Promotion of BACE1 mRNA Alternative Splicing Reduces Amyloid β-Peptide Production*

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Production of the amyloid β-peptide (Aβ) via sequential proteolytic cleavage of the amyloid precursor protein by β- and γ-secretases is strongly implicated in the pathogenesis of Alzheimer disease. The β-secretase that executes the first cleavage event is a transmembrane aspartyl protease known as β-site amyloid precursor protein-cleaving enzyme 1 (BACE1). BACE1 pre-mRNA is alternatively spliced through the use of alternative splice sites in exons 3 and 4, although the significance of these splicing events is unclear. Here, we quantitatively measured relative levels of BACE1 transcripts and identified a novel splice variant of BACE1. We found a subtle but significant difference in BACE1 splicing between brain and pancreas, indicating the cellular environment can affect BACE1 alternative splicing. Furthermore, we have shown that BACE1 proteins translated from alternatively spliced transcripts have dramatically reduced β-secretase activity and promotion of BACE1 alternative splicing reduces Aβ production. These findings illustrate the importance of BACE1 alternative splicing in affecting the level of Aβ produced in cells and suggest that targeting regulation of BACE1 alternative splicing is a potential therapeutic strategy for lowering β-secretase activity.

Deposition of extracellular plaques in the brain is a hallmark of Alzheimer disease (AD) pathology. The major component of these plaques is the amyloid β-peptide (Aβ), a hydrophobic peptide usually 40 or 42 amino acids in length and prone to aggregation (1, 2). Aβ is produced by sequential proteolysis of the amyloid precursor protein (APP) by β- and γ-secretases (3, 4). β-site APP-cleaving enzyme 1 (BACE1) is the primary transmembrane aspartyl protease responsible for β-secretase activity in the brain and carries out the first cleavage step leading to Aβ production (5–9). Moreover, BACE1 protein and activity levels are elevated in AD brains relative to controls, further suggesting its involvement in AD pathogenesis (10–14).

Elimination or reduction of BACE1 activity in order to slow Aβ production is an attractive therapeutic strategy for AD. Although the brains of Bace1 knock-out mice do not produce Aβ and these mice appear relatively healthy (15–17), there is evidence that complete absence of BACE1 may cause reduced myelination as well as some cognitive deficits in mice (18–20). However, partial reduction of BACE1 has been shown to dramatically reduce plaque deposition and synaptic deficits in APP transgenic mouse models (20–22). Although a number of BACE1 inhibitors have been developed, identification of a selective compound that is potent in vivo and practical for clinical development has proven challenging (23, 24). The BACE1 gene is composed of nine exons, and its pre-mRNA was previously found to undergo alternative splicing (25–27). BACE1 mRNA is most highly expressed in brain and pancreas (5–9), and alternatively spliced transcripts of BACE1 have been identified in both tissues (26, 27). Normal splicing of BACE1 results in production of the full-length 501-amino acid active protein (BACE1 501). Alternative splicing of BACE1 occurs through the use of an alternative 5′-splice site within exon 3 and/or an alternative 3′-splice site within exon 4 (Fig. 1A). The use of the normal 5′-splice site in combination with the alternative 3′-splice site in exon 4 causes an in-frame deletion of 75 base pairs at the 5′-end of exon 4, resulting in production of a protein isoform 476 amino acids in length (BACE1 476). Similarly, the use of the alternative 5′-splice site in exon 3 in combination with the normal 3′-splice site causes an in-frame deletion of 132 base pairs at the 3′-end of exon 3. This deletion results in a protein isoform 457 amino acids in length (BACE1 457). Finally, the use of both alternative splice sites results in an in-frame deletion of 207 base pairs and production of a 432-amino acid protein isoform (BACE1 432).

These alternative splicing events delete a portion of the protein between the two catalytic aspartates located in exon 2 and exon 6. The activities of BACE1 protein isoforms produced by alternative splicing are not well established. Past studies suggest that BACE1 476 and BACE1 457 have reduced activity or are completely inactive against APP, although the activities of BACE1 476, BACE1 457, and BACE1 432 have never been measured directly and the issue remains largely unresolved (25–27).

