ISSN 1672-9145 Acta Biochimica et Biophysica Sinica 2004, **36**(12): 787–792 **CN** 31-1940/Q

Minireview

Structural Features of Human Memapsin 2 (β-Secretase) and Their Biological and Pathological Implications

Lin HONG, Xiangyuan HE, Xiangping HUANG, Wanpin CHANG, and Jordan TANG*

Protein Studies Program, Oklahoma Medical Research Foundation, University of Oklahoma Health Science Center, Oklahoma City, Oklahoma 73104, USA

Abstract Memapsin 2 (β-secretase) is the membrane-anchored aspartic protease that initiates the cleavage of β-amyloid precursor protein (APP) leading to the production of amyloid-β (Aβ), a major factor in the pathogenesis of Alzheimer's disease (AD). Since memapsin 2 is a major target for the development of inhibitor drugs for AD, it has been intensively studied during the past five years. Here we discuss the structural features of the catalytic/specificity apparatus, transmembrane domain, cytosolic domain and the implications of these features in the physiological and pathological roles of this protease.

Key words memapsin 2; β-secretase; Alzheimer's disease; β-amyloid; β-amyloid precursor protein; aspartic protease; endocytosis; cellular trafficking.

Alzheimer's disease (AD), which is characterized by the progressive destruction of brain functions in older people, was first recognized in the early 20th century. Since then, modern medicine has further increased the number of people living to old age. AD has become the third leading cause of death for adults in the United States with the number of patients estimated to increase from 4. 5 million currently to about 12 million in 2025. There is no effective treatment for this disease at present.

Scientific investigation during the last two decades has pointed to the leading role of brain amyloid-β (Aβ) in the pathogenesis of Alzheimer's disease [1]. Aβ is a 40- or 42-residue peptide generated from the degradation of β-amyloid precursor protein (APP), a membrane protein, by two proteases known as β-secretase and γ-secretase [Fig. 1(A & B)]. In this pathway, β-secretase initiates the APP cleavage which is then followed by γ-secretase, a multiprotein complex, to produce Aβ. The excess level of the neurotoxic Aβ in the brain over a long time leads to the death of neurons, brain inflammation and other harmful events that mark the progression of AD. Either secretase is an obvious target for the development of inhibitor drugs for the treatment of AD. However, γ-secretase is known to participate in many important physiological functions, such as 'Notch' recognition in cell differentiation, which accounts for the toxicity observed in the clinical trials of γ-secretase inhibitors.

β-secretase was independently cloned and identified about five years ago in five laboratories [2–6] and was named BACE, ASP-2 or memapsin 2. We will use the name "memapsin 2" throughout this article since it conforms with IUB guideline for enzyme nomenclature [7]. Mature memapsin 2 is a Type I membrane protein that has three distinct domains [Fig. 1(C)]. The N-terminal ectodomain, or catalytic domain, is essentially an aspartic protease with sequence and conformation homologous to other mammalian aspartic proteases such as pepsin [8]. In the middle is a 26-residue transmembrane domain and at the C-terminus is a 21-residue cytosolic domain. The protease is synthesized as promemapsin 2 which is processed by furin to remove the pro peptide during transit through the secretory pathway. Memapsin 2 has become a major target for drug design owing not only to its key role in the production of Aβ but also that it is an aspartic protease for which inhibitor drugs were successfully developed in the case of the protease of human immunodeficiency virus. There is therefore much current interest in the structure-function relationships of memapsin 2 for rational design of inhibitor drugs and also for the understanding of the physiological and pathological functions of this protease.

