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Review Article

Inhibition of BACE1 for therapeutic use in Alzheimer's disease

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Abstract: Since BACE1 was reported as the β-secretase in Alzheimer's disease (AD) over ten years ago, encouraging progress has been made toward understanding the cellular functions of BACE1. Genetic studies have further confirmed that BACE1 is essential for processing amyloid precursor protein (APP) at the β-secretase site. Only after this cleavage can the membrane-bound APP C-terminal fragment be subsequently cleaved by γ-secretase to release so-called AD-causing Aβ peptides. Hence, in the past decade, a wide variety of BACE1 inhibitors have been developed for AD therapy. This review will summarize the major historical events during the evolution of BACE1 inhibitors designed through different strategies of drug discovery. Although BACE1 inhibitors are expected to be safe in general, careful titration of drug dosage to avoid undesirable side effects in BACE1-directed AD therapy is also emphasized.

Keywords: Alzheimer's disease, amyloid plaques, BACE1, aspartyl protease, drug discovery

Introduction

AD, a progressive neurodegenerative disorder, is the most common form of dementia that affects 4-8% of the elderly population worldwide. The neuropathological features of AD are the presence of extracellular amyloid plaques and intracellular neurofibrillary tangles (NFT) in the hippocampus and cortical grey matter of the AD brain. The amyloid plaques, also called senile plaques, result from the deposition of aggregated Aβ peptides [1, 2]. Although the naturally produced forty amino acid $A\beta_{40}$ is the predominant product (~90% abundance), the slightly longer product $A\beta_{42}$ tends to aggregate faster and is the major form of Aβ peptides that are linked to AD pathogenesis [3, 4]. This notion is further supported by the finding that most familial AD (FAD) mutations lead to overproduction of $A\beta_{42}$ years before AD symptoms appear [5]. Formation of NTF is via a totally different route, and NTF are composed of hyperphosporylated microtubule binding protein tau [6, 7]. The occurrence of NTF is likely a downstream event to amyloid deposition [8], an observation that further highlights the importance of reducing brain levels of Aβ peptides, especially of $A\beta_{42}$, as a promising strategy for AD therapy. β-secretase acts on the rate-limiting first step in Aβ generation and has therefore been a focus of intense research as a prime drug target. The gene encoding β-secretase was discovered a decade ago, and is now more commonly known as BACE1 for Beta-site APP-Cleaving Enzyme 1, Asp2 or memapsin 2 [9-13]. The most exciting finding is from the studies using BACE1-null mice, which demonstrate that genetic deletion of BACE1 abolishes the production of Aβ [14-16]. Hence, inhibition of BACE1 activity could reduce the production of all forms of Aβ, thereby diminishing plaque formation.

Structure and cellular trafficking of BACE1

To inhibit BACE1 effectively, knowledge of the structure and function of BACE1 is essential. BACE1 is one of the only two known aspartyl proteases that are localized on the cell membrane due to the presence of a membrane-anchoring domain. Although BACE1 and BACE2 share 59% homology and adopt a similar structural organization, BACE2 preferentially cleaves APP within the Aβ region between F19 and F20 and between F20 and A21 [17, 18]. Mice with

a deficiency in BACE2 are fertile and healthy [19], making cross inhibition of BACE2 by BACE1 inhibitors less of a concern.

Like other aspartyl proteases, BACE1 has a classical bilobal structure, with each of its two active aspartate motifs (D_{93}TG and D_{289}SG) located in a separate lobe, and mutation of either aspartate residue renders the enzyme inactive. BACE1 is first synthesized in the endoplasmic reticulum (ER) as a 501-aa immature precursor protein (proBACE1). Interestingly, proBACE1 exhibits considerable enzymatic activity, unlike other aspartic zymogens, because its prodomain fails to block the entrance of its substrate into the active pocket of BACE1 [20, 21]. This unique property of proBACE1 suggests that inhibition of its prodomain shedding would be ineffective in reducing APP processing and Aβ production in AD brains.

As a type I transmembrane protein, BACE1 becomes mature in the Golgi compartment by extensive posttranslational modifications which include glycosylation [22], the removal of the prodomain by furin-like proprotein convertases [23-26], reversible acetylation on seven Lys residues [27] and palmitoylation [25]. Even though BACE1 is transported to all secretory compartments after maturation, its transmembrane domain preferentially promotes the retention of this enzyme in the late Golgi and trans-Golgi network (TGN) compartments [28], where luminal BACE1 protease domain will encounter and cleave the type I transmembrane APP at the β-site.

BACE1 enzymatic activity is affected by the environmental pH, with its maximum proteolytic activity at ~pH 4.5 as shown by *in vitro* assays [29, 30]. Thus, BACE1 likely processes APP more efficiently in the relatively more acidic subcellular compartments (i.e., TGN and endosomes) than in the more neutral ER compartment [31-33]. Our recent studies suggest that increased expression of the BACE1-interacting protein reticulon 3 (RTN3) significantly increases retention of BACE1 in the ER, and this altered cellular trafficking can partially contribute to the reduced cleavage of APP by BACE1 [34, 35]. On the other hand, BACE1 trafficking to the endosomes is regulated via the phosphorylation state on Ser_{498} [36] or dileucine residues ($L_{499}L_{500}$) in its short cytoplasmic tail [36, 37]. Golgi-localized γ-ear containing ADP ribosylation factor-binding (GGA) family proteins appear to mediate the retrograde transport of BACE1 from endosomes to TGN [38-42]. Depletion of GGA proteins by RNAi or disruption of phosphorylation on Ser_{498} increases retention of BACE1 in endosomes, whose favorable acidic environment enhances BACE1 activity [43, 44]. Palmitoylation of BACE1 facilitates its localization in the lipid rafts [45, 46], where APP is also enriched and efficiently processed by BACE1 [47, 48].

The ultimate evidence supporting BACE1 as a superior AD drug target is the finding that BACE1 knock-out mice do not produce Aβ [14-16]. BACE1-null mice are fertile and exhibit relatively mild phenotypes such as hypomyelination [49, 50] and schizophrenia-like behaviors [51]. This is in sharp contrast to mice with deficiencies in components of the γ-secretase complex [52]. For example, mice deficient in presenilin-1 [53], nicastrin [54], or APH-1 [55] all exhibit serious developmental defects and die at early embryonic stages because γ-secretase is required to process Notch, a signaling molecule that controls cell fate in embryonic and adult myelopoiesis [56-58]. Therefore, compared with γ-secretase, BACE1 is viewed as a better drug target whose inhibition should cause less serious biological dysfunction.