Because BACE1 occupies such an important position in the pathway toward Aβ generation and BACE1 alternative splicing could produce changes in overall β-secretase activity, a comprehensive and systematic investigation of this splicing event is necessary. In this study, we quantified the relative levels of BACE1 alternatively spliced transcripts using real-time PCR,

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and we identified a novel alternative splicing event. Furthermore, we characterized the activity of the resulting protein isoforms, and we showed that increasing BACE1 alternative splicing can reduce Aβ levels in cells.

**EXPERIMENTAL PROCEDURES**

**Plasmids**—The cDNA encoding BACE1 501, 476, and 457 cloned into the pGEM-T vector (Promega) were received as a gift from Dr. Patrick Keller. The cDNA sequences from these plasmids were then cloned into pcDNA4/myc-His (Invitrogen). Creation of BACE1 432, construction of a BACE1 501 active site mutant, and correction of pre-existing mutations were accomplished using QuickChange Multi-Site Mutagenesis (Stratagene). Mutagenesis primer sequences can be found in the primer sequences table (supplemental Fig. S1).

**Cell Culture and Immunoprecipitation**—Human embryonic kidney (HEK) 293 cells stably overexpressing Swedish-mutated (KM → NL) APP (HEKsw) (28) were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum (Invitrogen), 2 mM glutamine (Invitrogen), 1% penicillin-streptomycin (Invitrogen), and 200 μg/ml geneticin (Invitrogen). HEK cells were transfected with BACE1 cDNA pcDNA 4/myc-His expression vectors. Cell lysates were collected with M-PER Mammalian Protein Extraction Reagent (Pierce) supplemented with Mini Complete Protease Inhibitor Mixture tablets (Roche Applied Science). Myc-tagged proteins were immunoprecipitated with the ProFound Mammalian c-Myc Tag IP kit (Pierce). Relative concentrations of each purified protein were determined by electrophoresis of serial dilutions of each immunoprecipitated sample on an 8–16% Tris-glycine gel (Invitrogen) and Western blotting with a 1:5000 dilution of an anti-Myc horse radish peroxidase antibody (Invitrogen).

**β-Secretase Activity Assay**—Equal amounts of purified BACE1 protein isoforms were normalized and supplemented with Mini Complete Protease Inhibitor Mixture (Roche Applied Science). Each sample was incubated with 200 nM OM99-2 BACE1 inhibitor (29) (EMD Biosciences) in DMSO (Sigma) or DMSO alone. Samples were assayed for β-secretase activity using the SensoLyte 520 β-secretase assay kit (AnaSpec) and measuring fluorescent signal at Ex/Em = 485/535 nm following a 30-min incubation at 37 °C.

**Treatment of Cells with Antisense RNA Oligos**—HEKsw cells were plated in 6-well plates coated with poly-d-lysine hydrobromide (Sigma). Cells were then transfected with 25 nM 2′-O-methyl antisense RNA oligos with a phosphorothioate backbone (Dharmacon) using Lipofectamine 2000 (Invitrogen). Antisense RNA oligos contained either a randomized sequence, a sequence complementary to the normal splice site at the exon 3-intron 3 junction, or a sequence complementary to the normal splice site at the intron 3-exon 4 junction (supplemental Fig. S1). In cells treated with two antisense RNA oligos, each oligo was transfected at a final concentration of 25 nM. Twenty-four hours after transfection, medium on the transfected cells was changed and aliquots of the new medium were taken after 0, 4, and 8 h.

**Enzyme-linked Immunosorbent Assay (ELISA)**—Aβ-40 levels were measured by colorimetric human β-amyloid 1–40 ELISA (Invitrogen). Medium collected from treated cells was centrifuged at 6800 × g for 3 min and diluted 1:16 for the assay.

**Reverse Transcription**—Total RNAs from frontal lobe, cerebellum, hippocampus, and pancreas were purchased (Stratagene, Clontech, and Ambion), and samples were pooled by tissue type. Total RNA from HEK cells was isolated using RNAqueous (Ambion). For RT, concentrations of total RNAs were quantified, and RT was carried out on 1 μg of total RNA using QuantiTect Reverse Transcription (Qiagen). Following RT, sample volumes were doubled with H2O.