In this article, we review the structural features of

Received: October 13, 2004 Accepted: November 8, 2004

This work was supported by a grant from NIH (No. AG-18933) and an Alzheimer's Association Pioneer Award to Prof. Jordan TANG

*Corresponding author: Tel, +1-405-271-7291; Fax, +1-405-271-7249; E-mail, jordan-tang@omrf.ouhsc.edu

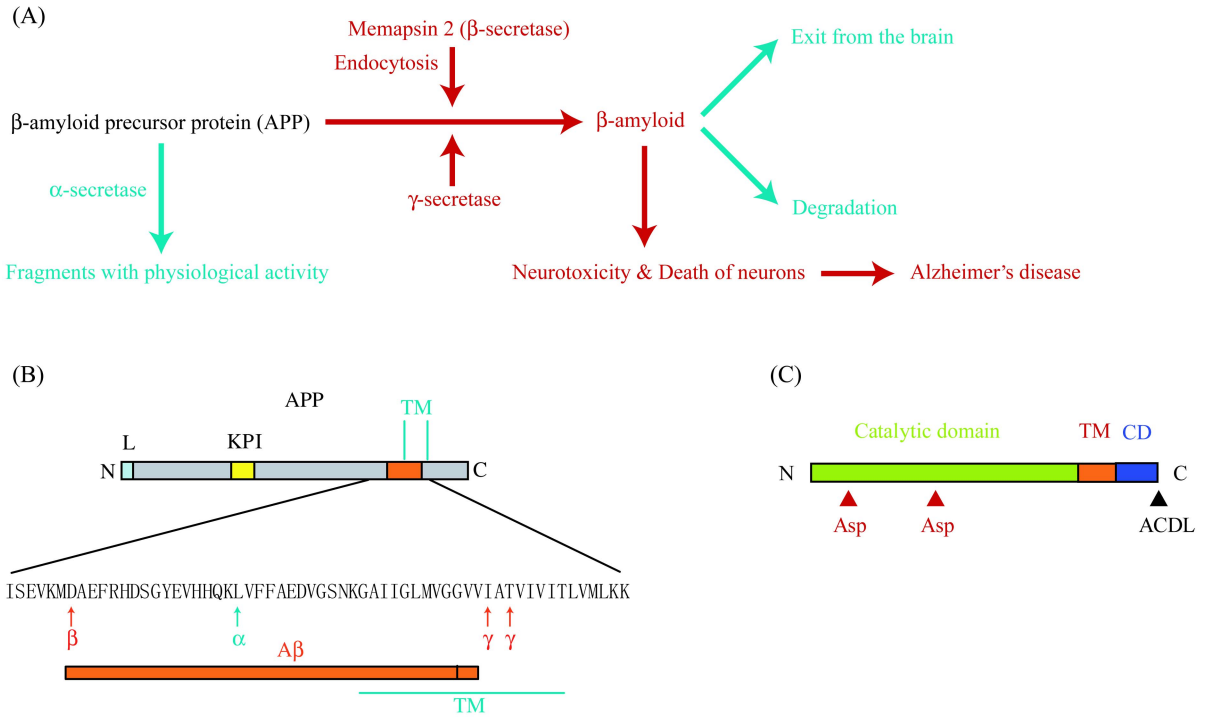

Fig. 1 Memapsin 2 in the pathogenesis of Alzheimer's disease

(A) Schematic presentation of the role of memapsin 2 (β-secretase) in the progression of Alzheimer's disease (AD). In the pathway to AD (red), β-amyloid precursor protein (APP) is proteolytically processed by memapsin 2 and γ-secretase to produce β-amyloid. An excess of Aβ in the brain ultimately leads to AD. The competing pathways that reduce Aβ are marked in blue. (B) Schematic presentation of the processing sites of APP by memapsin 2 (β), γ-secretase (γ) and α-secretase (α). Sequence of the processed region is shown below. (C) A diagram of the structural domains in memapsin 2. The catalytic, transmembrane and cytosolic domains are shown. The red triangles mark the positions of active-site aspartic acids and the black triangle marks the position of ACDL motif. TM, transmembrane domain; CD, cytosolic domain; KPI, Kunitz protease inhibitor domain; L, leader sequence; N, N-terminus; C, C-terminus.

human memapsin 2 and their functional implications.