BACE1 inhibitors as AD drug candidates

Developing potent BACE1 inhibitors in a hope to identify suitable AD drug candidates has been fiercely pursued for the past decade. Several strategies of drug discovery have been explored in the search for potent BACE1 inhibitors, *e.g.*, substrate-based design, high-throughput screening, and fragment-based lead generation approaches. In the following text, we will briefly review the history and current preclinical situations of BACE1 inhibitors being developed by these different approaches.

BACE1 Inhibitor Design by a Substrate-based Method

Substrate-based methods have often been used as the starting point for developing aspartyl protease inhibitors. The first substrate-based BACE1 inhibitor, P10–P4' StatVal (**Table 1, #1**), was developed by Elan Pharmaceuticals in order to purify β-secretase from human brain homogenates [11]. This peptidic inhibitor is a P1

Table 1. Substrate-based BACE1 Inhibitors

#	Inhibitor name	*in vitro* activity	Cellular activity	*in vivo* activity	Significance	Reference
1	P10–P4' StatVal	IC_{50} = ~30 nM	NA[a]	NA	First reported BACE1 inhibitor	Sinha et al., 1999 (Elan Pharmaceuticals)
2	0M99-2	K_i = 1.6 nM	NA	NA	The inhibitor used for BACE1 crystal structure determination	Hong et al., 2000 (Tang & Ghosh group)
3	KMI-429	IC_{50} = 3.9 nM	IC_{50}=42.8 nM in HEK 293 cells	2.5 nM intrahippocampal injection. Tg2576 mice, > 60% ↓ soluble Aβ; wild-type mice, > 30% ↓ soluble Aβ		Asai et al., 2006 (Kiso group)
4	5d	K_i = 1.1 nM	IC_{50}=39 nM in CHO cells	30% ↓ plasma Aβ after i.p. injected (8 mg/kg) into Tg2576 mice		Ghosh et al., 2007
5	24	K_i = 1.8 nM	IC_{50}=1 nM in CHO cells	65% ↓ plasma Aβ after i.p. injected (8 mg/kg) into Tg2576 mice		Ghosh et al., 2008
6	KMI-570	IC_{50} = 4.8 nM	NA	NA	Modified versions of KIMI-429 with predicted higher brain permeability	Kimura et al., 2006 (Kiso group)
7	KMI-684	IC_{50} = 1.2 nM	NA	NA		
8	GSK188909	IC_{50} = 5 nM	IC_{50}=5 nM in SHSY5Y-w.t. APP; IC_{50}=30 nM in SHSY5Y-APP Swedish mutation	>50% ↓ total Aβ of TASPTM mice (250 mg/kg oral)	First reported orally effective BACE1 inhibitor	Hussain et al., 2007 (GlaxoSmithKline)
9	compound 11	*Ki* = 0.7 nM	IC_{50}=21 nM in HEK-293 cells	CRND8 mice. Oral dose 10 mg/kg, 4% ↓ plasma Aβ40; 30 mg/kg, 25% ↓ plasma Aβ40; 100 mg/kg, 70% ↓ plasma Aβ40. Subcutaneous injection, 10 mg/kg, 58% ↓ plasma Aβ40; 30 mg/kg, 77% ↓ plasma Aβ40; 100 mg/kg, 88% ↓ plasma Aβ40.	Orally effective, good selectivity and pharmacokinetics	Iserloh et al., 2008 (Schering-Plough)
10	CTS-21166	NA	IC_{50}=1.2–3.6 nM (cell line details NA)	APP transgenic mice, 4mg/kg i.p. injection for 6 weeks, >35% ↓ brain Aβ, 40% ↓ plaque load. Human patients, 7.5–225 mg i.v. injection dose-dependant ↓ plasma Aβ, 80% ↓ at 225 mg	The only BACE1 passed Phase I clinical trial. Excellent pharmacokinetics, pharmacodynamics and oral availibility	http://www.alzforum.org/new/detail.asp?id=1790 (CoMentis)

[a] NA, not available

(S)-statine substituted substrate analogue with an *in vitro* half-maximal inhibitory concentration (IC_{50}) of ~30 nM. Shortly after the molecular cloning of memapsin 2 (BACE1), Tang (Oklahoma Medical Research Foudation) and Ghosh (University of Illinois at Chicago/Purdue University) teamed up and reported the octapeptidic, hydroxyethylene (HE) isotere-based transition-state analogue inhibitor OM99-2 (**Table 1, #2**) and the crystal structure of BACE1

with OM99-2 bound to its active site [59]. While OM99-2 showed excellent inhibitory potency *in vitro* (K_i = 1.6 nM), its bulky peptidic structure precluded its application *in vivo*. Nonetheless, the BACE1/OM99-2 co-crystal structure provided coveted molecular insight into the ligand binding interactions in the enzyme active site and significantly advanced the BACE1 inhibitor design by researchers around the globe. In parallel, Kiso's group (Kyoto Pharmaceutical University) developed their own octapeptidic BACE1 inhibitor KMI-008 (cellular IC_{50} = 413 nM) using a hydroxymethylcarbonyl (HMC) isostere as a transition-state mimic [60]. Further chemical modification of KMI-008 yielded more potent pentapeptidic BACE1 inhibitors KMI-420 (*in vitro* IC_{50} = 8.2 nM) and KMI-429 (*in vitro* IC_{50} = 3.9 nM) (**Table 1, #3**) [61]. KMI-429 appears to significantly reduce brain Aβ production when directly injected into the hippocampus of both wild-type mice and APP transgenic mice (Tg2576 mice) [62]. During this period of time, various substrate-based peptidomimetic inhibitors were also developed by large pharmaceutical companies and other academic research groups [63-65]. Although these peptidomimetic BACE1 inhibitors are highly potent *in vitro*, their inherent poor drug properties (i.e., large size, poor brain permeability, short half-life *in vivo,* and low oral availability) have made them unsuitable drug candidates. On the other hand, guided by a structure-based approach, these first-generation inhibitors have laid the foundation for the rational design of later generations of smaller, non-peptidic BACE1 inhibitors that have significantly improved drug properties.

The BACE1 inhibitors 5d and 24 (**Table 1, #4** and **#5**), developed via chemical modifications of OM99-2, are typical examples of less-peptidic BACE1 inhibitors. Both of them exhibited stronger potency (cellular IC_{50} equal to 39 nM and 1 nM, respectively) and impressive *in vivo* efficacy (reduction of plasma Aβ level by 30% and 65%, respectively) when intraperitoneally (i.p.) injected into Tg2576 mice [66, 67]. Further modifications of KMI-420 and KMI-429 produced tetrazole ring-containing compounds KMI-570 (**Table 1, #6**; *in vitro* IC_{50} = 4.8 nM) and KMI-684 (**Table 1, #7**; *in vitro* IC_{50} = 1.2 nM), both of which also display improved brain permeability [68].

