BRI2 regulates β-amylloid degradation by increasing levels of secreted insulin degrading enzyme (IDE)

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Background: The British Precursor Protein (BRI2) influences Amyloid Precursor Protein metabolism. Results: BRI2 lowers β-amylloid peptide levels by increasing levels of secreted insulin degrading enzyme (IDE) in both cells and mice. Conclusion: BRI2 as a receptor protein regulates IDE levels and in turn promotes β-amylloid degradation. Significance: Targeting the regulation of IDE may lead to new approaches to therapeutically address sporadic Alzheimer’s disease.

SUMMARY

The amyloid precursor protein (APP) is one of the major proteins involved in Alzheimer’s disease (AD). Proteolytic cleavage of APP gives rise to amyloid-β (Aβ) peptides which aggregate, and deposit extensively in the brain of AD patients. Although the increase in levels of aberrantly folded Aβ peptide is considered to be important to disease pathogenesis, the regulation of APP processing and Aβ metabolism is not fully understood. Recently, the British Precursor Protein (BRI2, ITM2B) has been implicated in influencing APP processing in cells, and Aβ deposition in vivo. Here we show that the wildtype BRI2 protein reduces plaque load in an AD mouse model, similar to its disease associated mutant form, ADα precursor protein (ADαPP), and analyze in more detail the mechanism of how BRI2 and ADαPP influence APP processing and Aβ metabolism in cells. We find that overexpression of either BRI2 or ADαPP reduces extracellular Aβ by increasing levels of secreted insulin degrading enzyme (IDE), a major Aβ degrading protease. This effect is also observed with BRI2 lacking its C-terminal 23 amino acid peptide sequence. Our results suggest that BRI2 might act as a receptor protein that regulates IDE levels which in turn influences APP metabolism in a previously unrecognized way. Targeting the regulation of IDE may be a promising therapeutic approach to sporadic AD.

Alzheimer’s disease (AD) is a disease of progressive dementia and neuron loss characterized by the deposition of β-amyloid plaques and the formation of neurofibrillary tangles in the brain (1). The main components of amyloid plaques, the amyloid-beta (Aβ) peptides, are derived by sequential proteolytic cleavage of the amyloid precursor protein (APP) (2). While mutations in the APP or the PS1 or PS2 genes, leading to alterations in APP processing, are genetically linked to
familial cases of AD (3), increased levels of amyloidogenic Aβ peptides are believed to play a major role in disease pathogenesis (4). However, besides Aβ generation, Aβ clearance is of equal importance in maintaining Aβ steady state levels. The metalloproteases nepriyin (NEP), endothelin-converting enzyme (ECE-1, ECE-2), and insulin-degrading enzyme (IDE) are the main Aβ-degrading enzymes in the brain (5,6) and reduced clearance of Aβ peptides may be more related to cases of sporadic AD (7).

Recently, the British Precursor Protein (BRI2, ITM2B, E25) (8-10) has been implicated to influence APP processing in cells, and Aβ aggregation in vivo (11-15). Although mutations in the BRI2 gene have been linked to familial Danish Dementia (FDD) and familial British Dementia (FBD) (10,16), the physiological function of wildtype BRI2 is not known. The 266 aa type-II transmembrane protein is processed by several proteases to shed a 23 aa peptide via furin-like cleavage (17), and a fragment including the BRICHOS domain (18) into the extracellular space (19). In FDD an 11 aa longer protein, ADan precursor protein (ADanPP), is expressed due to a 10 nucleotide duplication before the stop codon of the BRI2 gene causing a frameshift and elongation of the open reading frame (16). Furin-like cleavage of ADanPP yields a 34 aa peptide prone to amyloid formation (16). BRI2 can interact with APP and has been proposed to inhibit secretase cleavage of APP (11,13-15). Other studies report an interaction of the 23 aa BRI peptide, or the BRICHOS domain, with Aβ in vitro, and a potential inhibition of Aβ aggregation in vivo (12,20).

We previously showed that even the disease related form of BRI2, ADanPP, reduces plaque deposition in mouse models of AD (21). Given that these two dementia related proteins can interact and influence each other, this prompted us to further investigate the underlying mechanisms of the observed Aβ plaque reduction. We suspected that not only Aβ generation but also Aβ clearance by degrading proteases may be affected, since Aβ as well as BRI2-derived peptides can be degraded by IDE (22), one of the major Aβ-degrading enzymes in the brain.

EXPERIMENTAL PROCEDURES

Plasmids- The cDNA encoding the human wt form of BRI2 in vector pCR2.1 (provided by R. Vidal, Indianapolis, IN) was mutated by standard mutagenesis using PfuUltra High-Fidelity DNA Polymerase (Stratagene) to introduce the Danish mutation (10 nucleotide insertion TTTAATTGTG). BRI2 and ADanPP cDNA inserts were liberated by digest with BamHI, and introduced blunt-ended into the EcoRV cloning site of pcDNA3.1/Zeo(+) (Invitrogen). To generate the BRI2Δ construct a stop-codon was introduced following the triplet encoding aa 243 of BRI2 by site-directed mutagenesis of the pcDNA3.1/Zeo(+) construct. All cDNA constructs were verified by sequencing. The CMV wildtype βAPP695 cDNA construct (23) was used to express APP695 (24). A human PS1 cDNA mutated at L166P (25) was inserted into pcDNA3.1/Zeo(+) (Invitrogen). pEGFP-C1 (Clontech) was used to express EGFP.