Catalytic and Specificity Apparatus

In the crystal structure [8–11], memapsin 2 contains many features found in other aspartic proteases. These include the conformation of the catalytic aspartic residues, the location of the active-site cleft between the N- and C-terminal lobes, and the presence of a flap (residues 65–79) that covers the cleft [Fig. 2(A)]. However, the detailed structural determinants that render the substrate and inhibitor specificity are quite different from those in other proteases.

The active-site cleft of memapsin 2 accommodates eleven substrate residues (subsites), instead of eight substrate residues usually seen in other aspartic proteases. Subsites on the N-terminal side of the substrate, S_1 to S_4, and on the C-terminal side, S_1' to S_4', are distributed in a way similar to those of other proteases. Additional subsites, S_5 to S_7, which are found on the N-terminal side of the substrate [Fig. 2(B)], are located in part of the memapsin 2 that contains helix A and loops D and F [8], which are unique among aspartic proteases. Crystal structures of memapsin 2 bound to transition-state inhibitors defined the locations of these eleven subsites. Basically, the inhibitors, and presumably the substrates, are bound to the active-site cleft in an extended conformation with extensive interactions not only to the peptide backbone of the inhibitor/substrate, but also to each side chain in the subsite pockets [Fig. 2(C)]. The latter interactions, seen in crystal structures, agree mostly with the experimentally determined substrate/inhibitor specificity [11,12]. Together, these results have permitted design of potent inhibitors with K_i values in the sub-nM range [11–14].

Recent study of crystal structure of free memapsin 2 [10] revealed that the flap had moved significantly to provide access to the active-site cleft [Fig. 2(A)]. The flap movement was accomplished by the formation of new hydrogen bonds in a three-strand β-sheet including the