**Semi-quantitative PCR**—To simultaneously amplify BACE1 501, 476, 457, and 432 from RT products, PCR was performed using 400 nM forward primer in exon 3 and 400 nM reverse primer in exon 4 (both contain restriction endonuclease site 5′-overhangs). For amplification of BACE1 455, the exon 3 forward primer and an isoform-specific reverse primer designed to anneal to the exon 3-exon 5 junction were used. For an endogenous control, a forward primer and reverse primer were designed and each used at a final concentration of 100 nM to amplify a region of ribosomal protein L39 (RPL39). All PCR primer sequences are listed in the primer sequences table (supplemental Fig. S1). All PCR was carried out using Advantage 2 Polymerase (Clontech) and the following PCR program: 1 cycle of 95 °C for 1 min; 25 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 30 s, and extension at 68 °C for 1 min; 1 final extension cycle of 68 °C for 1 min. Following PCR, Novex Hi-Density Tris borate-EDTA (TBE) sample buffer (Invitrogen) was added to each sample. PCR products were separated by electrophoresis on a 20% TBE-acrylamide gel (Invitrogen) and visualized by post-staining with a 1:10,000 dilution of SYBR Gold (Invitrogen) in 1× TBE buffer (Invitrogen).

**Real-time PCR**—Real-time PCR primers were designed to specifically amplify each BACE1 isoform individually. Primer sequences are listed in the primer sequences table (supplemental Fig. S1). For 501, the forward primer anneals to the 3′-end of exon 3, and the reverse primer anneals to the 5′-end of exon 4. For 476, the forward primer anneals to the 3′-end of exon 3, and the reverse primer anneals to the isoform-specific exon 3–alternative exon 4 site junction. For 457, the forward primer anneals to the isoform-specific alternative exon 3 site-exon 4 junction, and the reverse primer used was the same as for 501. For 432, the forward primer anneals to the 5′-end of exon 3 and the reverse primer anneals to the alternative exon 3 site-alternative exon 4 site junction. For 455, the same forward primer used for 476 and the 455 isoform-specific reverse primer described in the semi-quantitative PCR methods were used. The forward and reverse primers used to amplify total BACE1 annealed within exon 5. For an endogenous control, a forward primer and reverse primer were designed to amplify a region of RPL39. Standards for each isoform were made by amplifying the region between exon 3 and exon 6 of each BACE1 cDNA pcDNA4/myc-His expression vector, electrophoresing products on an agarose gel, and purifying the amplified bands by MiniElute gel purification (Qiagen). 10-fold serial dilutions of each standard were made and used to produce standard curves for each isoform. Primer pairs were used at a final concentration of 800 nM, except for the RPL39 primers that were used at 200 nM. Real-time PCR was performed with Power SYBR Green
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Master Mix (Applied Biosystems) using the ABI 7500 Fast System (Applied Biosystems). Amounts of each isoform in each sample were normalized to RPL39 and in most cases expressed as an amount relative to BACE1 501. All quantified data represent an average of at least two independent experiments. Error bars represent S.E. of the mean. Data comparing differences between two samples were statistically analyzed using the unpaired Student’s t test. Differences were considered significant when p < 0.05.

RESULTS

BACE1 Alternative Splicing Differs between Brain and Pancreas—BACE1 has been previously shown to be most highly expressed in the brain and pancreas in humans (5–9). We wanted to confirm that alternatively spliced BACE1 mRNA exists in these tissues. The region of BACE1 between exon 3 and exon 4 was amplified by semi-quantitative RT-PCR in samples of total RNA from human cerebellum, frontal lobe, hippocampus, and pancreas. Amplification of this region produces four products corresponding in size to the four previously reported splice variants of BACE1. Multiple RNA samples from three different sources were pooled according to tissue type for analysis. Our PCR data indicate that each of the four splice variants is present in brain and pancreas (Fig. 1B). Full-length BACE1 501 is the predominantly expressed transcript in all of the tissue types analyzed, whereas levels of the alternatively spliced transcripts are relatively low and decrease corresponding to size. However, we observed higher levels of BACE1 476 in pancreas compared with the regions of the brain tested. To quantify these results, a real-time PCR assay was developed to measure levels of each alternatively spliced transcript in relation to BACE1 501. Although there are no significant differences in splicing detected between cerebellum, frontal lobe, or hippocampus, the ratio of BACE1 476 to BACE1 501 is about two times greater in pancreas than in these brain regions (Fig. 1C). Real-time PCR also shows an increase in the ratio of BACE1 432 to BACE1 501 in pancreas that is statistically significant, but there is no significant difference in the ratio of BACE1 457 to BACE1 501. We saw similar results when total RNA samples from these tissues were analyzed individually, instead of being pooled (data not shown). These results suggest that cellular factors in pancreas weaken the usage of the normal 3’-splice site at the intron 3-exon 4 junction or enhance the recognition of the alternative 3’-splice site in exon 4.

