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Age-dependent differential expression of BACE splice variants in brain regions of tg2576 mice

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Abstract

Plaques found in the brains of patients suffering from Alzheimer's disease (AD) mainly consist of β-amyloid (Aβ), which is produced by sequential cleaving of amyloid precursor protein (APP) by two proteolytic enzymes, β- and γ-secretases. Any change in the fine balance between these enzymes and their substrate may contribute to the etio-pathogenesis of AD. Indeed, the protein level and enzymatic activity of β-secretase (BACE), but not its mRNA level, were found elevated in brain areas of AD patients who suffer a high load of Aβ plaque formation. Similarly, increased BACE activity but no mRNA change was observed in a transgenic mouse model of AD, tg2576, in which over expression of the Swedish mutated human APP leads to Aβ plaque formation and learning deficits. Based on the recent demonstration of four BACE splice variants with different enzymatic activity, the discrepancy between BACE activity and mRNA expression may be explained by the altered BACE alternative splicing. To test this hypothesis, we studied the expression of all BACE splice variants in different brain areas of tg2576 mice at age of 4 months and 1 year old. We found developmental and regional differences between wild-type and tg2576 mice. Our results indicate that over expression of APP in tg2576 mice leads to the altered alternative splicing of BACE and the increase of its enzymatically more active splice variant (I-501).

Keywords: β-Secretase; Alternative splice variants; Brain regions; Age dependent; APP; β-Amyloid; tg2576 transgenic mice; Alzheimer disease

1. Introduction

Aggregates of β-amyloid peptides (Aβ) are the main constituent of senile plaques found in the brains of Alzheimer's disease (AD) patients [5,9,18]. Aβ is a product of sequential cleaving of amyloid precursor protein (APP) by two proteolytic enzymes β- and γ-secretases, which cleaves the APP at the N and C terminus of Aβ, respectively. Any change in the fine balance between these enzymes and their substrate may contribute to the etio-pathogenesis of AD [11]. Increase in Aβ production, indeed, may result from upregulation of the substrate itself (i.e., APP) as in the case of Down's syndrome (trisomy 21), or unbalanced production or activity of β- or γ-secretases.

β-Secretase is a type I transmembrane aspartic proteinase which belongs to the Asp2 family [13,15,20,24,27]. In mammals, BACE mRNA and protein are expressed mainly in the pancreas and the brain, but low expression levels can be found in many other body tissues [20,27,29]. BACE cleaves the APP at the N terminus of Aβ, splitting it into a 100 kD soluble segment (APPsβ) and a 12 kD membrane anchored segment (C99) [10,27]. Next, the C99 is cleaved by γ-secretase, creating the Aβ peptide. Three new alternative splice variants of the BACE gene were recently described [25]. These variants are the result of the alternative splicing of parts of exon

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3 and/or exon 4, which produces in frame deletion of 75 (I-476), 132 (I-457) and 207 (I-432) nucleotides, and encodes protein isoforms with different enzymatic activity [3,6,25] and expression [31].

Increased expression of BACE protein and enzymatic activity have been recently demonstrated in neocortical and hippocampal brain regions of AD patients [7,12,30]. However, the increase in BACE protein has not been associated with elevation of its mRNA levels [7,12].

Similar to AD patients, BACE activity and not mRNA levels were found elevated in transgenic mice, termed tg2576, which express the Swedish mutation of human APP and develop age-dependent Aβ plaques and learning deficits [1,16,23]. Brains of tg2576 show about a 10-fold increase in Aβ levels by the age of 1 year [4,14,17,28]. In light of the existence of different BACE splice variants, the discrepancy between BACE activity and mRNA expression may be the result of altered expression of specific BACE isoforms. To test this hypothesis, we have determined the age-dependent differential expression of BACE and its four known splice variants in six brain regions of young and old tg2576 mice and compared them to their wild-type littermates. We compared the expression of all BACE splice variants at two different ages and in different brain areas of tg2576 and wild-type mice.