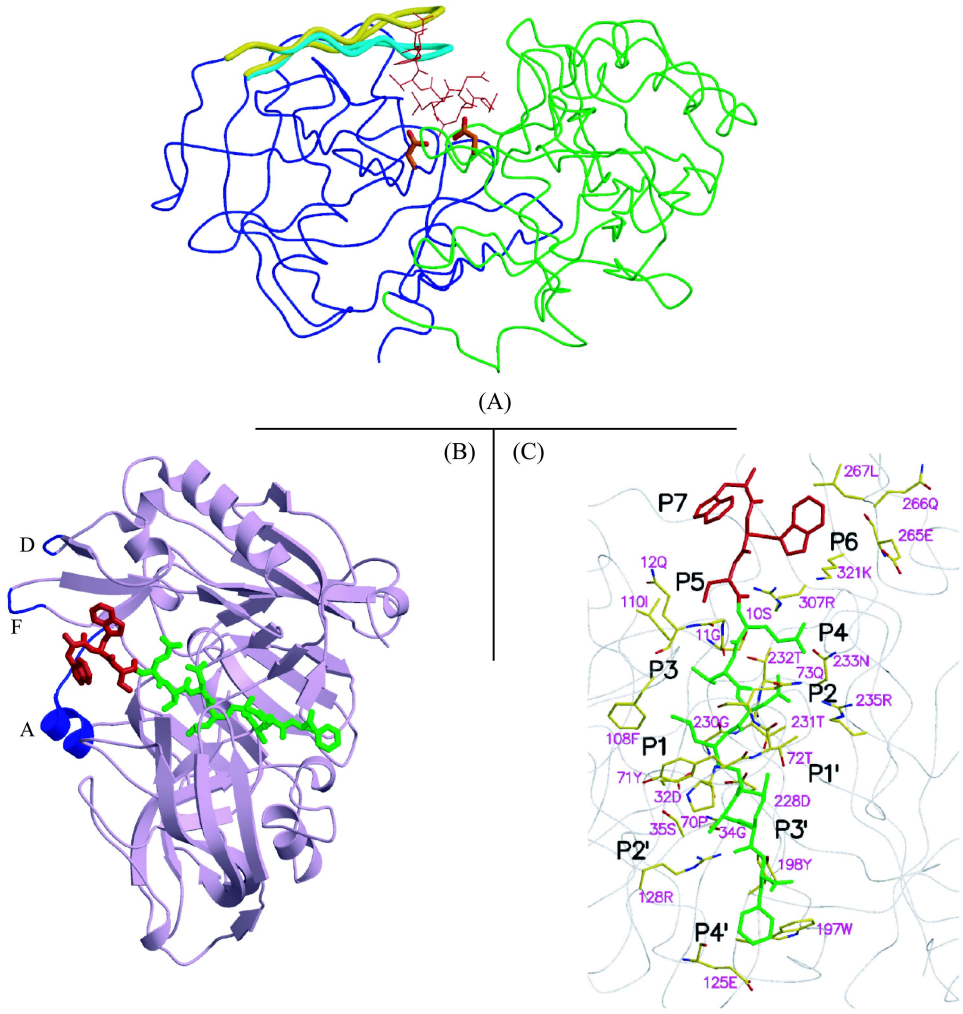

Fig. 2 Structures of memapsin 2 catalytic domain and its interactions with ligands

(A) Ribbon model of memapsin 2 three-dimensional structure and its catalytic apparatus. The active-site cleft, which is located between the N-terminal (green) and C-terminal (blue) lobes, is occupied by an eight-residue inhibitor (fine red sticks), OM00-3. The view into the elongated cleft is from the N-terminal end of the inhibitor. Two catalytic aspartic acids are shown in thick red sticks. The flap that covers over the cleft is shown in the closed (light blue) and open (gold) conformations. (B) Side view of memapsin 2 active-site cleft with part of an inhibitor that occupies eleven subsites. Eight inhibitor residues, from P_4 to P_4' (green), are common in other aspartic porteases. Three unique memapsin 2 subsites that bind residues P_5, P_6 and P_7 (red) are located near the structural regions (helix A and loops D & F, blue) that are unique for memapsin 2. (C) Interactions of memapsin 2 residues (gold with residue numbers in magenta) with inhibitor residues described in B above. The inhibitor subsites are mark in black.

flap itself, rendering the flap opening conformation energetically stable. When a substrate or an inhibitor is bound to the protease, the interactions between the protease, including the flap, and the ligand drive the flap conformation into a closed position. The opening to the cleft in free memapsin 2 is surprisingly narrow. The movement of substrates or inhibitors into the cleft would require conformational reorientation of several protein residues located at the narrowest point of the opening [Fig. 2(A)]. The 'narrowness' of the opening appears to have selective functions for memapsin 2 substrates. This is particularly true for subsite S_1', which prefers a much smaller side chain, such as alanine, than would have been predicted from the crystal structure of the S_1' pocket. The restricted access to the active-site cleft produces yet another factor to be considered in the design of memapsin 2 inhibitors.

Trans-membrane Domain

The hinge region between the transmembrane domain and the last disulfide bond in the catalytic domain contains

only six residues [8], suggesting that the catalytic domain is situated close to the membrane surface. Considering that the catalytic domain has two oligosaccharide units [15], the 'short leash' may function to limit the motion and orientation of the catalytic domain. The single-strand transmembrane domain of memapsin 2 is palmitoylated at three cysteine residues [16]. This type of modification is consistent with the observation that memapsin 2 is localized in the 'lipid raft' part of the plasma membrane [17,18]. The 'lipid raft' localization of memapsin 2 is apparently important for its function in APP hydrolysis. First, the 'lipid raft', where APP is also localized, is the membrane subdomain that facilitates the endocytosis of both proteins into endosomes where APP is hydrolyzed by memapsin 2. Second, many 'lipid raft' residents, such as glycosylphosphatidylinositol-anchored proteins [17,19], heparan sulfate [20] and cholesterol [18], have influence on the APP hydrolysis catalyzed by memapsin 2. It is not yet clear how these membrane components exert their influence and whether all of them directly interact with the transmembrane domain of memapsin 2. It seems likely, however, that the transmembrane domain plays a significant role in membrane subdomain localization and in the mechanism of memapsin 2 endocytosis.