Generation of transgenic mice expressing wildtype human BRI2 (wtBriPP)- BRI2 cDNA (see above) was introduced into the blunt-ended SalI cloning site of the cosmid-based Syrian Hamster prion protein expression vector (provided by S. Prusiner, San Francisco, CA). After removal of vector sequences by NotI digestion, microinjections of the purified construct into C57Bl/6 pronuclei yielded several putative founders C57Bl/6N-Tg(SHaPrP-BRI2). Two founders were further bred with C57Bl/6J mice to produce stable transgenic lines. All mice included in the analyses are of generation F2 or higher.

Transgenic mice used for cross-breeding- The APPPS1-21 mice used for cross-breeding with wtBriPP transgenic mice have been described previously (25). APPPS1-21 mice have been generated and maintained on a pure C57Bl/6J background. All mice analyzed were hemizygous for the transgene(s) of interest. All animal experiments were performed in accordance with the current German animal welfare law and licensed by the local
Histology and immunohistochemistry- Brains were removed upon sacrifice and immersion-fixed in 4% paraformaldehyde. Immunohistochemistry was done on 25μm thick coronally cut cryoprotected free-floating frozen sections, using standard immunoperoxidase procedures with Elite ABC kits (Vector Laboratories) with Vector SG (Vector Laboratories, Burlingame, CA, USA). The CN3 polyclonal antibody to Aβ was used as previously described (26).

Stereology and quantification of pathology- Stereological analysis was performed using a microscope equipped with a motorized x-y-z stage coupled to a video-microscopy system (Systems Planning and Analysis, Inc., Alexandria, VA). Neocortical brain regions were defined using a standard mouse brain atlas (Franklin and Paxinos). Quantification was done on the left hemisphere. Analysis of Aβ amyloid load was done on a series of coronally cut 25μm free-floating sections (every 24th section for the neocortex). Thus all analyses included 8-10 sections per animal. The amyloid load (percentage) was determined by calculating the areal fraction occupied by immunoreactive Aβ, in two-dimensional sectors at a single focal plane at 20x/0.45 numerical aperture.

Cell culture, Transfection and Treatment- HEK293 cells (ATCC), HEK293 cells stably expressing wildtype βAPP695 cDNA (HEK293-APPwt cells) (23), and HeLa cells (ATCC) were cultured in DMEM 4.5 g/L glucose with L-glutamine (Lonza) supplemented with 10% fetal calf serum (Invitrogen) and 1% penicillin/streptomycin (Invitrogen). Transient transfection of cells was carried out using FuGene HD (Roche) or Lipofectamine LTX with Plus Reagent (Invitrogen) according to the manufacturer's instructions. Cells transfected with pcDNA3.1/Zeo(+) vector served as a control. One day after transfection, cells were supplemented with fresh medium and incubated for 24h to obtain conditioned cell media. Insulin solution (human), 10mg/ml, cell culture tested (Sigma) was added to the cell culture medium in 10μM concentration during the incubation period as indicated. After gathering conditioned cell media, the relative number of cells was measured using alamarBlue® cell viability reagent and fluorescent detection according to the manufacturer's protocol (Invitrogen), to adjust for differences in cell numbers.

Antibodies and Western Blot Analysis- The following antibodies were used: monoclonal antibody 6E10 specific to human Aβ (crude ascites; Covance), anti-APP-C-terminal rabbit polyclonal antibody A8717 (Sigma-Aldrich), monoclonal antibody 8G4 to GAPDH (HyTest Ltd), chicken polyclonal antibody to ITM2B raised against amino acids 1-60 (Abcam), rabbit polyclonal antibody to Insulin degrading enzyme / IDE (ab25970, Abcam), used for western blot detection of IDE, rabbit polyclonal antibody IDE-1 (27), used for detection of IDE in CSF, monoclonal anti-IDE antibody (ab25733, Abcam), used for immunoprecipitation of IDE, SC6 anti-neprilysin monoclonal antibody (Novocastra), monoclonal antibody NT1 raised against the amino-terminal region of PS1 (28) and HRP-conjugated secondary antibodies (Santa Cruz). Synthetic CTF-50, a 50-amino acid peptide resulting from the γ-secretase cleavage of the C-terminus of β-amyloid precursor protein (APP) at Leu720-Val721 (Calbiochem) was used as a molecular weight control for AICD. For detection of proteins from mouse brains, brains were fresh-frozen, homogenized at 10% (w/v) in homogenation buffer (0.32M sucrose in PBS including complete protease inhibitor tablets (Roche Diagnostics)), aliquoted, and stored at -80 degrees Celsius until further use. For detection of proteins from cell lysates, cells were lysed in RIPA buffer supplemented with protease inhibitors (10mM Tris pH8.0, 150mM NaCl, 1% NP40, 0.5% deoxycholate, 0.1% SDS, 1x complete inhibitor mix (Roche), 5mM EDTA, 2mM 1,10-phenanthroline (Sigma)) followed by centrifugation at 16,000g for 15 min. For purification of cellular membranes, cells were incubated in hypotonic buffer (10mM Tris pH7.4, 1mM EDTA, 1mM EGTA, 1x complete inhibitor mix, 2mM 1,10-phenanthroline) for 10 min on ice. Cells were homogenized, nuclei and debris were removed by centrifugation at 1500g for 10 min. The supernatant was then centrifuged at 100,000g for 1h. The membrane pellet was resuspended.
in RIPA buffer. Protein concentrations were determined using BCA Protein Assay Reagent (Thermo Scientific) and equal amounts of protein were analyzed by SDS-PAGE. Conditioned cell media were centrifuged at 900g, 5min, 4°C to eliminate cells, supernatants were supplemented with 1x complete protease inhibitor mix (Roche) and stored at -80°C. APP, IDE, nephrilysin, wildtype and mutated forms of BR12 (ITM2B) were analyzed by 10% or 12% Tris-glycine SDS-PAGE. To detect total Aβ, equal amounts of conditioned cell media were analyzed on 12% NuPAGE gels using MES running buffer (Invitrogen). For analysis of APP C-terminal fragments, separation of Aβ40/Aβ42 and western blot detection of proteins was carried out as described previously (29). Representative blots from at least three independent experiments are shown. Densitometry was performed with ImageJ software in order to quantify the intensity of the individual bands.