Encouragingly, GlxoSmithKline reported the first orally available BACE1 inhibitor GSK188909, a small non-peptidic compound developed from substrate-based design (**Table 1, #8**). GSK188909 displayed a cellular IC_{50} of 5 nM and showed excellent selectivity over other aspartic proteases. When orally administered in TASTPM mice, which express both Swedish mutant APP and PS1 mutant M146V, GSK188909 effectively reduced brain Aβ levels [69]. Subsequently, Schering-Plough also reported an orally effective 4-phenoxypyrrolidine-based BACE1 inhibitor named compound 11 with good pharmacokinetics and selectivity (**Table 1, #9**; K_i = 0.7 nM, cellular IC_{50} = 21 nM) [70]. The most exciting news in the race of BACE1 drug discovery was perhaps the debut of CoMentis' CTS-21166 (**Table 1, #10;** cellular IC_{50} = 1.2–3.6 nM), which is the only BACE1 inhibitor that has passed Phase I clinical trial thus far. In 2008, CoMentis revealed this small compound as a transition-state analog inhibitor (structure is currently undisclosed) with excellent properties in brain penetration, selectivity, metabolic stability, and oral availability; all of these have met the requirements of an ideal oral drug candidate [71]. When i.p. injected (4 mg/kg over six weeks) into an aggressive APP transgenic mouse (expressing both the Swedish and London mutations), CTS-21166 reduced brain Aβ levels by over 35% and plaque load by 40%. The data from human Phase I studies suggested that this compound appeared safe at dose as high as 225mg, and when intravenously (i.v.) injected into AD patients, it caused a dose-dependant reduction of plasma Aβ levels for an extended period of time (http://www.alzforum.org/new/detail.asp?id=1790). More thorough clinical evaluation of CTS-21166 is underway by CoMentis in a partnership with Astellas Pharma in Japan. Following this trend, several companies such as Merck, Eli Lilly, and Takeda are also considering Phase I human testing with their own BACE1 inhibitors (http://www. alzforum.org/new/detail.asp?id=1790). Interesting clinical data will likely be available for these inhibitors in the near future.

BACE1 inhibitor design by a high-throughput screening strategy

Though many BACE1 inhibitors were successfully designed as substrate-based analogues, the majority of non-peptidic BACE1 inhibitors were developed using high-throughput screening (HTS) or a fragment-based lead generation

Table 2. Non-substate-based BACE1 inhibitors

#	Inhibitor name	Design method	*in vitro* activity	Cellular activity	*in vivo* activity	Significance	Reference
11	10d	HTS[a]	IC_{50} = 0.11 μM	NA	NA	85 fold selectivity over cathepsin D	Cole et al., 2006 (Wyeth)
12	3a	HTS	K_i = 11 nM	Very efficiently inhibit Aβ40 secretion in CHO cells transfected with Swedish mutant APP	3 hours after oral dosing at 30 mg/kg, 40–70% ↓ plasma Aβ40 in rats	Excellent brain permeability	Baxter et al., 2007 (Johnson & Johnson)
13	compound 29	FBDD[b]	IC_{50} = 80 nM	IC_{50} = 470 nM	NA		Edwards et al., 2007 (AstraZeneca)

[a] HTS, high throughput screening

approach. HTS has been conducted by many pharmaceutical companies to identify BACE1 inhibiting "hit" compounds from various chemical libraries. In 2001, Takeda reported the first series of non-peptidic BACE1 inhibitors with an *in vitro* IC_{50} of 0.35–2.93 μM using this approach [72]. Subsequently, Wyeth reported their hit compound WY-25105 (*in vitro* IC_{50} = 3.7 μM, cellular IC_{50} = 20 μM) containing an acylguanidine moiety that can form key interactions with the two catalytic Asp residues (Asp_{93} and ASP_{289}) at the BACE1 active site [73]. Structure optimization of this compound has led to the development of a more potent BACE1 inhibitor named 10d with an *in vitro* IC_{50} of 0.11 μM (**Table 2, #11**), and this compound showed an 85-fold selectivity over another aspartic protease, cathepsin D [73]. Using the similar approach, Johnson & Johnson developed the BACE1 inhibitor 3a with a stronger potency (K_i = 11 nM; **Table 2, #12**) [74]. More importantly, this compound exhibits excellent brain permeability and oral availability. When orally administered at 30 mg/kg to rats, 3a was able to lower plasma Aβ40 levels by 40–70% 3 hours postdosing. Despite all of these successes, none of these HTS-based inhibitors has entered a clinical trial.

BACE1 inhibitor design by a fragment-based lead generation approach

Recently, "fragment-based drug discovery (FBDD)" has emerged as a novel alternative to the traditional HTS method in identifying potent BACE1 inhibitory drugs. In contrast to HTS, which uses libraries of relatively high-molecular-weight compounds, the FBDD approach takes advantage of libraries comprising more diverse and smaller-sized compounds (fragments) to identify hits that can be efficiently developed into potent leads with drug-like properties. After the hits are identified, medicinal chemistry will come into play to further optimize these leads into suitable drugs. Compared with the traditional HTS approach, a significantly higher hit ratio can be obtained using a FBDD approach even though the binding affinity between the lower-molecular-weight fragments and the large active site of BACE1 will be weaker. A variety of biophysical techniques (NMR, X-ray crystallography, surface plasmon resonance (SPR), etc.), computational tools and biochemical assays can be coupled in fragment screening. By a fragment screening approach assisted with NMR followed by X-ray crystallography and SPR assays, AstraZeneca reported a FBDD-based BACE1 inhibitory compound 29 with a cellular IC_{50} near 470 nM (**Table 2, #13**) [75, 76]. Astex, in a partnership with AstraZeneca, reported several BACE1 inhibitors developed by fragment screening through X-ray crystallography [77]. Because this approach provides detailed binding mode of the fragments to the BACE1-active pocket, further optimization of these inhibitors by medicinal chemistry can be easily performed. Huang *et al.* (University of Zürich) reported non-peptidic BACE1 inhibitors using fragment screenings by a computer-assisted docking simulation method [78, 79]. Very recently, Godemann *et al.* (Evotec AG) identified a series of BACE1 inhibitors by a novel fluorescence-polarization-assay-based fragment screening method in conjunction with X-ray crystallography

[80]. The integration of various techniques in the fragment screening has made FBDD an increasingly popular method for designing potent small-molecule BACE1 inhibitors.