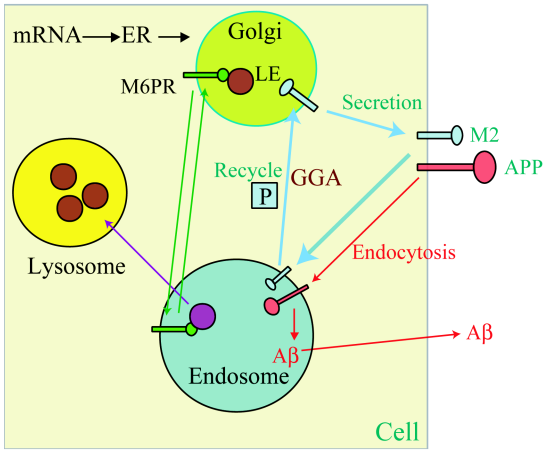

Fig. 3 Schematic presentation of cellular transport of memapsin 2 and APP in the production of Aβ

Memapsin 2 is synthesized in cells as promemapsin 2 which is processed to memapsin 2 (M2) during transit through the secretory pathway. Both memapsin 2 and APP are endocytosed from cell surface into endosomes, where the acidic condition supports the hydrolysis of APP by memapsin 2. Memapsin 2 is then recycled through Golgi back to the cell surface. The endosome to Golgi transport of memapsin 2 is mediated by cytosolic GGA interaction with phosphorylated (marked P) memapsin 2 cytosolic domain. The pathways for memapsin 2 endocytosis and recycle are marked in light blue. The generation of Aβ from APP is marked in red. Memapsin 2 transport and recycling system are similar to those for mannose-6-phosphate receptors (M6PR, green) that are involved in the transport of lysosomal enzymes (purple) from Golgi to endosomes.

Cytosolic Domain and Intracellular Transport

Like other Type I membrane proteins, the cytosolic domain of memapsin 2 contains signals that provide destinations for the cellular transport of this protease, which is important in the production of Aβ. Memapsin 2 and APP, which reach the cell surface via the secretory pathway, are endocytosed into endosomes where APP is cleaved by memapsin 2. The protease then is recycled back to the cell surface (Fig. 3). From mutagenesis experiments, a dileucine motif was shown to be needed for the endocytosis of memapsin 2 [21,22]. More recently, a motif with the sequence DISLL in the cytosolic domain of memapsin 2 was shown to mediate its cellular trafficking [23,24]. This motif belongs to the acidic-cluster-dileucine (ACDL) type of sorting signals (DXXLL, where X denotes a nonconserved residue [25]) that binds to the VHS (Vps-27, Hrs, and STAM) domains of GGA (Golgi-localized γ-ear-containing ARF binding) proteins. GGA proteins are known to bind to the ACDL sorting signal in the cytosolic region of mannose-6-phosphate receptors (MPRs) [26, 27] and other lysosomal membrane proteins [28] as the initial sorting recognition in the transport of these proteins

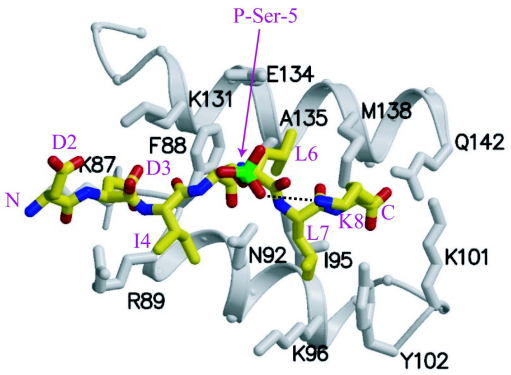

Fig. 4 Crystal structure of a memapsin 2 cytosolic peptide (gold) binding to the VHS domain (grey) of GGA1