**Immunodepletion of IDE from conditioned cell media**- A mouse monoclonal antibody against IDE (ab25733, Abcam) was coupled to M-280 Sheep anti-Mouse IgG Dynabeads (Invitrogen). Conditioned cell media from BR12 transfected HEK293 cells were incubated with IDE coupled Dynabeads (IDE-IP) or uncoupled Dynabeads as a control (co-IP). Immunodepletions were carried out according to the manufacturer’s instructions. Subsequently IDE in cell media and precipitated IDE were analyzed by western blot. Media were transferred to HEK293-APPwt cells for 24h and secreted Aβ was analyzed by western blot and immunoassay.

**Immunoblot assay to measure human Aβ in conditioned cell media**- Conditioned cell media were analyzed for Aβ38, Aβ40 and Aβ42 levels where indicated by an electrochemiluminescent-based sandwich immunoassay using MS6000 Human (6E10) Abeta-3-Plex Kit and the Sector® Imager 6000 (Meso Scale Discovery) according to the manufacturer’s instructions. Each sample was measured in duplicate and the mean was taken. Values were adjusted for the relative number of viable cells measured by alamarBlue® cell viability assay. The concentration obtained for controls was set to 100%, and %Aβ relative to controls was calculated. To measure human Aβ in wtBriPP/APPS1 mice, frozen brain hemispheres were homogenized in 50mM Tris pH8, 150mM NaCl, 4mM EDTA containing protease inhibitors complete (Roche) and 2mM 1,10-phenanthroline (Sigma). Aβ was extracted with DEA as described previously (21) and Aβ levels were measured by immunoassay as described above.

**Aβ degradation in vitro**- Conditioned cell medium from BR12 or pcDNA3.1/Zeo(+) control transfected HEK-APPwt cells was prepared as described above, mixed with 10µM Aβ1-40 (American Peptide Company) and incubated for 1h at 37°C as described earlier (30). Aliquots were taken prior to (0h) and following the incubation period. For degradation of Aβ with recombinant IDE, 9µM Aβ1-40 was incubated with 0.6 ng/µl IDE (R&D systems) in Tris/NaCl buffer (50mM Tris, 1M NaCl, pH 7.5) for 1h.

**Mass Spectrometry Analysis**- Molecular masses of intact Aβ1-40 peptides and the proteolytic products upon degradation of Aβ by recombinant or cell-derived IDE were determined using the matrix-assisted laser desorption ionization-time of flight mass spectrometer (MALDI-TOF MS, Biflex III, Bruker Daltonics GmbH, Germany). Snap-frozen samples from Aβ degradation assays were thawed and desalted using C18 ZipTips (Millipore) following manufacturer’s instructions. Samples were mixed with α-cyano-4-hydroxy cinnamic acid (Sigma) spotted onto a ground steel MALDI target plate (Bruker Daltonics GmbH, Germany). Spectra were recorded in the linear mode at a laser frequency of 20Hz within a mass range from 1,000 to 6,000 Da. Each spectrum is the result from an average outcome of at least 300 laser shots collected in 30 shot steps. FlexAnalysis 1.0 software (Bruker Daltonics GmbH, Germany) was used for visual estimation of the mass spectra performing smoothing and baseline subtraction.

**Statistical Analysis**- Results were analyzed by ANOVA followed by Bonferroni’s multiple comparison test to compare multiple groups or unpaired t-test to compare two groups using GraphPad Prism 5 statistical software. The
level of statistical significance was set at p=0.05, two-tailed. In all graphs included, errors bars indicate one SEM.

