Target of γ -secretase modulators, presenilin marks the spot

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The EMBO Journal (2011) 30, 4696–4698. doi:10.1038/emboj.2011.410

γ -Secretase modulators, or GSMs, which reduce the production of A β 42, have emerged as a promising class of compounds for the treatment of Alzheimer's disease (AD). The target and mechanism of action of GSMs have been controversial as of late, with some evidence suggesting that GSMs function by binding the substrate, amyloid precursor protein (APP), and others claiming action through direct modulation of the enzyme, γ -secretase. In this issue of *The EMBO Journal*, Ohki *et al* (2011) show that piperidine acetic acid-based GSMs directly bind to presenilin, the catalytic subunit of γ -secretase; and GSM binding induces conformational changes in γ -secretase, leading to a shift in the cleavage site specificity of APP from longer to shorter peptide fragments.

Amyloid β peptides are the predominant species found in the amyloid plaques associated with AD. Upon γ -secretase cleavage within the transmembrane region of the APP, A β peptides of varying length are released. Several studies have shown that the longer and more hydrophobic A β peptides, such as A β 42, are more prone to aggregate and are likely the major toxic species. Moreover, it appears that it is the ratio of A β 42 to A β 40, rather than total A β levels, which is the key determinant for disease progression (Karran *et al*, 2011). Consequently, γ -secretase has become an appealing drug target for the reduction of A β production. However, γ -secretase is thought to process a plethora of substrates in addition to APP—most notably, Notch receptor proteins. Therefore, the importance of selective γ -secretase inhibition has become increasingly obvious in recent years, and was demonstrated by the failure during phase III clinical trials of Eli-Lilly's Semagacestat, a non-selective γ -secretase inhibitor (GSI). Semagacestat treatment resulted in slightly worse cognition scores and an increase in the risk of skin cancer, likely due to notch-related toxicities. This failure forced the field to question the validity of γ -secretase as a target for AD therapy, and current pharmaceutical strategies strive towards the abrogation of any notch-related toxicities. Considerable efforts have been made towards the advancement of notch-sparing GSIs such as BMS-708163, which is currently in clinical trials.

The discovery that some non-steroidal anti-inflammatory drugs (NSAIDs), for example, ibuprofen and sulindac sulphide, could selectively inhibit the production of A β 42 while favouring A β 38 production and leaving A β 40 and notch cleavage virtually intact, was very promising (Weggen *et al*,

2001). These molecules seem to alter the cleavage site specificity within the APP substrate rather than simply being selective inhibitors. However, these compounds have very weak potency for A β 42 cleavage, greatly limiting their use as AD therapeutics. Initial studies using a biotinylated photoaffinity probe derived from flurbiprofen suggested that the NSAID GSM incurred selectivity by binding to the APP substrate, rather than the enzyme (Kukar *et al*, 2008). This finding was unexpected and exciting; however, this has since been disputed as non-selective binding (Barrett *et al*, 2011) and others have claimed that NSAID GSMs instead bind to an

Figure 1 Pharmacological regulation of γ -secretase. Proteolytic activity is abrogated for all substrates upon γ -secretase inhibitor (GSI) binding (top panel). Upon γ -secretase modulator (GSM) binding to PS-NTF (bottom panel), a conformational change occurs in presenilin and the cleavage site preference is switched from A β 42 to A β 38. The red symbol represents a decrease in production, the green + sign signifies an increase in production, and the grey equal sign denotes no or little change in substrate cleavage. The D letters represent the two catalytic aspartates.

allosteric subsite within the γ -secretase complex (Behr *et al*, 2004). More recently, another study suggested that ibuprofen binds to an allosteric site within the enzyme, but that substrate docking is first required (Uemura *et al*, 2010).

Meanwhile, pharmaceutical companies continued the search for more potent GSMs and two structurally distinct series of second-generation GSMs have emerged: (1) the acetic acid GSMs derived from NSAIDs, as exemplified by GSM-1, and (2) the imidazole-based GSMs. Recently, it was shown that a second-generation imidazole GSM could pull down both PS1-NTF and Pen2 (Kounnas *et al*, 2010). However, until now, the target by which GSMs modulate γ -secretase was elusive and controversial.