The peptide shown here has the sequence of DDISLLK which contains the ACDL motif. The phosphorylated serine interacts with lysine in a hydrogen bond (dotted line). These interactions provide the recognition of the cytosolic domain of memapsin 2 by GGA proteins in the recycling pathway of memapsin 2 from endosomes to Golgi.

from trans-Golgi to endosomes. However, memapsin 2 is not known to transport from trans-Golgi to endosomes. GGA proteins do not appear to participate in memapsin 2 endocytosis because they are not found in significant

amount on the plasma membrane. Also, the weak binding of the VHS domain of GGA proteins to the native ACDL signal appears to be below the threshold required for cellular function [24]. The phosphorylated ACDL (at serine 498 within the DISLL sequence) of memapsin 2, however, binds to GGA proteins at an affinity similar to the interaction of MPR's with GGA proteins [28]. The depletion of GGA biosynthesis with siRNA also resulted in the accumulation of memapsin 2 in endosomes (data not shown). These results suggest that phosphorylated ACDL to GGA interaction mediates the recycling of memapsin 2 from endosomes back to the cell surface. This is in good agreement with the previous observation that the phosphorylation of memapsin 2 is required in the recycling pathway [29]. The increase of GGA interacting affinity by means of the phosphorylation of memapsin 2 ACDL is apparently an entropic effect derived from the change of the peptide conformation upon phosphorylation to permit a favorable interaction with VSH/GGA (Fig. 4) [24].

It is not clear at present how the dileucine motif in the cytosolic domain is involved in the endocytosis of memapsin 2. The ectodomain of memapsin 2 devoid of both the transmembrane and cytosolic domain has been found to be internalized by cells and the process is dependent on the endocytic signal in the cytosolic domain of APP [19]. The probable mechanism here appears to be the interaction between the ectodomain of APP and that of memapsin 2. This is supported by the co-immunoprecipitation of the full-length memapsin 2 and APP from cell lysates [19]. These observations support the idea that the endocytosis of memapsin 2 is mediated by multiple mechanisms, including APP mediated internalization and a dileucine mediated endocytosis. How these different processes are regulated is unclear at present. The presence of redundant internalization mechanisms is perhaps not surprising since memapsin 2 is likely involved in the processing of membrane proteins other than APP, so there is a need for close regulation of these processes.

Perspectives

Many structural features that determine the specificity and mechanism have been identified from the crystal structure of memapsin 2 catalytic domain and its interactions with ligands. Information derived from these studies has formed the basis for the rational design of inhibitor drugs to treat Alzheimer's disease. The structural studies have also created some new questions. For example, why does memapsin 2 have the unique subsites S_5 to S_7, while the *in vitro* substrates without binding to these subsites appear to be quite efficient? Is it possible to utilize these new subsites for the design of new inhibitor drugs? Also, what is the advantage of the narrow opening to the active-site cleft created by flap movement? Is there a substrate-induced further opening of the cleft? These and other intriguing questions are important for a full understanding of the cellular functions of memapsin 2 and would require future studies to answer.

Although the structural features of the transmembrane strand in 'lipid raft' localization and the cytosolic domain in mediating intracellular trafficking are becoming clear, the regulations of these events are poorly understood. Recent evidence suggests that memapsin 2 activity is regulated by the neuronal activity and synaptic functions [30]. In this scheme, neuronal activities would relate directly to the activity of memapsin 2 in the cleavage of APP to produce Aβ, which in turn suppresses synaptic transmission as a feedback inhibition of synaptic activity. These results suggest that memapsin 2 activity is finely regulated *in vivo*. It is yet unclear how memapsin 2 activity is regulated in terms of the structure-function relationships discussed above. These questions are not only scientifically intriguing but also have impact on the understanding of the limitations in the memapsin 2 targeted drug therapy.