RESULTS

**BRI2 reduces plaque load in the APPPS1 mouse model**- We have recently established and described a mouse model for familial Danish dementia overexpressing ADanPP, a mutant form of BRI2. When these mice were crossed to the APPPS1 mouse model of β-amyloid deposition (25) we found a significant reduction in plaque load in the double transgenic mice expressing ADanPP as compared to control APPPS1 single tg littermates (21). These results suggest that ADanPP expression may influence Aβ generation or deposition. A potential influence of the wildtype BRI2 protein on APP processing has been described by other groups (11,13,14). To compare the effect of BRI2 and ADanPP in the same AD mouse model, we crossed transgenic mice expressing ADanPP to APPPS1 mice. We found that BRI2 lead to a 53.3% reduction in Aβ plaque load (Figure 1A-C), similar to the 68% reduction previously found with ADanPP (21). Western blot analyses of whole brain homogenates confirmed a decrease in total Aβ in double-tg mice compared with single-tg APPPS1 mice, while levels of APP and the APP-C-terminal fragment C99 were not significantly changed (Figure 1D, E). In a next step we analyzed if BRI2 affects the metabolism of APP and Aβ in 1.4 month old APPPS1 mice prior to amyloid deposition. While levels of APP and C99 were similar in single and double-tg predisposing mice (Figure 1 F, G) the analysis of Aβ by immunoassay revealed decreased levels of Aβ40, Aβ42 and Aβ38 in the BRI2 expressing double-tg APPPS1 predisposing mice (Figure 1I). In double-tg compared to single-tg APPPS1 mice Aβ40 was reduced by 41.5% ±16.8 (p=0.0298); Aβ42 was reduced by 62.5% ±23.3 (p=0.0280) and Aβ38 was reduced by 42.8% ±17.0 (p=0.0273). No changes in α- or β-cleaved APP-C-terminal fragments (CTFs) or AICD were detected by western blot with an APP-C-terminal specific antibody (Figure 1H). Taken together these results suggest an influence of BRI2 on Aβ generation or turnover which occurs prior to amyloid deposition. Since levels of C99, C83 and AICD remained unchanged, and levels of all Aβ species were reduced, we suspected that rather than having an effect on APP processing by secretases, BRI2 reduced Aβ levels post-processing, potentially by enhanced Aβ clearance.

To further investigate the molecular mechanism of the influence of BRI2 and ADanPP on APP, and Aβ metabolism in particular, we conducted cell culture experiments. **BRI2 and its mutant form ADanPP reduce levels of secreted Aβ and increase levels of C99** in HEK293-APPwt cells- HEK293 cells stably expressing APP⁰⁸⁵ (HEK293-APPwt cells) were transfected with constructs to co-express either BRI2 or ADanPP. We found that both BRI2 and ADanPP clearly reduced levels of secreted Aβ in transfected cells, while levels of APP remained unchanged (Figure 2A). Quantification of Aβ peptides from conditioned cell media by immunoassay revealed a decrease in both Aβ40 and Aβ42. BRI2 and ADanPP significantly reduced Aβ40 to levels 46.0% and 52.2% to that of controls, and Aβ42 to levels 27.1% and 31.0% to that of controls, respectively (Figure 2B). No significant differences were found between the two constructs in their effect on Aβ. To test the influence of both proteins on APP processing, C-terminal fragments of APP were analyzed. We found a strong increase in the β-cleavage derived C99 fragment with both constructs, in line with previously published results for BRI2 in cell culture (11,13). No major changes in levels of the α-secretase cleavage product C83, nor in levels of the γ-secretase cleavage product AICD were observed (Figure 2C and supplemental Figure 1). Therefore the decrease in secreted Aβ does not seem to result from an inhibition of γ-secretase cleavage of the C99 fragment. Still BRI2 might modulate γ-secretase activity specifically, such that Aβ and AICD generation are affected differently. As an example, certain non-steroidal anti-inflammatory drugs (NSAIDS) selectively
lower Aβ42 and increase Aβ38 levels without inhibition of AICD generation (31,32). To investigate this possibility, we specifically analyzed levels of all three of the major secreted Aβ forms by immunoassay in conditioned cell media of BRI2 and control transfected cells. BRI2 lead to a decrease of all three Aβ forms. Levels of Aβ38 in BRI2 transfected cells dropped below the detection limit and no selective increase in Aβ38 was observed (Figure 2D). Taken together these results do not point to an inhibition or modulation of γ-secretase cleavage of APP by BRI2.

The effects of BRI2 on Aβ and C99 are independent of the BRI peptide- In investigating plaque load reduction by BRI2, it has been proposed that the shed 23 aa BRI peptide may inhibit extracellular Aβ aggregation and deposition (12). We therefore asked whether the reduction in Aβ levels observed in cells was due to the involvement of the BRI peptide. BRI2 was cloned and modified, such that a shorter BRI2 protein lacking the peptide sequence (aa 244-266) was expressed. In transient transfection experiments, this BRI2 variant, BRI2Δ, led to a similar decrease in secreted Aβ and increase in C99 as seen for the expression of full-length BRI2 and ADanPP (Figure 3). These results suggest an influence of BRI2 on Aβ levels, which is independent of its secreted peptide.