It should be emphasized that each of the aforementioned strategies of drug discovery has its pros and cons. While substrate-based BACE1 inhibitors usually show high potency and selectivity, their poor oral availability and permeability across the blood-brain barrier frequently make them unsuitable drug candidates. By contrast, the HTS method has the advantage of generating "hits" with high diversity, smaller size, and more drug-like properties (*i.e.*, oral availability and brain penetration). However, the hit ratio of HTS tends to be extremely low and the "hits" generally have lower potency and selectivity than substrate-based inhibitors. Compared with the traditional HTS method, however, fragment-based screening enjoys much higher hit ratios, and like HTS, can identify leads with favorable drug properties. On the other hand, fragment leads are too small to exhibit satisfactory potency and selectivity, thus requires considerable subsequent modifications by medicinal chemists. Overall, a combinatorial approach that carefully integrates the strengths of different design strategies may find its successful application in the future design of more applicable BACE1 inhibitory drugs.

Potential mechanism-based toxicity from BACE1 inhibition

At the current stage, whether strong inhibition of BACE1 activity will cause untoward mechanism-based toxicity is less of a concern due to the mild phenotypes of BACE1-null mice. However, it has become increasingly clear that this enzyme also cleaves membrane-bound substrates other than APP. One of these demonstrated substrates is neuregulin-1 (Nrg-1) [49, 50], an essential neurotrophic factor that signals through the ErbB family of receptor-tyrosine kinases to regulate various neurological processes, including synaptic functions, neuronal migration, myelination, and neurotransmitter functions, among many others [81-83]. In BACE1-null mice, abolished cleavage of Nrg1 by BACE1 disrupted BACE1-dependant Nrg1/ErbB signaling, causing hypomyelination (thinner myelin) of both peripheral nerves [49, 50] and central nerves [49] as well as schizophrenia-like phenotypes in these mice [51]. Furthermore, we showed that BACE1-null mice exhibit impaired remyelination in adult mice [84]. Another important BACE1 substrate is the voltage-gated sodium channel β2 (Na_vβ2) subunit [85, 86]. Voltage-gated sodium channel proteins are composed of a pore-forming α subunit and auxiliary β subunits [87]. We recently demonstrated that BACE1–null mice display epileptic seizures presumably arising from elevated neuronal surface expression of sodium channel proteins and the resulted increased neuronal excitability [88]. It is highly plausible that the abolished cleavage of Navβ2 by BACE1 leads to these observed alterations, because the disruption of this cleavage in BACE1-null mice will potentially affect both the expression and cellular trafficking of sodium channel proteins. The absence of BACE1 will reduce the shedding of Navβ2 intracellular domain that can regulate gene expression of the sodium channel α subunit [85]; disruption of BACE1-dependant cleavage of Navβ2 will also increase the level of full length Navβ2 protein, which is capable of binding to the α subunit and mediating its trafficking to the membrane surface [89]. Moreover, aged BACE1 –null mice were found to exhibit hippocampal neurodegeneration that is likely attributable to the sustained asynchronous neuronal stimulation in these mice [88]. Together, these important findings strongly suggest that BACE1 performs diverse physiological functions through processing of different substrates. Therefore, careful titration of drug dosage may still be a needed precaution to avoid the potential mechanism-based toxicity incurred by BACE1-directed AD therapy.

Concluding remarks

In the past decade, massive research efforts have been directed toward understanding BACE1 as a critical target for AD therapy. The crystal structure of BACE1 and our increasing knowledge of the cell biology of this enzyme have paved the way for the development of a wide variety of BACE1 inhibitors. During the preparation of this manuscript, several new classes of BACE1 inhibitors were reported. However, this article is not meant to be an all-inclusive summary of BACE1 inhibitors revealed to date. Instead, we have selectively presented some BACE1 inhibitors that have made pioneering contributions to the field. Although some orally effective BACE1 inhibitors have been reported and at least one (CTS-21166 from

CoMentis) of these inhibitors have entered a human clinical trial, it remains a daunting challenge to find small-molecule BACE1 inhibitors with satisfactory pharmacological properties. Due to the inherent disadvantages of each drug design method, combinatorial strategies that integrate the strengths of individual methods might boost the success rate in the design of future generations of more viable BACE1 drugs. Finally, the accumulating knowledge regarding the physiological functions of BACE1 further supports that BACE1 is still a highly viable AD therapeutic target. The optimal inhibition of BACE1 in humans will likely answer whether a so-called "amyloid hypothesis"-based strategy is the best way for improving the cognitive function of AD patients.

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References

[1] Selkoe DJ. Alzheimer disease: mechanistic understanding predicts novel therapies. Ann Intern Med 2004; 140: 627-638.

[2] Tanzi RE and Bertram L. Twenty years of the Alzheimer's disease amyloid hypothesis: a genetic perspective. Cell 2005; 120: 545-555.

[3] Hutton M, Perez-Tur J and Hardy J. Genetics of Alzheimer's disease. Essays Biochem 1998; 33: 117-131.

[4] Younkin SG. The role of A beta 42 in Alzheimer's disease. J Physiol Paris 1998; 92: 289-292.

[5] Hardy J. Amyloid double trouble. Nat Genet 2006; 38: 11-12.

[6] Grundke-Iqbal I, Iqbal K, Quinlan M, Tung YC, Zaidi MS and Wisniewski HM. Microtubule-associated protein tau. A component of Alzheimer paired helical filaments. J Biol Chem 1986; 261: 6084-6089.

[7] Ihara Y, Nukina N, Miura R and Ogawara M. Phosphorylated tau protein is integrated into paired helical filaments in Alzheimer's disease. J Biochem 1986; 99: 1807-1810.

[8] LaFerla FM and Oddo S. Alzheimer's disease: Abeta, tau and synaptic dysfunction. Trends Mol Med 2005; 11: 170-176.

[9] Vassar R, Bennett BD, Babu-Khan S, Kahn S, Mendiaz EA, Denis P, Teplow DB, Ross S, Amarante P, Loeloff R, Luo Y, Fisher S, Fuller J, Edenson S, Lile J, Jarosinski MA, Biere AL, Curran E, Burgess T, Louis JC, Collins F, Treanor J, Rogers G and Citron M. Beta-secretase cleavage of Alzheimer's amyloid precursor protein by the transmembrane aspartic protease BACE. Science 1999; 286: 735-741.