References

1 Selkoe DJ, Schenk D. Alzheimer's disease: Molecular understanding predicts amyloid-based therapeutics. Annu Rev Pharmacol Toxicol, 2003, 43: 545–584

2 Lin X, Koelsch G, Wu S, Downs D, Dashti A, Tang J. Human aspartic protease memapsin 2 cleaves the β-secretase site of β-amyloid precursor protein. Proc Natl Acad Sci USA, 2000, 97(4): 1456–1460

3 Vassar R, Bennett BD, Babu-Khan S, Kahn S, Mendiaz EA, Denis P, Teplow DB *et al*. β-Secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. Science, 1999, 286 (5440): 735–741

4 Yan R, Bienkowski MJ, Shuck ME, Miao H, Tory MC, Pauley AM, Brashier JR *et al*. Membrane-anchored aspartyl protease with Alzheimer's disease β-secretase activity. Nature, 1999, 402(6761): 533–537

5 Hussain I, Powell D, Howlett DR, Tew DG, Meek TD, Chapman C, Gloger IS *et al*. Identification of a novel aspartic protease (Asp 2) as beta-secretase. Mol Cell Neurosci, 1999, 14(6): 419–427

6 Sinha S, Anderson JP, Barbour R, Basi GS, Caccavello R, Davis D, Doan M *et al*. Purification and cloning of amyloid precursor protein beta-secretase from human brain. Nature, 1999, 402(6761): 537–540

7 Nomenclature of multiple forms of enzymes. Recommendations (1976) IUPAC-IUB Commission on Biochemical Nomenclature (CBN). J Biol Chem, 1977, 252(17): 5939–5941

8 Hong L, Koelsch G, Lin X, Wu S, Terzyan S, Ghosh AK, Zhang XC *et al*. Structure of the protease domain of memapsin 2 (β-secretase) complexed with inhibitor. Science, 2000, 290(5489): 150–153