2. Methods

2.1. Reverse transcription real-time PCR

Subjects were tg2576 mice and their wild-type littermates in two age groups, 4 months or 1 year (young transgenic (Ytg); old transgenic (Otg); young wild type (Ywt); old wild type (Owt)). Transgenic mice were generated by crossing tg2576 female with tg2576 males, both derived from C57Bl6/SJL F2 backcrossed to C57B6 breeders [14]. Mice were genotyped by PCR using DNA obtained from tail clippings. According to standard convention, N10 and beyond are considered fully congenic. Therefore, the mice examined had little or no variation in strain background. Mice were housed 2–6 per cage with ad libitum food and water, and maintained on a 12:12 light/dark cycle in a constant temperature (23 °C). Animals were sacrificed by halothane overdose, decapitated, and the brain regions were rapidly removed and freezed in liquid nitrogen. Following the dissection, total RNA from the various brain regions was extracted using Trizol (Invitrogen, Carlsbad, CA). BACE primer pairs were designed to fit the *Mus musculus* BACE sequence (GenBank accession no. NM_011792.2). Single strand cDNA was synthesized by incubating total RNA (5 μg) with the reverse primer P4 (5′-CCAATGATCATGCTCCCTCC-3′, corresponding to bases 1121–1140 of BACE; for positioning relative to the splice junction, see Fig. 1), 0.5 mM dNTP mix, 10 mM DTT, 1× first strand buffer and 50 U of SuperScript™ II RT (Invitrogen, Carlsbad, CA), at 42 °C for 50 min and then incubating

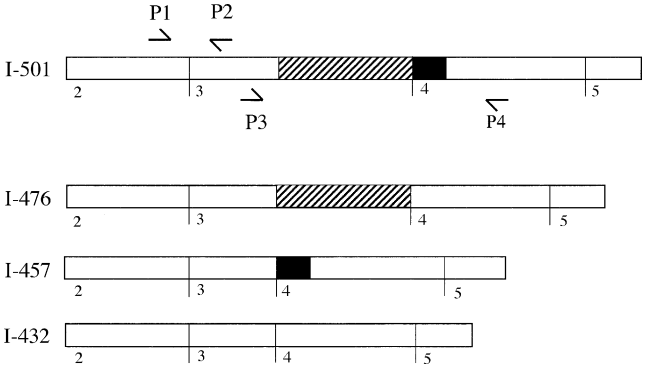

Fig. 1. Schematic drawing of BACE splice variants, their exons and the positioning of primers P1–P4 relative to the splice junctions. The vertical lines denote the splice junctions. The hedged and full areas denote the splice deletions (hedge exon 3, full exon 4).

at 70 °C for 15 min. For real-time quantitative PCR, triplicate aliquots of the brain regions' reverse transcribed RNA (0.4 μg), together with known amounts of external standards (purified PCR products, 10^4–10^8 copies) were amplified with BACE primers. One reaction containing water instead of template was used as negative control. To quantify BACE expression, we amplified a region upstream to the alternative spliced site (P1, 5′-AGACGCTCAACATCCTGGTG-3′, forward primer corresponding to bases 684–703 and P2, 5′-CCTGGGTGTAGGGCACATAC-3′, reverse primer corresponding to bases 811–830; Fig. 1). To control for RNA integrity and for differences attributable to errors in experimental manipulation from tube to tube, we normalized the data by dividing it by the expression level of S18 ribosomal RNA (rS18-15′-TCACCAAGAGGGCTGGAGAA-3′, rS18-25′-CAGTGGTCTTGGTGTGCTGA-3′). Each PCR reaction (final volume 20 μl) contained 0.5 μM of either BACE or rS18 primer pair, 10 μl of 2× QuantiTect SYBR Green PCR (Qiagen, Valencia, CA) and either 0.4 μg of reverse transcribed RNA, known amounts of external standards, or water. The following four step program was used for both BACE and S18 rRNA: (i) denaturation of cDNA (1 cycle: 95 °C for 15 min); (ii) amplification (40 cycles: 94 °C for 15 s, 58 °C for 20 s, 72 °C for 20 s); (iii) melting curve analysis (1 cycle: 95 °C for 1 s, 65 °C for 10 s, 95 °C for 1 s); (iv) cooling (1 cycle: 40 °C for 30 s). Temperature transition rate was 20 °C/s except for the third segment of the melting curve analysis where it was 0.2 °C/s. Fluorimeter gain value was 7. Real-time detection of SYBR Green I fluorescence intensity, indicating the amount of PCR products formed, was measured at the end of each elongation phase. Quantification of the amplification products and melting curve analysis were done using the LightCycler software (LightCycler, Roche, Indianapolis, IN). The log-linear phase of the curves of the unknown PCR products was fitted using the second derivative method, and compared to those of the known standards. Specificity of the PCR products was verified by melting curve analysis followed by gel electrophoresis (Fig. 2) and sequencing. Differences between the BACE expression levels in the