BRI2 increases levels of secreted IDE- The observed Aβ reduction did not seem to result from inhibition or modulation of γ-secretase cleavage by BRI2, or sequestration of soluble Aβ by the BRI peptide. The balance between anabolism and catabolism of Aβ determines its steady-state levels, such that a reduction in Aβ may also be caused by enhanced Aβ degradation. Two major Aβ-degrading enzymes in the brain are neprilysin (33) and insulin-degrading enzyme (IDE) (34). Thus, we next analyzed the expression of neprilysin and IDE in BRI2 transfected cells, to test for a possible involvement in increased Aβ-degradation. Levels of neprilysin were not significantly changed in the membrane fraction of HEK293-APPwt cells transfected with BRI2 compared to controls (Figure 4A). IDE can be secreted from cells, where it can degrade Aβ (35) and also stay in the cytoplasm, where it is involved in the degradation of AICD (34). To discriminate between these different pools, we analyzed IDE levels in cell supernatants and cell lysates of cells transfected with the different BRI2 constructs. Expression of BRI2, ADanPP and BRI2Δ led to a significant increase in secreted IDE in conditioned cell media by 2.6-fold, 3.2-fold, and 2.9-fold, respectively, compared to controls. Levels of intracellular IDE were not significantly affected by expression of these constructs (Figure 4B,C).

As an additional control, for overexpression of an unrelated transmembrane protein, we transfected cells with the presenilin 1 mutant PS1L166P. This mutant associated with familial AD leads to increased Aβ42 generation (36). In contrast to BRI2 the overexpression of PS1L166P did not lead to increased IDE secretion (Supplemental Figure 2A,B) or decrease in Aβ (Supplemental Figure 2C) showing that this effect is specific for BRI2 proteins. Expression and functionality of PS1L166P was confirmed by its expected effect of increased Aβ42 generation (Supplemental Figure 2C). Cell viability was not affected by overexpression of BRI2, ruling out a possible increase in levels of IDE due to cell death (Supplemental Figure 2D). As a second control, overexpression of an unrelated protein, enhanced green fluorescent protein (EGFP), did not influence levels of secreted IDE or Aβ (Supplemental Figure 2E,F). Taken together these results suggest that the BRI2-mediated reduction in Aβ may be due to increased degradation by IDE.

Insulin treatment inhibits the BRI2-mediated Aβ decrease- To test if the BRI2-mediated Aβ decrease could indeed be caused by increased levels of IDE, we analyzed if the effect could be blocked by the addition of insulin. Since insulin is a substrate with higher affinity for IDE than Aβ, its addition in excess inhibits Aβ-degradation by IDE (35). To this end we transfected HEK293-APPwt cells with BRI2 or vector control and compared Aβ levels in conditioned cell media with or without the addition of insulin during the incubation period. We found that the presence of insulin in the culture medium of BRI2
transfected cells significantly blocked the BRI2-mediated reduction in Aβ, as shown by western blot and immunoblot measurements of Aβ40 and Aβ42 (Figure 5A,B). BRI2 transfection significantly reduced secreted Aβ40 and Aβ42 to levels 11.3% and 11.2% to that of the control (p<0.0001, n=5). Insulin significantly blocked this reduction (p<0.0001, n=5), such that only a minor reduction to 80.6% and 66.7%, Aβ40 and Aβ42 was observed (p=0.0019 / p=0.0015). Thus, inhibition of Aβ degradation by IDE repressed in large part the Aβ-lowering effect of BRI2 expression. The addition of insulin did not change levels of APP or APP-C-terminal fragments (Figure 5 A,C). Interestingly, the BRI2 mediated increase in C99 was not blocked by insulin (Figure 5C), indicating that the Aβ decrease and C99 increase are not linked to each other.

Transfer of conditioned cell medium from BRI2 transfected HEK293 cells to untransfected HEK293-APPwt cells causes a decrease in secreted Aβ— The above results strongly suggested a major role for secreted IDE in the Aβ-reduction by BRI2. The secreted form of IDE is contained in the cell medium. Thus, we asked if the transfer of conditioned cell medium from BRI2 transfected cells would have a similar effect on HEK293-APPwt cells as overexpression of BRI2 in these cells (for an overview of the experiment see Figure 6A). To do so, we first transfected HEK293 cells with BRI2 or control vector and harvested conditioned cell media 48h post transfection. BRI2 expression in these cells and an increase in secreted IDE in the conditioned cell medium were confirmed by western blot (Figure 6B). Equal amounts of conditioned cell medium from control or BRI2 transfected HEK293 cells was then transferred to HEK293-APPwt cells and incubated for 24h to allow Aβ secretion. In addition, insulin was added to one set of cells to inhibit Aβ-degradation by IDE. Secreted Aβ was clearly reduced in the BRI2-media treated cells compared to the controls (Figure 6C,D). Quantification by immunoblot revealed a significant reduction in secreted Aβ40 and Aβ42 to 55.9% and 63.0% that of control levels (p<0.0001, n=4). Thus the transfer of conditioned cell medium of BRI2 transfected cells (BRI2-CM) leads to a similar effect as BRI2 overexpression. In addition, when insulin was added to the conditioned cell medium during incubation with the HEK293-APPwt cells, its Aβ reducing effect was completely blocked (p<0.0001, n=4) (Figure 6C,D). APP expression in HEK293-APPwt cells remained unchanged by the addition of BRI2-CM or insulin (Figure 6H). In a second set of experiments, we immunodepleted IDE from the conditioned cell medium prior to its addition to HEK293-APPwt cells. Immunoprecipitation with an IDE-specific antibody effectively removed secreted IDE from the conditioned cell medium of BRI2 transfected cells (BRI2-CM) compared to the control immunoprecipitation (Figure 6E). While BRI2-CM following control immunoprecipitation reduced secreted total Aβ to the same extent as untreated BRI2-CM, immunodepletion of IDE significantly prevented the BRI2-CM-mediated Aβ decrease (Figure 6 F,G).