In this issue of *EMBO*, Iwatsubo, Tomita and colleagues show quite convincingly that their biotinylated photoactivatable GSM-1 probe, termed GSM-1-BpB, labels the TMD1 region of PS1-NTF (Ohki *et al*, 2011). Importantly, they are able to specifically label PS1-NTF from mouse brain membranes. Moreover, they were unable to show specific labelling of any other component of γ -secretase or endogenous APP. They also show that GSM-1-BpB labelling is not affected by pre-incubation with the active site-directed GSI L685,458, allosteric GSI Compound E, substrate mimic Peptide11, or by overexpression of APP substrate C99. Additionally, they show that GSM-1-BpB is able to bind the zymogen form of PS1 as evidenced by labelling of full-length PS1 and the inactive D385A mutant, which is distinct from L685,458 derived probes (Li *et al*, 2000).

The null effect of Peptide 11 or C99 expression suggests that GSM-1 binding could be independent of substrate binding. Further corroborating this theory is the fact that GSM-1 photoprobes also specifically labelled signal peptide peptidase (SPP), an altogether unrelated aspartyl intramembrane protease with active site homology, but no known common substrates. These claims are further supported by a recent and similar study done in our laboratory, which used clickable photoaffinity-based GSM-1 probes to not only label PS1-NTF in membranes, but also label PS1- Δ E9 proteoliposomes in the absence of substrates (Crump *et al*, 2011).

By combining a series of labelling and mutagenesis experiments, Ohki *et al* (2011) were able to relegate the GSM-1-BpB binding site to the C-terminal region of TMD1, and found that GSM binding altered the accessibility of the N-terminal cytosolic region of TMD1 upon binding. Furthermore, our group applied a multi-active site-based photoaffinity probe approach and demonstrated that GSM-1 binding could dose dependently enhance the labelling of a probe that targets the S1 subsite, illustrating a conformational change to this region of the active site of γ -secretase (Crump *et al*, 2011). Taken

together, this suggests a unique binding site for GSMs, which induces conformational changes in PS1 and allows for a shift in the cleavage preference of γ -secretase (see Figure 1).

Recently, Ebke *et al* (2011) have found that probes derived from the imidazole GSM RO-57, specifically labelled both PS1-NTF and PS2-NTF, but none of the other γ -secretase components or endogenous APP, which further supports that the second-generation GSMs target presenilin within the γ -secretase complex. Therefore, three groups (Crump *et al*, 2011; Ebke *et al*, 2011; Ohki *et al*, 2011) have taken advantage of the increased potency of second-generation GSMs and designed crosslinking probes that have independently identified PS1-NTF as a specific target of these GSMs. In addition, it has been shown through a series of competition studies that GSMs have distinct binding sites from allosteric GSIs.

While it is clear that both the piperidine acetic acid-based GSMs, demonstrated by GSM-1, and the imidazole GSM RO-57 bind to the N-terminal fragment of presenilin, a few outstanding questions remain. (1) Both Ohki *et al* and Crump *et al* show specific labelling of SPP by GSM-1 photoprobes, and Ohki *et al* show that GSM-1 has no effect on the cleavage of a synthetic SPP substrate. Nevertheless, if GSMs are to be used chronically to treat AD patients, it will be essential to know what potential side effects could result from SPP binding. Furthermore, do GSMs target additional proteins that could lead to off-target activity? (2) How similar are the mechanisms of NSAID GSMs to second-generation GSMs? Do the structurally distinct piperidine acetic acid GSMs work by a common mechanism as the imidazole GSMs? And what are the precise binding sites within PS1-NTF? (3) Lastly, and most intriguingly, how does a conformational change in presenilin upon GSM binding modify the enzyme's preference for one cleavage site over another? However, this last question will likely require high-resolution structural information before it can be resolved.

Identifying presenilin as the true target for second-generation GSMs revalidates γ -secretase as a viable target for AD therapy. This study shows that the enzyme γ -secretase itself can be modulated, rather than inhibited, and in such a way that can lower the ratio of A β 42/40, which is thought to be so integral in AD onset and progression. Simultaneously, GSMs have less of an effect on Notch or other substrates, presumably resulting in fewer adverse side effects. Discovery of potent and safe GSMs is a key step towards the development of a γ -secretase-based therapy for AD.

Conflict of interest

The authors declare that they have no conflict of interest.

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