Compelling evidence, most importantly, dominantly inherited mutations that cause early-onset AD, support Aβ as the pathogenic initiator of a cascade of events that lead to synaptic toxicity and neuronal cell death. For this reason, Aβ has been the primary target toward developing potential AD therapeutics. Blocking the enzymes that produce Aβ from its protein precursor (APP) has been a favored strategy [2, 3], but these two proteases, β- and γ-secretases, have many other substrates, and directly blocking their activity can be toxic. In a new report in *Cell Discovery* [4], Cui and colleagues demonstrate that these two secretases can directly interact and describe the discovery of compounds that lower Aβ by disrupting this interaction, potentially avoiding toxicity associated with direct protease inhibition.

β-secretase is a membrane-tethered protease that cleaves APP in the lumenal/extracellular space, shedding the large ectodomain of APP and leaving membrane-bound C-terminal fragments (Figure 1). These APP fragments are then cut within their transmembrane domains (TMDs) to produce Aβ by γ-secretase, a four-protein complex with the multi-pass membrane protein presenilin as the catalytic component. Several previous reports have suggested that β- and γ-secretases could interact [5-7], perhaps working closely together to cut APP to form Aβ. The new study built on those earlier findings by generating a convenient reporter assay that would give a clear signal — luciferase activity — upon direct interaction. Strong evidence is provided for a direct and specific interaction between the two secretases, including important positive and negative controls, although future studies should confirm this interaction with endogenous proteins.

The reporter assay was also convenient for screening compounds that could potentially block the interaction between the two secretases. Testing over 10 000 small molecules resulted in seven compounds that specifically reduced interaction between the proteases, without affecting cell viability or *in vitro* secretase activity. Only one of these seven, however, 3-α-akebonoic acid (3AA), significantly reduced Aβ production in cells. 3AA is a steroidal natural product available in very limited quantities. Therefore, the search for active analogs began with the structurally related and more available betulin acid, ultimately identifying XYT472B as a conveniently synthesized compound with similar activity to 3AA.

Other APP proteolytic products besides Aβ — shed APP ectodomain and APP C-terminal fragments — were said not to be affected by 3AA and XYT472B. As reducing Aβ production, no matter how it is accomplished, must also alter other APP proteolytic products, these findings would instead suggest that the compounds affect Aβ secretion or degradation. However, inspection of the data suggests some inhibitory effects on β-secretase cleavage of APP in cells. Thus, disrupting the β-/γ-secretase interaction in cells may result in reduced processing of APP by β-secretase. Another possibility — and one that should be explored in future studies — is that the compounds affect β-secretase trafficking or maturation, consistent with a previous study supporting an interaction between γ-secretase and the inactive, immature form of β-secretase [6].

The discovery of XYT472B allowed its modification into an affinity labeling reagent, capable of crosslinking directly to its protein target with subsequent coupling to biotin to allow isolation and identification of this target. These experiments identified presenilin as the secretase component containing the binding site for 3AA and XYT472B. The inability of another type of inhibitor that interacts with the γ-secretase active site to prevent affinity labeling demonstrated that the new compounds bind elsewhere on presenilin. Addition of peptides representing the various TMDs of presenilin gave results consistent with TMD 6 as the binding site for 3AA and XYT472B. Presenilin's TMD 6 contributes to the γ-secretase active site and is likely involved in gating of substrates into the active site [8, 9]. Thus, the new compounds would have to bind TMD 6 in a manner that does not interfere with these important functions, which would

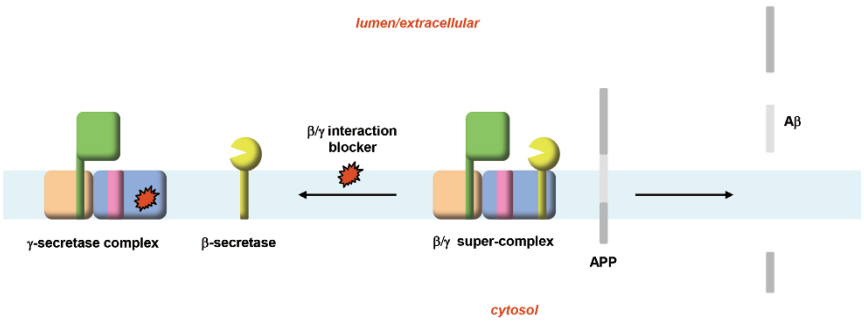

Figure 1 Interaction between β- and γ-secretases and disruption of this interaction by small molecules. γ-secretase is composed of four membrane components with presenilin (blue) as the catalytic component. β-secretase apparently interacts with γ-secretase via presenilin, and these two enzymes then work together to efficiently cleave APP to Aβ and other proteolytic products. Small molecules such as 3AA bind to presenilin to putatively disrupt interaction with β-secretase and reduce Aβ production without directly inhibiting either protease's enzymatic activity.

otherwise inhibit γ-secretase activity directly. Future studies will be needed to identify the precise location of the compound binding site, with mutagenesis of the involved presenilin residues to determine effects on interaction with β-secretase.

Regardless of the exact mechanism, the compounds proved to be effective *in vivo*. Administration of XYT472B directly into the brain in an AD mouse model reduced Aβ plaques and rescued cognitive deficits. Further study is required to show that β- and γ-secretase solo activity is not unduly affected under these conditions, as direct secretase inhibitors have failed in clinical trials in large part due to toxic consequences of interfering with critical substrates other than APP [10]. In addition, more potent analogs will be needed that allow peripheral administration, and compound metabolic stability will likely require optimization for long-term studies of efficacy and safety.

The discovery of this new class of Aβ-lowering agents is provocative and promising. Future studies should help clarify their mechanism and address other key questions. For instance, what is the effect of the compounds on other substrates for β-secretase? For γ-secretase? Will medicinal chemistry improve on the micromolar activity of these early lead compounds? Do other γ-secretase inhibitors or modulators compete for binding on presenilin? Do these compounds avoid the toxic consequences of shutting down β- or γ-secretase? If so, then cutting in on these two secretase dance partners may be an effective move.

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References

1 Goedert M, Spillantini MG. *Science* 2006; **314**:777-781.
2 Ghosh AK, Gemma S, Tang J. *Neurotherapeutics* 2008; **5**:399-408.
3 Wolfe MS. *J Neurochem* 2011; **120 Suppl 1**:89-98.
4 Cui J, Wang X, Li X, *et al. Cell Discov* 18 Aug 2015; **1**:15021. doi:10.1038/celldisc.2015.21
5 Hattori C, Asai M, Oma Y, *et al. Biochem Biophys Res Commun* 2002; **293**:1228-1232.
6 Hebert SS, Bourdages V, Godin C, *et al. Neurobiol Disease* 2003; **13**:238-245.
7 Kuzuya A, Uemura K, Kitagawa N, *et al. J Neurosci Res* 2007; **85**:153-165.
8 Sun L, Zhao L, Yang G, *et al. Proc Natl Aca Sci USA* 2015; **112**:6003-6008.
9 Watanabe N, Takagi S, Tominaga A, *et al. J Biol Chem* 2010; **285**:19738-19746.
10 De Strooper B. *Cell* 2014; **159**:721-726.