Taken together these results suggest that a secreted factor in the medium of BRI2 transfected cells confers the BRI2-mediated Aβ decrease, and that this soluble factor is IDE, because the effect can be blocked by insulin, and more specifically, by immunodepletion of IDE. Next we were interested if the transfer of conditioned cell medium would also lead to an increase in levels of the C99-fragment in the recipient cells. As shown in Figure 6H, C99 levels were not changed in these cells although Aβ was reduced. Thus, as seen in the experiment above, the observed Aβ decrease and C99 increase are independent effects.

BRI2-mediated Aβ decrease by IDE in HeLa cells- Next, we used HeLa cells to confirm our results in a different cell line. HeLa cells had been used previously to study the effects of BRI2 on APP processing (13). We co-transfected APPwt and BRI2 into HeLa cells and analyzed levels of APP cleavage products and IDE. Again, BRI2 led to a reduction in secreted Aβ (Supplemental Figure 3A) paralleled by an increase in secreted IDE, with no changes in cell viability (Supplemental Figure 3C,D). Similar to that in
HEK293-APPwt cells an increase in C99 levels was observed, while AICD levels remained the same (Supplemental Figure 3B,H). To test if the increased levels of secreted IDE also caused the Aβ decrease in this cell line, we included the addition of insulin as variable in the experiment. As in the HEK293-APPwt cells, in HeLa cells almost all of the Aβ lowering effect was blocked when Aβ degradation by IDE was inhibited by the addition of insulin (Supplemental Figure 3E,F). BRI2 transfection significantly reduced secreted Aβ40 and Aβ42 to levels 38.1% and 12.7% that of the control, as measured by immunoaassay (p=0.0021 /p=0.0019, n=3). Insulin significantly blocked this reduction (p=0.0002 /p=0.0003, n=3), as only a minor reduction to 79.4% and 82.5% Aβ40 and Aβ42 compared to control levels was observed (p=0.0029 /p=0.0301) (Supplemental Figure 3E,F). As a control, levels of APP and APP-C-terminal fragments were examined, and shown to remain the same following the addition of insulin (Supplemental Figure 3G,H). These results confirm the major influence of IDE in the BRI2-mediated Aβ decrease.

Enhanced IDE mediated degradation of Aβ1-40 in cell media of BRI2 transfected HEK293-APPwt cells- To specifically test for IDE mediated degradation of Aβ we compared the Aβ proteolysis pattern obtained after its degradation by recombinant IDE and by conditioned cell media from control and BRI2 transfected HEK293-APPwt cells. Following incubation with synthetic Aβ1-40, very similar MALDI-TOF spectra were obtained for conditioned cell media from BRI2 transfected cells (Figure 7E,F) and recombinant IDE (Figure 7A,B). Both contained peaks corresponding to individual Aβ fragments generated after its degradation by IDE (table 1)(30). Incubation with cell media from control transfected cells did not show an increase in IDE-specific Aβ-degradation products (Figure 7C,D). This evidence strongly supports that BRI2 mediates increased Aβ degradation through secreted IDE.

Increased IDE levels in CSF of wtBriPP mice- Next, we wanted to test if the BRI2-mediated increase in secreted IDE may also be involved in the observed Aβ decrease and plaque load reduction in the transgenic mice. As expected, no significant differences in IDE levels were detected in the total brain homogenates of wtBriPP-tg mice compared to controls (Figure 8A,B). Here, both the secreted and the intracellular pools of IDE are mixed together such that changes in only the secreted form may be masked by the relatively overwhelming level of intracellular IDE. To specifically analyze secreted IDE, we collected CSF from wtBriPP-tg and non-tg littermates. Western blot detection of IDE revealed significantly higher levels of IDE in the CSF of tg mice compared to their non-tg littermate controls (Figure 8C,D).

DISCUSSION

The British Precursor Protein BRI2 is argued to influence APP processing and Aβ deposition, in cells and in vivo, by varying mechanisms (11-15). While the previous studies have suggested that mutations in the BRI2 protein may relate to a loss of BRI2 function and its effect on APP (37,38), we have recently described that the mutant form of BRI2, ADanPP, decreases Aβ plaque load in transgenic mouse models of AD (21). In the present study we show that wildtype BRI2 causes a similar plaque load reduction in the APPPS1 mouse model. We also confirmed previous results that overexpression of BRI2 decreases levels of cell-secreted Aβ (11,13) and showed in addition that the mutated form ADanPP reduces Aβ levels to a similar extent in HEK293-APPwt cells.