BACE1 pre-mRNA Undergoes Exon 4 Skipping—Because our findings support the possibility that usage of the normal 3’-splice site is not recognized by splicing machinery as robustly in pancreas as in brain, we reasoned that exon 4 skipping might also occur in pancreas (Fig. 2A). A reverse primer was designed to anneal to the exon 3-exon 5 junction to more simply detect only an exon 4-skipped transcript. Using the isoform-specific primer pair, semi-quantitative RT-PCR was performed on total RNA from human cerebellum, frontal lobe, hippocampus, and pancreas. Surprisingly, we found a low level of transcript lacking exon 4 in all tissues tested (Fig. 2B). An isoform-specific RT-PCR designed to identify an exon 3-skipped transcript did not yield any detectable products (data not shown). Because exon 4 skipping results in an in-frame deletion that would produce a protein 455 amino acids in length, the transcript completely lacking exon 4 will subsequently be referred to as BACE1 455 (Fig. 2A). This splice variant was not detected in our previous experiments because the reverse primer used for PCR amplification in those experiments was designed to anneal to exon 4. Furthermore, exclusion of exon 4 by alternative splicing produces a product with only six fewer base pairs than the BACE1 457 transcript, meaning it would be difficult to distinguish these two transcripts from each other based on RT-PCR product size. Real-time PCR shows BACE1 455 is expressed in tissues at low levels relative to BACE1 501 (Fig. 2C). Interestingly, the level of BACE1 455 mRNA is about three times greater in pancreas compared with regions of the brain tested (Fig. 2B and C). This, along with the observation that BACE1 476 and BACE1 432 also exist at higher levels of each alternatively spliced transcript in relation to BACE1 501.

A

B

C

FIGURE 1. Alternative splicing of BACE1 within exon 3 and exon 4 in brain and pancreas. A, the use of normal splice sites and alternative splice sites of BACE1 produces four RNA transcripts (BACE1 501, BACE1 476, BACE1 457, and BACE1 432). The alternative 5’-splice site (ALT 5’ SS) is in exon 3, and the alternative 3’-splice site (ALT 3’ SS) is in exon 4. Use of the normal 5’-splice site (NORM 5’ SS) and the normal 3’-splice site (NORM 3’ SS) results in production of the full-length BACE1 501 transcript. B, total RNA samples were pooled by tissue type (cerebellum, n = 24; frontal lobe, n = 4; hippocampus, n = 20; pancreas, n = 3) and amplified. Transcripts of BACE1 (501, 476, 457, and 432) were detected in cerebellum (C), frontal lobe (FL), hippocampus (H), and pancreas (P) by semi-quantitative RT-PCR between exons 3 and 4. C, real-time PCR revealed a statistically significant increase in BACE1 476 (black) and BACE1 432 (white) relative to BACE1 501 in pancreas compared with regions of the brain tested (*, p < 0.05 compared with pancreas). No significant difference in the ratio of BACE1 457 (gray) relative to BACE1 501 was detected. Amplification of RPL39 was used as an endogenous control. For real-time PCR data analysis, quantities were normalized to RPL39 and then expressed as a proportion relative to BACE1 501. n = 2. Error bars represent S.E. of the mean.
levels in pancreas, suggests that usage of the normal 3′-splice site at the intron 3-exon 4 junction is weaker in pancreas than in brain. Because we found that exon 4 skipping occurs, we reasoned that a transcript resulting from use of the alternative 5′-splice site in exon 3 and the 3′-splice site at the intron 4-exon 5 junction may exist. However, an RT-PCR designed to amplify such a transcript did not produce a detectable product from tissue RNA (data not shown).

