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Neuroscience Letters 428 (2007) 103–108
Neuroscience Letters
www.elsevier.com/locate/neulet

A novel beta-site amyloid precursor protein cleaving enzyme (BACE) isoform regulated by nonsense-mediated mRNA decay and proteasome-dependent degradation

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Received 24 May 2007; received in revised form 15 September 2007; accepted 15 September 2007

Abstract

Proteolytic cleavage of amyloid beta-peptide (Aβ) from amyloid precursor protein (APP) is a key event in the pathogenesis of Alzheimer's disease. Beta-site amyloid precursor protein cleaving enzyme (BACE) cleaves the APP at the N-terminus of Aβ. We investigated whether particular stress conditions modify the expression and activity of BACE, and found that treatment of human neuroblastoma cells with protein synthesis inhibitors induced expression of a novel splice variant of BACE. This unusual transcript, I-127, is produced by usage of an internal splicing donor site in exon 3. The splicing event leads to a premature termination codon, as well as elimination of one of two conserved aspartic protease active sites, a transmembrane domain, and a C-terminal cytoplasmic tail from BACE. Low levels of this mRNA were found in the human brain. When expressed in cells, I-127 had no effect on Aβ secretion and was retained in the endoplasmic reticulum without propeptide removal. It was also unstable with a turnover $t_{1/2}$ of ∼2 h; normal BACE had a turnover $t_{1/2}$ of ∼8 h. Finally, I-127 was degraded in a proteasome-dependent manner. Thus, I-127 is regulated by both nonsense-mediated mRNA decay (NMD) and proteasome-dependent degradation.

Keywords: Alternative splicing; Alzheimer's disease; BACE; mRNA surveillance; Nonsense-mediated mRNA decay; Proteasome-dependent degradation

Alzheimer's disease (AD) is the most common type of senile dementia and characterized by the progressive formation in the brain of insoluble amyloid plaques consisting of the 4-kD Aβ protein. Aβ is sequentially cleaved from APP by two proteolytic enzymes, the β- and γ-secretases. BACE has been shown to have all the properties of β-secretase [18]. The BACE open reading frame encodes a protein of 501 amino acids containing an N-terminal 21-amino acid signal peptide, a 24-amino acid propeptide, two conserved aspartic protease active site domains, a transmembrane domain (residues 461–477), and a 24-amino acid C-terminal cytoplasmic tail (Fig. 1A). Immunostaining has demonstrated predominant localization of BACE to the Golgi and endosomes [18]. BACE is expressed initially as a preproprotein and then cotranslationally *N*-glycosylated. This process produces a ∼60-kDa immature BACE in the endoplasmic reticulum (ER), when then undergoes propeptide cleavage and further complex glycosylation, producing a final ∼70-kDa mature BACE in the Golgi [3,5,7]. Three alternatively spliced variants of the BACE gene have been described [2,16]. These variants are the result of the alternative splicing of parts of exon 3 and/or exon 4, which produces in-frame deletions of 75 (I-476), 132 (I-457), and 207 (I-432) nucleotides, and results in protein isoforms with different enzymatic activities [2,16]. In contrast to BACE, I-476 and I-457 are retained in the ER in an immature state [2,4,16]. We have investigated whether particular stress conditions modify the expression and activity of BACE. Here we report a novel splicing variant I-127 induced by treatment of human neuroblastoma cells with protein synthesis inhibitors.

Tissue samples were taken from the frozen frontal lobes of patients (AD AD1; male, age 74, onset 69, AD2; female, age 82, onset unknown, AD3; female, age 76, onset unknown, AD4; male, age 82, onset 80; AD5, female, age 65, onset 50; Non-AD control, Ctr1; male, age 65, Ctr2; female, age 78, Ctr3;

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doi:10.1016/j.neulet.2007.09.033