[10] Yan R, Bienkowski MJ, Shuck ME, Miao H, Tory MC, Pauley AM, Brashier JR, Stratman NC, Mathews WR, Buhl AE, Carter DB, Tomasselli AG, Parodi LA, Heinrikson RL and Gurney ME. Membrane-anchored aspartyl protease with Alzheimer's disease beta-secretase activity. Nature 1999; 402: 533-537.

[11] Sinha S, Anderson JP, Barbour R, Basi GS, Caccavello R, Davis D, Doan M, Dovey HF, Frigon N, Hong J, Jacobson-Croak K, Jewett N, Keim P, Knops J, Lieberburg I, Power M, Tan H, Tatsuno G, Tung J, Schenk D, Seubert P, Suomensaari SM, Wang S, Walker D, Zhao J, McConlogue L and John V. Purification and cloning of amyloid precursor protein beta-secretase from human brain. Nature 1999; 402: 537-540.

[12] Hussain I, Powell D, Howlett DR, Tew DG, Meek TD, Chapman C, Gloger IS, Murphy KE, Southan CD, Ryan DM, Smith TS, Simmons DL, Walsh FS, Dingwall C and Christie G. Identification of a novel aspartic protease (Asp 2) as beta-secretase. Mol Cell Neurosci 1999; 14: 419-427.

[13] Lin X, Koelsch G, Wu S, Downs D, Dashti A and Tang J. Human aspartic protease memapsin 2 cleaves the beta-secretase site of beta-amyloid precursor protein. Proc Natl Acad Sci U S A 2000; 97: 1456-1460.

[14] Luo Y, Bolon B, Kahn S, Bennett BD, Babu-Khan S, Denis P, Fan W, Kha H, Zhang J, Gong Y, Martin L, Louis JC, Yan Q, Richards WG, Citron M and Vassar R. Mice deficient in BACE1, the Alzheimer's beta-secretase, have normal phenotype and abolished beta-amyloid generation. Nat Neurosci 2001; 4: 231-232.

[15] Cai H, Wang Y, McCarthy D, Wen H, Borchelt DR, Price DL and Wong PC. BACE1 is the major beta-secretase for generation of Abeta peptides by neurons. Nat Neurosci 2001; 4: 233-234.

[16] Roberds SL, Anderson J, Basi G, Bienkowski MJ, Branstetter DG, Chen KS, Freedman SB, Frigon NL, Games D, Hu K, Johnson-Wood K, Kappenman KE, Kawabe TT, Kola I, Kuehn R, Lee M, Liu W, Motter R, Nichols NF, Power M, Robertson DW, Schenk D, Schoor M, Shopp GM, Shuck ME, Sinha S, Svensson KA, Tatsuno G, Tintrup H, Wijsman J, Wright S and McConlogue L. BACE knockout mice are healthy despite lacking the primary beta-secretase activity in brain: implications for Alzheimer's disease therapeutics. Hum Mol Genet 2001; 10: 1317-1324.

[17] Farzan M, Schnitzler CE, Vasilieva N, Leung D and Choe H. BACE2, a beta -secretase homolog, cleaves at the beta site and within the amyloid-beta region of the amyloid-beta precursor protein. Proc Natl Acad Sci U S A 2000; 97: 9712-9717.
[18] Yan R, Munzner JB, Shuck ME and Bienkowski MJ. BACE2 functions as an alternative alpha-secretase in cells. J Biol Chem 2001; 276: 34019-34027.
[19] Dominguez D, Tournoy J, Hartmann D, Huth T, Cryns K, Deforce S, Serneels L, Camacho IE, Marjaux E, Craessaerts K, Roebroek AJ, Schwake M, D'Hooge R, Bach P, Kalinke U, Moechars D, Alzheimer C, Reiss K, Saftig P and De Strooper B. Phenotypic and biochemical analyses of BACE1- and BACE2-deficient mice. J Biol Chem 2005; 280: 30797-30806.
[20] Shi XP, Chen E, Yin KC, Na S, Garsky VM, Lai MT, Li YM, Platchek M, Register RB, Sardana MK, Tang MJ, Thiebeau J, Wood T, Shafer JA and Gardell SJ. The pro domain of beta-secretase does not confer strict zymogen-like properties but does assist proper folding of the protease domain. J Biol Chem 2001; 276: 10366-10373.
[21] Hong L, Turner RT, 3rd, Koelsch G, Shin D, Ghosh AK and Tang J. Crystal structure of memapsin 2 (beta-secretase) in complex with an inhibitor OM00-3. Biochemistry 2002; 41: 10963-10967.
[22] Haniu M, Denis P, Young Y, Mendiaz EA, Fuller J, Hui JO, Bennett BD, Kahn S, Ross S, Burgess T, Katta V, Rogers G, Vassar R and Citron M. Characterization of Alzheimer's beta -secretase protein BACE. A pepsin family member with unusual properties. J Biol Chem 2000; 275: 21099-21106.
[23] Bennett BD, Denis P, Haniu M, Teplow DB, Kahn S, Louis JC, Citron M and Vassar R. A furin-like convertase mediates propeptide cleavage of BACE, the Alzheimer's beta -secretase. J Biol Chem 2000; 275: 37712-37717.
[24] Capell A, Steiner H, Willem M, Kaiser H, Meyer C, Walter J, Lammich S, Multhaup G and Haass C. Maturation and pro-peptide cleavage of beta-secretase. J Biol Chem 2000; 275: 30849-30854.
[25] Benjannet S, Elagoz A, Wickham L, Mamarbachi M, Munzer JS, Basak A, Lazure C, Cromlish JA, Sisodia S, Checler F, Chretien M and Seidah NG. Post-translational processing of beta-secretase (beta-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: 10879-10887.
[26] Creemers JW, Ines Dominguez D, Plets E, Serneels L, Taylor NA, Multhaup G, Craessaerts K, Annaert W and De Strooper B. Processing of beta-secretase by furin and other members of the proprotein convertase family. J Biol Chem 2001; 276: 4211-4217.
[27] Costantini C, Ko MH, Jonas MC and Puglielli L. A reversible form of lysine acetylation in the ER and Golgi lumen controls the molecular stabilization of BACE1. Biochem J 2007; 407: 383-395.
[28] Yan R, Han P, Miao H, Greengard P and Xu H. The transmembrane domain of the Alzheimer's beta-secretase (BACE1) determines its late Golgi localization and access to beta -amyloid precursor protein (APP) substrate. J Biol Chem 2001; 276: 36788-36796.
[29] Turner RT, 3rd, Loy JA, Nguyen C, Devasamudram T, Ghosh AK, Koelsch G and Tang J. Specificity of memapsin 1 and its implications on the design of memapsin 2 (beta-secretase) inhibitor selectivity. Biochemistry 2002; 41: 8742-8746.
[30] Tomasselli AG, Qahwash I, Emmons TL, Lu Y, Leone JW, Lull JM, Fok KF, Bannow CA, Smith CW, Bienkowski MJ, Heinrikson RL and Yan R. Employing a superior BACE1 cleavage sequence to probe cellular APP processing. J Neurochem 2003; 84: 1006-1017.
[31] Haass C, Lemere CA, Capell A, Citron M, Seubert P, Schenk D, Lannfelt L and Selkoe DJ. The Swedish mutation causes early-onset Alzheimer's disease by beta-secretase cleavage within the secretory pathway. Nat Med 1995; 1: 1291-1296.
[32] Koo EH and Squazzo SL. Evidence that production and release of amyloid beta-protein involves the endocytic pathway. J Biol Chem 1994; 269: 17386-17389.
[33] Vassar R, Kovacs DM, Yan R and Wong PC. The beta-secretase enzyme BACE in health and Alzheimer's disease: regulation, cell biology, function, and therapeutic potential. J Neurosci 2009; 29: 12787-12794.
[34] Murayama KS, Kametani F, Saito S, Kume H, Akiyama H and Araki W. Reticulons RTN3 and RTN4-B/C interact with BACE1 and inhibit its ability to produce amyloid beta-protein. Eur J Neurosci 2006; 24: 1237-1244.
[35] He W, Lu Y, Qahwash I, Hu XY, Chang A and Yan R. Reticulon family members modulate BACE1 activity and amyloid-beta peptide generation. Nat Med 2004; 10: 959-965.
[36] Walter J, Fluhrer R, Hartung B, Willem M, Kaether C, Capell A, Lammich S, Multhaup G and Haass C. Phosphorylation regulates intracellular trafficking of beta-secretase. J Biol Chem 2001; 276: 14634-14641.
[37] Huse JT, Pijak DS, Leslie GJ, Lee VM and Doms RW. Maturation and endosomal targeting of beta -site amyloid precursor protein-cleaving enzyme. The Alzheimer's disease beta-secretase. J Biol Chem 2000; 275: 33729-33737.
[38] He X, Chang WP, Koelsch G and Tang J. Memapsin 2 (beta-secretase) cytosolic domain binds to the VHS domains of GGA1 and GGA2: implications on the endocytosis mechanism of memapsin 2. FEBS Lett 2002; 524: 183-187.
[39] Shiba T, Kametaka S, Kawasaki M, Shibata M,