**BACE1 Protein Isoforms Produced from Alternatively Spliced Transcripts Have Reduced Activity**—After examining BACE1 splice variants in tissues, we sought to determine the effect of alternative splicing on BACE1 protease activity. The amino acids deleted by alternative splicing lie between the two active site aspartates in exons 2 and 6. The crystal structure of the catalytic domain of full-length BACE1 501 was previously solved (30). Analysis of the catalytic domain structure reveals that the alternatively spliced region has well defined structure and is in close proximity to the active site residues of the folded protein (Fig. 3A). It is reasonable to predict that the absence of this region would be detrimental to the structural integrity of the catalytic domain and, therefore, the activity of the enzyme.

In light of the structural data, we directly measured the activity of individual BACE1 protein isoforms. Each BACE1 transcript was cloned into an expression vector with a C-terminal Myc tag. Myc-tagged BACE1 isoforms were then individually expressed and purified. Relative concentrations of the purified proteins were determined by Western blotting (Fig. 3B). Equal amounts of each purified protein were tested in an in vitro β-secretase activity assay that detects cleavage of a fluorescent substrate. As expected, the active site mutant was catalytically inactive (data not shown). Although the four alternatively spliced protein isoforms show a low level of BACE1 activity in relation to the catalytically inactive mutant, the activity of these isoforms is severely reduced compared with that of BACE1 501 (Fig. 3C). Addition of a previously characterized β-secretase inhibitor (OM99-2) (29) further reduces activity in all samples. Taken together, these results suggest that the alternative splicing region has well defined structure and is in close proximity to the active site residues of the folded protein. The crystal structure of the catalytic domain of full-length BACE1 501 was previously solved (30).
results show that alternative splicing of BACE1 pre-mRNA results in the deletion of regions required for robust catalytic activity and suggest that an increase in BACE1 alternative splicing might result in a reduced level of overall β-secretase activity.

**Increasing BACE1 Alternative Splicing Reduces Aβ Production**—Because alternative splicing of BACE1 produces protein isoforms with severely reduced activity, we wanted to determine whether BACE1 splicing could be altered in such a way as to increase production of these isoforms in cells. Alternative splicing of endogenous BACE1 in a HEK cell line stably overexpressing Swedish-mutated APP (HEKswé) (28) is similar to that seen in brain and pancreas (Figs. 1B and 4A, lane 1). Antisense RNA oligonucleotides with sequences complementary to normal splice sites can be used to physically prevent splicing factors from accessing these sites, thereby promoting usage of alternative splice sites. Antisense RNA oligos were designed to target either the normal 5′-splice site at the exon 3-intron 3 junction or the normal 3′-splice site at the intron 3-exon 4 junction of BACE1. These antisense RNA oligos were transfected into HEKswé cells to increase production of alternatively spliced BACE1 isoforms. As expected, both semi-quantitative RT-PCR and real-time PCR indicate that blocking the normal 3′-splice site with antisense RNA results in a decrease in usage of this site, therefore significantly increasing levels of BACE1 476 and BACE1 432 mRNA while decreasing levels of full-length BACE1 501 (Fig. 4, A and B). Similarly, blocking only the normal 5′-splice site results in an increase in BACE1 457 and BACE1 432 production, although the effect of this oligo appears to be more modest, having little effect on the level of BACE1 501. Treatment of cells with both antisense RNA oligos targets both normal splice sites and has the expected effect of increasing production of all alternatively spliced transcripts, especially BACE1 432. A faint fifth PCR product detected when the normal 5′-splice site is blocked corresponds in size to a recently discovered splice variant of BACE1 thought to be a target of nonsense-mediated mRNA decay, and its levels would be expected to increase under these conditions (31) (Fig. 4A, asterisk).