Not only Aβ production but also Aβ degradation plays an important role in the regulation of Aβ levels (5,6). Since we found no evidence for changes in APP processing in mice, we investigated the potential role of BRI2 and ADanPP in regulating Aβ degradation. While neprilysin levels were not changed in BRI2 transfected cells, we found that the reduction in Aβ was strikingly paralleled by an increase in cell secreted IDE. This effect was specific for BRI2 proteins since it was not found with the overexpression of PS1-L166F or EGFP used as controls. With additional experiments we demonstrated that
the observed increase in secreted IDE was responsible for most, if not all, of the Aβ decrease. By simply transferring conditioned cell medium of BRI2 transfected HEK293 cells, the Aβ reduction could be induced in non-transfected HEK293-APPwt cells to a similar degree as with BRI2 transfection. This shows that a factor secreted from BRI2 transfected cells, which is most probably IDE, must mediate the effect. This is strongly supported by the result that addition of insulin, which inhibits Aβ-degradation by competing for IDE (35), completely abolished the Aβ-lowering effect of both BRI2 transfection and the transfer of conditioned cell media from BRI2 transfected HEK293 cells. Furthermore, the more specific removal of IDE, by immunoprecipitation from the conditioned media, abolished the Aβ-lowering effect on the recipient cells, ruling out a major contribution of other soluble factors from the conditioned cell media. In addition, incubation of synthetic Aβ1-40 with conditioned cell medium from BRI2 transfected cells showed enhanced Aβ-derived degradation fragments very similar in their MALDI-TOF spectrum to those derived from incubation with recombinant IDE, strongly supporting that BRI2 mediates Aβ degradation by secreted IDE.

As an alternative mechanism, it has been proposed that BRI2 could lower Aβ levels through direct inhibition of γ-secretase cleavage of the APP C99 fragment (11,13,14). In line with this hypothesis, we also found increased amounts of the C99 fragment in cells. However, in our experiments levels of the γ-secretase cleavage product AICD remained unchanged. These results are contradictory to previously published results (13) and may be due to the direct detection of the AICD fragment in our experiments, as opposed to use of an indirect method of measurement (13). In our cell experiments, the main factor responsible for the decrease in Aβ was contained in conditioned media and could be inhibited by insulin. Our results indicate that the increase in C99 is independent of the Aβ reduction, since it was not observed in cells that received the conditioned cell medium from BRI2 transfected cells. In addition, we did not observe changes in C99 levels in vivo, in APPPS1 mice crossed with wtBriPP mice. This is in line with the described increase in Aβ in BRI knockout mice without changes in APP-C-terminal fragments C83 and C99 (14).

In mouse models, the 23 amino acid long BRI peptide, which is shed by furin-like cleavage, is proposed to inhibit Aβ aggregation and thus reduce plaque load in transgenic mice (12). With a shorter BRI2 protein lacking the peptide sequence (BRI2Δ244-266) we still observed increased levels of secreted IDE and decreased Aβ. Thus, this function of BRI2 seems to be independent of the BRI peptide sequence. This observation is in line with results from others reporting that aa 46-106 of BRI2 were sufficient for the reduction of Aβ40 levels in vitro (11).

IDE is a metalloprotease that cleaves insulin as well as other peptide substrates (39). The enzyme is primarily located in the cytosol, but a fraction of the enzyme is found in peroxisomes and on the plasma membrane. In addition, the enzyme is secreted by an unconventional pathway (40), can be found in the cell media of several cell lines, and is shown to degrade extracellular Aβ (27,35). Here, we show that BRI2 influences only the cell secreted form of IDE, while intracellular levels of IDE remain relatively unchanged. To date, it is not known exactly how these different pools of IDE are regulated. A potential association with exosomes is discussed (30,41) and further work will be needed to investigate the involvement of BRI2 in these processes in more detail.

Aβ levels are elevated in the brains of IDE-deficient mice (34,42) and in mouse models of amyloid deposition increased levels of IDE, through overexpression, reduce amyloid plaque load (43). Since 95% of IDE is contained in the cytosol and only a small fraction is secreted (39), small changes within this pool prove to be difficult to detect in the mouse brain. We did not find significant differences in IDE levels in total brain homogenates of wtBriPP tg mice compared to control mice. To specifically analyze secreted IDE in the brain, we collected CSF and found...
that IDE was significantly increased in the CSF of wtBriPP tg mice compared to non-tg littermates. These results suggest that BRI2 can mediate an increase specifically in secreted IDE, in both cells and mice.