9 Hong L, Turner RT 3rd, Koelsch G, Shin D, Ghosh AK, Tang J. Crystal structure of memapsin 2 (β-secretase) in complex with an inhibitor OM00-3. Biochemistry, 2002, 41(36): 10963–10967
10 Hong L, Tang J. Flap position of free memapsin 2 (β-secretase), a model for flap opening in aspartic protease catalysis. Biochemistry, 2004, 43(16): 4689–4695
11 Turner RT, Hong L, Koelsch G, Ghosh AK, Tang J. Structural locations and functional roles of new subsites S5, S6 and S7 in memapsin 2 (β-secretase). Biochemistry, 2004, in press
12 Turner RT 3rd, Koelsch G, Hong L, Castanheira P, Ermolieff J, Ghosh AK, Tang J *et al*. Subsite specificity of memapsin 2 (β-secretase): Implications for inhibitor design. Biochemistry, 2001, 40(34): 10001–10006
13 Ghosh AK, Bilcer G, Harwood C, Kawahama R, Shin D, Hussain KA, Hong L *et al*. Structure-based design: potent inhibitors of human brain memapsin 2 (β-secretase). J Med Chem, 2001, 44(18): 2865–2868
14 Ghosh AK, Hong L, Tang J. Beta-secretase as a therapeutic target for inhibitor drugs. Curr Med Chem, 2002, 9(11): 1135–1144
15 Charlwood J, Dingwall C, Matico R, Hussain I, Johanson K, Moore S, Powell DJ *et al*. Characterization of the glycosylation profiles of Alzheimer's β-secretase protein Asp-2 expressed in a variety of cell lines. J Biol Chem, 2001, 276(20): 16739–16748
16 Benjannet S, Elagoz A, Wickham L, Mamarbachi M, Munzer JS, Basak A, Lazure C *et al*. Post-translational processing of beta-secretase (β-amyloid-converting enzyme) and its ectodomain shedding. The pro- and transmembrane/cytosolic domains affect its cellular activity and amyloid-beta production. J Biol Chem, 2001, 276(14): 10879–10887
17 Tun H, Marlow L, Pinnix I, Kinsey R, Sambamurti K. Lipid rafts play an important role in A beta biogenesis by regulating the beta-secretase pathway. J Mol Neurosci, 2002, 19(1-2): 31–35
18 Ehehalt R, Keller P, Haass C, Thiele C, Simons K. Amyloidogenic processing of the Alzheimer β-amyloid precursor protein depends on lipid rafts. J Cell Biol, 2003, 160(1): 113–123
19 Huang XP, Chang WP, Koelsch G, Turner RT 3rd, Lupu F, Tang J. Internalization of exogenously added memapsin 2 (β-secretase) ectodomain by cells IS mediated by amyloid precursor protein. J Biol Chem, 2004, 279(36): 37886–37894
20 Scholefield Z, Yates EA, Wayne G, Amour A, McDowell W, Turnbull JE. Heparan sulfate regulates amyloid precursor protein processing by BACE1, the Alzheimer's β-secretase. J Cell Biol, 2003, 163(1): 97–107
21 Huse JT, Pijak DS, Leslie GJ, Lee VM, Doms RW. Maturation and endosomal targeting of β-site amyloid precursor protein-cleaving enzyme. The Alzheimer's disease β-secretase. J Biol Chem, 2000, 275(43): 33729–33737
22 Pastorino L, Ikin AF, Nairn AC, Pursnani A, Buxbaum JD. The carboxyl-terminus of BACE contains a sorting signal that regulates BACE trafficking but not the formation of total Aβ. Mol Cell Neurosci, 2002, 19(2): 175–185
23 He X, Chang WP, Koelsch G, Tang J. Memapsin 2 (β-secretase) cytosolic domain binds to the VHS domains of GGA1 and GGA2: Implications on the endocytosis mechanism of memapsin 2. FEBS Lett, 2002, 524(1-3): 183–187
24 He X, Zhu G, Koelsch G, Rodgers KK, Zhang XC, Tang J. Biochemical and structural characterization of the interaction of memapsin 2 (β-secretase) cytosolic domain with the VHS domain of GGA proteins. Biochemistry, 2003, 42(42): 12174–12180
25 Bonifacino JS. The GGA proteins: Adaptors on the move. Nat Rev Mol Cell Biol, 2004, 5(1): 23–32
26 Puertollano R, Aguilar RC, Gorshkova I, Crouch RJ, Bonifacino JS. Sorting of mannose 6-phosphate receptors mediated by the GGAs. Science, 2001, 292(5522): 1712–1716
27 Zhu Y, Doray B, Poussu A, Lehto VP, Kornfeld S. Binding of GGA2 to the lysosomal enzyme sorting motif of the mannose 6-phosphate receptor. Science, 2001, 292(5522): 1716–1718
28 Takatsu H, Katoh Y, Shiba Y, Nakayama K. Golgi-localizing, γ-adaptin ear homology domain, ADP-ribosylation factor-binding (GGA) proteins interact with acidic dileucine sequences within the cytoplasmic domains of sorting receptors through their Vps27p/Hrs/STAM (VHS) domains. J Biol Chem, 2001, 276(30): 28541–28545
29 Walter J, Fluhrer R, Hartung B, Willem M, Kaether C, Capell A, Lammich S *et al*. Phosphorylation regulates intracellular trafficking of β-secretase. J Biol Chem, 2001, 276(18): 14634–14641
30 Kamenetz F, Tomita T, Hsieh H, Seabrook G, Borchelt D, Iwatsubo T, Sisodia S *et al*. APP processing and synaptic function. Neuron, 2003, 37(6): 925–937

Edited by
You-Shang ZHANG