Waguri S, Uchiyama Y and Wakatsuki S. Insights into the phosphoregulation of beta-secretase sorting signal by the VHS domain of GGA1. Traffic 2004; 5: 437-448.
[40] von Arnim CA, Tangredi MM, Peltan ID, Lee BM, Irizarry MC, Kinoshita A and Hyman BT. Demonstration of BACE (beta-secretase) phosphorylation and its interaction with GGA1 in cells by fluorescence-lifetime imaging microscopy. J Cell Sci 2004; 117: 5437-5445.
[41] He X, Li F, Chang WP and Tang J. GGA proteins mediate the recycling pathway of memapsin 2 (BACE). J Biol Chem 2005; 280: 11696-11703.
[42] Wahle T, Prager K, Raffler N, Haass C, Famulok M and Walter J. GGA proteins regulate retrograde transport of BACE1 from endosomes to the trans-Golgi network. Mol Cell Neurosci 2005; 29: 453-461.
[43] Koh YH, von Arnim CA, Hyman BT, Tanzi RE and Tesco G. BACE is degraded via the lysosomal pathway. J Biol Chem 2005; 280: 32499-32504.
[44] Tesco G, Koh YH, Kang EL, Cameron AN, Das S, Sena-Esteves M, Hiltunen M, Yang SH, Zhong Z, Shen Y, Simpkins JW and Tanzi RE. Depletion of GGA3 stabilizes BACE and enhances beta-secretase activity. Neuron 2007; 54: 721-737.
[45] Vetrivel KS, Meckler X, Chen Y, Nguyen PD, Seidah NG, Vassar R, Wong PC, Fukata M, Kounnas MZ and Thinakaran G. Alzheimer disease Abeta production in the absence of S-palmitoylation-dependent targeting of BACE1 to lipid rafts. J Biol Chem 2009; 284: 3793-3803.
[46] Kalvodova L, Kahya N, Schwille P, Ehehalt R, Verkade P, Drechsel D and Simons K. Lipids as modulators of proteolytic activity of BACE: involvement of cholesterol, glycosphingolipids, and anionic phospholipids in vitro. J Biol Chem 2005; 280: 36815-36823.
[47] Tun H, Marlow L, Pinnix I, Kinsey R and Sambamurti K. Lipid rafts play an important role in A beta biogenesis by regulating the beta-secretase pathway. J Mol Neurosci 2002; 19: 31-35.
[48] Cordy JM, Hussain I, Dingwall C, Hooper NM and Turner AJ. Exclusively targeting beta-secretase to lipid rafts by GPI-anchor addition up-regulates beta-site processing of the amyloid precursor protein. Proc Natl Acad Sci U S A 2003; 100: 11735-11740.
[49] Hu X, Hicks CW, He W, Wong P, Macklin WB, Trapp BD and Yan R. Bace1 modulates myelination in the central and peripheral nervous system. Nat Neurosci 2006; 9: 1520-1525.
[50] Willem M, Garratt AN, Novak B, Citron M, Kaufmann S, Rittger A, DeStrooper B, Saftig P, Birchmeier C and Haass C. Control of peripheral nerve myelination by the beta-secretase BACE1. Science 2006; 314: 664-666.
[51] Savonenko AV, Melnikova T, Laird FM, Stewart KA, Price DL and Wong PC. Alteration of BACE1-dependent NRG1/ErbB4 signaling and schizophrenia-like phenotypes in BACE1-null mice. Proc Natl Acad Sci U S A 2008; 105: 5585-5590.
[52] De Strooper B, Vassar R and Golde T. The secretases: enzymes with therapeutic potential in Alzheimer disease. Nat Rev Neurol 6: 99-107.
[53] De Strooper B, Saftig P, Craessaerts K, Vanderstichele H, Guhde G, Annaert W, Von Figura K and Van Leuven F. Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein. Nature 1998; 391: 387-390.
[54] Li J, Fici GJ, Mao CA, Myers RL, Shuang R, Donoho GP, Pauley AM, Himes CS, Qin W, Kola I, Merchant KM and Nye JS. Positive and negative regulation of the gamma-secretase activity by nicastrin in a murine model. J Biol Chem 2003; 278: 33445-33449.
[55] Ma G, Li T, Price DL and Wong PC. APH-1a is the principal mammalian APH-1 isoform present in gamma-secretase complexes during embryonic development. J Neurosci 2005; 25: 192-198.
[56] Kopan R and Turner DL. The Notch pathway: democracy and aristocracy in the selection of cell fate. Curr Opin Neurobiol 1996; 6: 594-601.
[57] Wolfe MS. Gamma-secretase: structure, function, and modulation for Alzheimer's disease. Curr Top Med Chem 2008; 8: 2-8.
[58] Sisodia SS and St George-Hyslop PH. gamma-Secretase, Notch, Abeta and Alzheimer's disease: where do the presenilins fit in? Nat Rev Neurosci 2002; 3: 281-290.
[59] Hong L, Koelsch G, Lin X, Wu S, Terzyan S, Ghosh AK, Zhang XC and Tang J. Structure of the protease domain of memapsin 2 (beta-secretase) complexed with inhibitor. Science 2000; 290: 150-153.
[60] Shuto D, Kasai S, Kimura T, Liu P, Hidaka K, Hamada T, Shibakawa S, Hayashi Y, Hattori C, Szabo B, Ishiura S and Kiso Y. KMI-008, a novel beta-secretase inhibitor containing a hydroxymethylcarbonyl isostere as a transition-state mimic: design and synthesis of substrate-based octapeptides. Bioorg Med Chem Lett 2003; 13: 4273-4276.
[61] Kimura T, Shuto D, Hamada Y, Igawa N, Kasai S, Liu P, Hidaka K, Hamada T, Hayashi Y and Kiso Y. Design and synthesis of highly active Alzheimer's beta-secretase (BACE1) inhibitors, KMI-420 and KMI-429, with enhanced chemical stability. Bioorg Med Chem Lett 2005; 15: 211-215.
[62] Asai M, Hattori C, Iwata N, Saido TC, Sasagawa N, Szabo B, Hashimoto Y, Maruyama K, Tanuma S, Kiso Y and Ishiura S. The novel beta-secretase inhibitor KMI-429 reduces amyloid beta peptide production in amyloid precursor protein transgenic and wild-type mice. J Neurochem 2006; 96: 533-540.
[63] Hu J, Cwi CL, Smiley DL, Timm D, Erickson JA, McGee JE, Yang HC, Mendel D, May PC, Shapiro M and McCarthy JR. Design and synthesis of statine-containing BACE inhibitors. Bioorg Med Chem Lett 2003; 13: 4335-4339.
[64] Tung JS, Davis DL, Anderson JP, Walker DE,