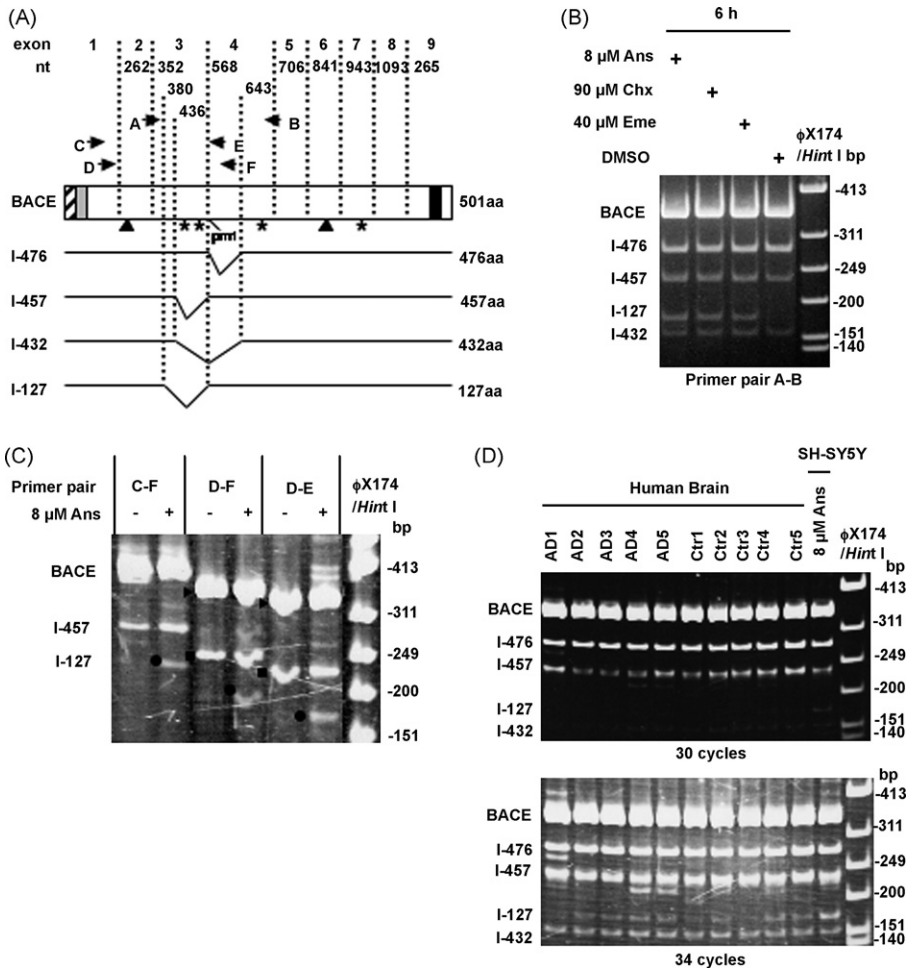

Fig. 1. Detection of alternatively spliced transcripts by PCR. (A) Schematic representation of the coding exons and alternatively spliced transcripts of BACE. The translation initiation site, 5′ nucleotide sequence of each exon, end of the coding sequence, and the internal splice site in exons 3 and 4 are shown. The signal peptide, propeptide, and transmembrane domains are indicated by a hatched rectangle, a grey box, and a black box, respectively. Triangles and asterisks denote protease aspartic active sites and *N*-glycosylation sites, respectively. pmt; premature termination codon. The positions of primers A (nt 344–363), B (antisense of nt 711–692), C (nt 180–199), D (nt 220–239), E (antisense of nt 590–570) and F (antisense of nt 614–595) are indicated by arrows. (B) cDNA from SH-SY5Y cells treated with vehicle (DMSO), 8 μM anisomycin (Ans), 90 μM cycloheximide (Chx), or 40 μM emetine hydrochloride (Eme) for 6 h was subjected to PCR amplification (30 cycles) using primers A and B [16]. (C) Presence of exons 1–4 in I-127. cDNA from SH-SY5Y cells treated with 8 μM Ans was amplified by PCR (30 cycles) using the indicated primer pairs. The arrowhead, square, and circle denote the PCR products derived from BACE, I-457, and I-127, respectively. (D) Detection of I-127 mRNA in the human brain. cDNA from SH-SY5Y cells treated with 8 μM Ans and from cortical tissues were subjected to PCR amplification using primers A and B under the indicated cycling conditions. AD1 to AD5 were AD samples. Ctr1 to 5 were non-AD control samples.

female, age 68, Ctr4; male, age 82, Ctr5; male, age 58). All brain samples were approved to be used for research by informed consent from the family of each patient.

The entire coding sequence of BACE or I-127 cDNA, tagged with a hemagglutinin (HA)-epitope sequence at the C-terminus, was amplified and cloned into the pcDNAZeo vector (Invitrogen). HEK293 cells were transfected with pcDNAZeo, pcDNA/BACE-HA or pcDNA/I-127-HA using LipofectAMINE^{TM} 2000 transfection reagent (Invitrogen). At 5 h after transfection, the medium was changed and at 48 h post-transfection, cells and culture media were harvested for subcellular fractionation. Metabolic labeling and pulse-chase experiments were performed as described in [16] and the cells were chased in MEM supplemented with 10% FBS for the times indicated in the figure. For treatment with proteasome or lysosomal degradation inhibitors, 50 μM MG132 (Merck), 20 μM *clasto*-lactacystin β-lactone (Merck), 20 mM ammonium chloride (Sigma) or 100 μM chloroquine (Sigma) were added at 6 h before cell harvesting at 30 h post-transfection.

Cells were washed with ice-cold PBS and removed in the presence of removing medium [12]. For the preparation of total cell lysate, the cells were sonicated in RIPA buffer [16]. For subcellular fractionation, the cells were homogenized in homogenization medium [12] by repeated aspiration: 10 times each through 22- and 25-gauge needles, respectively. The homogenates were spun at $1000 \times g$ for 10 min and the supernatants were spun at $3000 \times g$ for 10 min. The obtained supernatants were further centrifuged at $100{,}000 \times g$ for 30 min, and the resulting supernatants and pellets dissolved in SDS sample buffer were used as cytosol and membrane fractions, respectively. The collected culture media were precleared with UltraLink^{TM} immobilized protein A/G (Pierce).