We also wanted to determine the effect of antisense RNA treatment on BACE1 455 transcript levels (i.e., exon 4 skipping). Furthermore, we reasoned that treatment with the antisense RNA oligo targeted against the normal 5′-splice site might promote exon 3 skipping. Although no significant amount of exon 3 skipping is detected under any conditions, we were unable to detect this transcript in tissues, RT-PCR using a forward primer designed to anneal in exon 2 and a reverse primer designed to anneal in exon 5 indicates that this transcript exists at very low levels in untreated HEKswé cells (data not shown). Production of this transcript increases upon antisense RNA treatment, although under all conditions it remains at levels lower than that of BACE1 432. The changes in alternative splicing we observed are not due to nonspecific effects of trans-
fected antisense RNA oligos because no effect is seen in cells treated with an antisense RNA oligo with a randomized sequence. An increase in alternative splicing should result in increased levels of inactive BACE1 protein isoforms and a corresponding decrease in the level of full-length catalytically active BACE1 protein. Therefore, increasing BACE1 alternative splicing with antisense treatment did not affect total BACE1 mRNA levels. For real-time PCR data analysis, the quantity of total BACE1 in each sample was normalized to RPL39 and then expressed as a proportion relative to MOCK. n = 2. Error bars represent S.E. of the mean.

FIGURE 5. Effect of antisense RNA oligo treatment on Aβ production. A, media from oligo-treated cells were tested for Aβ-40 levels by ELISA. After 4 (gray) or 8 (white) hours, a statistically significant reduction in Aβ-40 was seen with the 3′ AS oligo (⁎, p < 0.05 compared with MOCK). The 5′ AS oligo alone was not effective, consistent with its inability to substantially reduce BACE1 501, but it did enhance the Aβ-40-lowering effect of the 3′ AS oligo after 8 h (⁎⁎, p < 0.05). n = 2. B, real-time PCR using primers in exon 5 showed antisense treatment did not affect total BACE1 RNA levels. For real-time PCR data analysis, the quantity of total BACE1 in each sample was normalized to RPL39 and then expressed as a proportion relative to MOCK. n = 2. Error bars represent S.E. of the mean.

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cells is due specifically to changes in alternative splicing and not a reduction in total BACE1 expression. Western blot analysis of cell lysates shows no decrease in APP protein levels upon treatment with antisense RNA oligos (data not shown). Additionally, lactate dehydrogenase cytotoxicity assays indicate that transfection of cells with antisense RNA oligos did not induce cell death (data not shown). Taken together, these results demonstrate that BACE1 splicing can be manipulated in cells and show that differences in BACE1 alternative splicing can influence the level of Aβ produced by cells.

**DISCUSSION**

BACE1 mRNA levels are highest in brain and pancreas (5–9), and alternative splicing has been shown to occur in both tissues (26, 27). Here, we have for the first time used real-time PCR to quantify the levels of each splice variant in relation to full-length BACE1 501 in both brain and pancreas, allowing us to detect subtle differences in BACE1 alternative splicing. Although robust β-secretase activity exists in the brain, no activity is observed in the pancreas (7). This cannot be due to tissue-specific differences in alternative splicing, because BACE1 501 mRNA was previously found to be the most abundant transcript in both brain and pancreas (26, 27). Our semi-quantitative and real-time PCR results in tissues support this conclusion. Instead, the difference in activity between brain and pancreas may be due to varying levels of endoproteolysis within the catalytic domain of BACE1 (32).

Although BACE1 457 was previously cloned from human brain (26), some reports have suggested that BACE1 457 is a pancreas-specific BACE1 isoform (25, 27) or is expressed in frontal cortex but not in cerebellum (33). Using real-time PCR, we found levels of BACE1 457 mRNA in human cerebellum, frontal lobe, and hippocampus comparable with those seen in human pancreas. Furthermore, we have shown clearly that BACE1 501, BACE1 476, BACE1 457, and BACE1 432 mRNAs are all present in human brain and pancreas tissues to some degree. Although our data indicate that alternatively spliced transcripts of BACE1 constitute a relatively low proportion of total BACE1 mRNA, it is possible that these transcripts exist at higher levels in tissue types not tested here, or under certain physiological conditions.