Mamo S, Jewett N, Hom RK, Sinha S, Thorsett ED and John V. Design of substrate-based inhibitors of human beta-secretase. J Med Chem 2002; 45: 259-262.

[65] Tamamura H, Kato T, Otaka A and Fujii N. Synthesis of potent beta-secretase inhibitors containing a hydroxyethylamine dipeptide isostere and their structure-activity relationship studies. Org Biomol Chem 2003; 1: 2468-2473.

[66] Ghosh AK, Kumaragurubaran N, Hong L, Kulkarni SS, Xu X, Chang W, Weerasena V, Turner R, Koelsch G, Bilcer G and Tang J. Design, synthesis, and X-ray structure of potent memapsin 2 (beta-secretase) inhibitors with isophthalamide derivatives as the P2-P3-ligands. J Med Chem 2007; 50: 2399-2407.

[67] Ghosh AK, Kumaragurubaran N, Hong L, Kulkarni S, Xu X, Miller HB, Reddy DS, Weerasena V, Turner R, Chang W, Koelsch G and Tang J. Potent memapsin 2 (beta-secretase) inhibitors: design, synthesis, protein-ligand X-ray structure, and in vivo evaluation. Bioorg Med Chem Lett 2008; 18: 1031-1036.

[68] Kimura T, Hamada Y, Stochaj M, Ikari H, Nagamine A, Abdel-Rahman H, Igawa N, Hidaka K, Nguyen JT, Saito K, Hayashi Y and Kiso Y. Design and synthesis of potent beta-secretase (BACE1) inhibitors with P1' carboxylic acid bioisosteres. Bioorg Med Chem Lett 2006; 16: 2380-2386.

[69] Hussain I, Hawkins J, Harrison D, Hille C, Wayne G, Cutler L, Buck T, Walter D, Demont E, Howes C, Naylor A, Jeffrey P, Gonzalez MI, Dingwall C, Michel A, Redshaw S and Davis JB. Oral administration of a potent and selective non-peptidic BACE-1 inhibitor decreases beta-cleavage of amyloid precursor protein and amyloid-beta production in vivo. J Neurochem 2007; 100: 802-809.

[70] Iserloh U, Pan J, Stamford AW, Kennedy ME, Zhang Q, Zhang L, Parker EM, McHugh NA, Favreau L, Strickland C and Voigt J. Discovery of an orally efficaceous 4-phenoxypyrrolidine-based BACE-1 inhibitor. Bioorg Med Chem Lett 2008; 18: 418-422.

[71] (a) Hey JA, Koelsch G, Bilcer G, Jacobs A, Tolar M, Tang J, Ghosh A K, Hsu HH. Single Dose Administration of the β-Secretase Inhibitor CTS21166 (ASP1702) Reduces Plasma Aβ40 in Human Subjects. Presented at the International Conference on Alzheimer's Disease (ICAD), Chicago, IL, 2008.

(b) Hsu HH, Jacobs A, Yu J, Li A, Koelsch G, Bilcer G, Tolar M, Grove C, Ghosh AK, Tang J, Hey JA. Phase 1 Safety and Pharmacokinetic Profile of Single Doses of CTS21166 (ASP1702) in Healthy Males. Presented at the International Conference on Alzheimer's Disease (ICAD), Chicago, IL, 2008.

(c) Yu J, Li A, Koelsch G, Bilcer G, Hsu HH, Jacobs A, Tolar M, Tang J, Ghosh AK, Hey JA. Pharmacokinetic/Pharmacodynamic Analysis of Plasma Aβ40 Reduction in Human Subjects Produced by the β-Secretase Inhibitor CTS21166 (ASP1702). Presented at the International Conference on Alzheimer's Disease (ICAD), Chicago, IL, 2008.

(d) Koelsch G, Tang J, Ghosh AK, Bilcer G, Hey JA, Windisch M. BACE1 Inhibitor CTS21166 (ASP1702) Penetrates Brain and Reduces Aβ Pathology in a Transgenic Mouse Model of Advanced AD. Presented at the International Conference on Alzheimer's Disease (ICAD), Chicago, IL, 2008.