Our data indicate that there are no differences in BACE1 alternative splicing between cerebellum, frontal lobe, and hippocampus. However, our findings do show a subtle but significant difference in BACE1 splicing between pancreas and the regions of the brain we tested. The increased level of BACE1 476 and BACE1 432 in pancreas suggests less usage of the normal 3′-splice site in exon 4 (7). This finding also illustrates that BACE1 splicing can be sensitive to the cellular environment, raising the possibility that cellular perturbations, endogenous or pharmacological, can modulate BACE1 splicing.

We also found that exon 4 skipping occurs endogenously and, when translated, would produce a BACE1 isoform 455 amino acids in length. Interestingly, we found that the level of BACE1 455 relative to BACE1 501 is higher in pancreas compared with that seen in cerebellum, frontal lobe, and hippocampus, further supporting the idea that usage of the normal
3′-splice site at the intron 3-exon 4 junction is slightly impaired in pancreas compared with brain. BACE1 455 has not previously been described, possibly due to its low expression level in tissues and its similarity in size to BACE1 457. Because of the apparent complexity of BACE1 alternative splicing, we cannot rule out the possibility that alternative splicing also results in other BACE1 RNA transcripts that either exist at very low levels or are products of alternative splicing outside of the region between exon 3 and exon 4. Additional alternative splicing of BACE1 RNA could result in the production of protein isoforms with varying levels of activity or rapid degradation of an unstable transcript. Indeed, a splice variant of BACE1 regulated by nonsense-mediated mRNA decay has recently been identified (31).

Previous attempts to determine the activity of BACE1 476 and BACE1 457 have been inconclusive (25–27). Furthermore, there are no data addressing the activity of BACE1 432 or BACE1 455. Analysis of the structure of the catalytic domain of BACE1 suggests that alternative splicing of BACE1 RNA would severely affect the protease activity of the protein. We purified and tested BACE1 protein isoforms in an in vitro β-secretase assay and found that alternative splicing of BACE1 produces proteins with very low catalytic activity against the APP substrate. Because our assay measured activity exclusively against an APP-based substrate, it is possible that BACE1 isoforms translated from alternatively spliced transcripts exhibit different substrate selectivity than BACE1 501. However, the effective protease activity of these shortened BACE1 protein isoforms in vivo may be virtually non-existent. In BACE1 457, BACE1 455, and BACE1 432, the deleted region includes glycosylation sites thought to be required for proper enzymatic maturation (25–27). Additionally, there is some evidence that BACE1 476 and BACE1 457 may be retained in the endoplasmic reticulum due to improper protein folding, thereby resulting in proteasome-mediated endoplasmic reticulum-associated degradation (27, 34).

It remains unclear whether proteins translated from alternatively spliced BACE1 transcripts play a role in vivo. We acknowledge that alternatively spliced variants of BACE1 may not yet have a specific physiological function. Instead, these splicing events may simply represent a mechanism for enhancing proteomic diversity that could lead to the development of new functions independent of protease activity. However, the effect of alternative splicing on BACE1 activity is intriguing because the brains of AD patients exhibit increased BACE1 protein levels and activity compared with healthy individuals (10–14). Therefore, it is possible that variations in BACE1 splicing could have either pathogenic or protective effects in the human brain.

BACE1 inhibition has been considered a possible therapeutic strategy in treatment or prevention of AD (23, 24). Knock out of Bace1 rescues cognitive deficits seen in APP transgenic mice (20–22, 35). However, it may not be desirable to completely inhibit BACE1 activity (18–20). We have shown that treatment of cells with specific antisense RNA oligonucleotides can increase alternative splicing of BACE1 RNA, decrease levels of full-length BACE1, and correspondingly reduce Aβ-40 production. Modulation of pre-mRNA splicing by antisense oligos is an increasingly promising approach for treatment of diseases such as Duchenne muscular dystrophy (36). Therefore, targeting normal splicing of BACE1 with antisense RNA oligos in order to increase alternative splicing could be a useful therapeutic strategy for achieving partial inhibition of BACE1. Additionally, unique secondary structure of the RNA molecule itself or protein factors that specifically regulate BACE1 alternative splicing would be attractive pharmacological targets.

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