(e) Bilcer G, Koelsch G, Swanson L, Yu J, Li A, Turner R, Weerasena V, Dehdarani M, Tang J, Ghosh AK, Hey JA. Pharmacological Profile of Bace1 Inhibitor CTS21166 (ASP1702). Presented at the International Conference on Alzheimer's Disease (ICAD), Chicago, IL, 2008.

(f) Shitaka Y, Yarimizu J, Nagakura A, Mitani Y, Takami S, Miyake A, Hamakawa N, Hey JA, Koelsch G, Bilcer G, Matsuoka N. Oral Administration of the BACE1 Inhibitor CTS21166 (ASP1702) Improves Cognition and Reduces Brain Aβ in Tg2576 Transgenic Mice. Presented at the International Conference on Alzheimer's Disease (ICAD), Chicago, IL, 2008.

[72] Miyamoto M, Matsui J, Fukumoto H, Tarui N. Preparation of 2-[2-amino- or 2-(N-heterocyclyl) ethyl]-6-(4-biphenylylmethoxy)tetralin derivatives as β-secretase inhibitors. PCT Int Appl WO2001087293A1; 2001.

[73] Cole DC, Manas ES, Stock JR, Condon JS, Jennings LD, Aulabaugh A, Chopra R, Cowling R, Ellingboe JW, Fan KY, Harrison BL, Hu Y, Jacobsen S, Jin G, Lin L, Lovering FE, Malamas MS, Stahl ML, Strand J, Sukhdeo MN, Svenson K, Turner MJ, Wagner E, Wu J, Zhou P and Bard J. Acylguanidines as small-molecule beta-secretase inhibitors. J Med Chem 2006; 49: 6158-6161.

[74] Baxter EW, Conway KA, Kennis L, Bischoff F, Mercken MH, Winter HL, Reynolds CH, Tounge BA, Luo C, Scott MK, Huang Y, Braeken M, Pieters SM, Berthelot DJ, Masure S, Bruinzeel WD, Jordan AD, Parker MH, Boyd RE, Qu J, Alexander RS, Brenneman DE and Reitz AB. 2-Amino-3,4-dihydroquinazolines as inhibitors of BACE-1 (beta-site APP cleaving enzyme): Use of structure based design to convert a micromolar hit into a nanomolar lead. J Med Chem 2007; 50: 4261-4264.

[75] Geschwindner S, Olsson LL, Albert JS, Deinum J, Edwards PD, de Beer T and Folmer RH. Discovery of a novel warhead against beta-secretase through fragment-based lead generation. J Med Chem 2007; 50: 5903-5911.

[76] Edwards PD, Albert JS, Sylvester M, Aharony D, Andisik D, Callaghan O, Campbell JB, Carr RA, Chessari G, Congreve M, Frederickson M, Folmer RH, Geschwindner S, Koether G, Kolmodin K, Krumrine J, Mauger RC, Murray CW, Olsson LL, Patel S, Spear N and Tian G. Application of fragment-based lead generation to the discovery of novel, cyclic amidine beta-secretase inhibitors

with nanomolar potency, cellular activity, and high ligand efficiency. J Med Chem 2007; 50: 5912-5925.
[77] Congreve M, Aharony D, Albert J, Callaghan O, Campbell J, Carr RA, Chessari G, Cowan S, Edwards PD, Frederickson M, McMenamin R, Murray CW, Patel S and Wallis N. Application of fragment screening by X-ray crystallography to the discovery of aminopyridines as inhibitors of beta-secretase. J Med Chem 2007; 50: 1124-1132.
[78] Huang D, Luthi U, Kolb P, Edler K, Cecchini M, Audetat S, Barberis A and Caflisch A. Discovery of cell-permeable non-peptide inhibitors of beta-secretase by high-throughput docking and continuum electrostatics calculations. J Med Chem 2005; 48: 5108-5111.
[79] Huang D, Luthi U, Kolb P, Cecchini M, Barberis A and Caflisch A. In silico discovery of beta-secretase inhibitors. J Am Chem Soc 2006; 128: 5436-5443.
[80] Godemann R, Madden J, Kramer J, Smith M, Fritz U, Hesterkamp T, Barker J, Hoppner S, Hallett D, Cesura A, Ebneth A and Kemp J. Fragment -based discovery of BACE1 inhibitors using functional assays. Biochemistry 2009; 48: 10743-10751.
[81] Michailov GV, Sereda MW, Brinkmann BG, Fischer TM, Haug B, Birchmeier C, Role L, Lai C, Schwab MH and Nave KA. Axonal neuregulin-1 regulates myelin sheath thickness. Science 2004; 304: 700-703.
[82] Nave KA and Salzer JL. Axonal regulation of myelination by neuregulin 1. Curr Opin Neurobiol 2006; 16: 492-500.
[83] Mei L and Xiong WC. Neuregulin 1 in neural development, synaptic plasticity and schizophrenia. Nat Rev Neurosci 2008; 9: 437-452.
[84] Hu X, He W, Diaconu C, Tang X, Kidd GJ, Macklin WB, Trapp BD and Yan R. Genetic deletion of BACE1 in mice affects remyelination of sciatic nerves. FASEB J 2008; 22: 2970-2980.
[85] Kim DY, Carey BW, Wang H, Ingano LA, Binshtok AM, Wertz MH, Pettingell WH, He P, Lee VM, Woolf CJ and Kovacs DM. BACE1 regulates voltage-gated sodium channels and neuronal activity. Nat Cell Biol 2007; 9: 755-764.
[86] Wong HK, Sakurai T, Oyama F, Kaneko K, Wada K, Miyazaki H, Kurosawa M, De Strooper B, Saftig P and Nukina N. beta Subunits of voltage-gated sodium channels are novel substrates of beta-site amyloid precursor protein-cleaving enzyme (BACE1) and gamma-secretase. J Biol Chem 2005; 280: 23009-23017.
[87] Catterall WA. From ionic currents to molecular mechanisms: the structure and function of voltage-gated sodium channels. Neuron 2000; 26: 13-25.
[88] Hu X, Zhou X, He W, Yang J, Xiong W, Wong P, Wilson CG and Yan R. BACE1 deficiency causes altered neuronal activity and neurodegeneration. J Neurosci 2010; 30: 8819-8829.
[89] Isom LL. Beta subunits: players in neuronal hyperexcitability? Novartis Found Symp 2002; 241: 124-138; discussion 138-143, 226-132.