A second line of evidence, that indicates that enhanced IDE-mediated degradation may be responsible for the plaque load reduction in mice, comes from the fact that levels of the major secreted Aβ forms, Aβ40, Aβ42 and Aβ38, are equally reduced in wtBriPP/APPPS1 mice compared to single transgenic APPPS1 mice early, prior to amyloid deposition. The reduced plaque load in older mice may thus be caused by lower Aβ levels, rather than by an inhibition of Aβ aggregation. Taken together, our results suggest that BRI2-mediated changes in the secreted form of IDE in the brain are responsible for the observed decrease in amyloid deposition. It remains possible, that in vivo, other proposed mechanisms (11-14) may work in concert with this previously undescribed function of BRI2.

At present, the physiological role of the BRI2 protein is not known. It is cleaved by several proteases, whereby extracellular parts are shed and an intracellular domain is generated (19), similar to proteins involved in signal transduction by regulated intramembrane proteolysis (RIP) (44). Having these properties, BRI2 resembles receptor proteins involved in signal transduction. Like many receptors BRI2 can form homodimers (45). Thus BRI2 signaling may regulate levels of extracellular IDE. Several pathways can regulate IDE levels in different experimental systems. The PI3K pathway (46), insulin receptor signaling (47), the transcription factor PPAR-γ (48,49), palmitic acid and docosahexaenoic acid (50), as well as upregulation of exosome secretion dependent on protein isoprenylation (30) have been implicated. Future work will investigate the activation of these pathways by BRI2.

The regulation of Aβ levels is of great interest for potential therapies for Alzheimer’s disease. Apart from interference with Aβ generation, a promising alternative may be the enhancement of Aβ degradation by targeting Aβ-degrading enzymes (6). Recently published results indicate the contribution of decreased CNS β-amyloid clearance in late onset AD (7). Thus, a better understanding of naturally occurring Aβ regulation may lead to new approaches to therapeutically address sporadic AD.

REFERENCES

FOOTNOTES

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FIGURE LEGENDS

Figure 1. BRI2 reduces amyloid plaque deposition in the APPPS1 mouse model of AD. (A,B) Aβ immunostaining of 4 month-old wtBriPP+/APPSS1+ double tg mice compared to single tg
wtBriPP+/APPPS1+ littersmates reveals a decrease in Aβ plaque deposition. Higher magnification (right panels). (C) Stereological quantification of neocortical Aβ load reveals a remarkable decrease in Aβ deposition in the double tg mice (**p<0.001; all females; n=11 single tg, n=7 double tg). (D) Western blotting of APP, C99 and Aβ in wtBriPP+/APPPS+ and wtBriPP+/APPPS+ mice reveals no change in levels of APP and C99, but a decrease in Aβ in double transgenic mice. Shown are three mice for each genotype. GAPDH serves as a loading control. (E) Quantification of C99 levels by densitometric analysis of band intensities normalized to GAPDH (p=0.683 ns; n=5 single tg, n= 5 double tg). Scale bars are 500 and 100 µm. (F-I) Analysis of APP, C99 and Aβ levels in 1.4 month old pre-depositing wtBriPP+/APPPS1+ double tg mice compared to single tg wtBriPP-/APPPS1+ littersmates. (F) Western blot analysis with antibody 6E10 reveals no differences in APP or C99 levels. Shown are three mice for each genotype, all females. GAPDH serves as a loading control. (G) Quantification of C99 levels by densitometric analysis of band intensities normalized to GAPDH and APP (p=0.2201 ns; n=5 single tg, n= 5 double tg, all females). (H) Western blot analysis with antibody A8717 reveals no differences in α− or β-cleaved APP-C-terminal fragments (APP-CTFs) (upper panel) or AICD (lower panel, stronger exposure of the same blot). The two bands represent the nonphosphorylated and phosphorylated form of AICD (AICD / p-AICD). (I) Immunoassay of DEA extracted brain homogenates shows a significant decrease in Aβ40, Aβ42 and Aβ38 comparing double-tg with single-tg littersmates (*p<0.05; n=9 wtBriPP+/APPPS1+; n=5 wtBriPP-/APPPS1+, all females).

**Figure 2.** BRI2 and ADanPP reduce levels of secreted Aβ and increase levels of the C99 fragment in cells. HEK293-APPwt cells were transfected with BRI2 or ADanPP expression plasmids or pcDNA3.1 vector as a control (co). (A) Secreted Aβ was prominently decreased by BRI2 and ADanPP, while APP levels remained unchanged. Expression of BRI2 constructs was confirmed by western blot with the ITM2B antibody, detection of GAPDH served as a loading control. (B) Quantification of secreted Aβ40 and Aβ42 by immunoassay from four independent experiments reveals a significant reduction in BRI2 and ADanPP transfected cells relative to controls (***p<0.0001; BRI2 vs ADanPP p>0.05). (C) C-terminal fragments of APP were analyzed in cell lysates. An increase in levels of C99 fragments (middle panel, short exposure) but no change in levels of AICD (lower panel, longer exposure of the same blot) are observed. APP levels remain unchanged. (D) HEK293-APPwt cells were transfected with BRI2 or pcDNA3.1 vector as a control (co). Aβ40, Aβ42 and Aβ38 from conditioned cell media were analyzed by immunoassay from three independent experiments. All three Aβ forms are decreased in cell media of BRI2 transfected cells. Secreted Aβ38 from BRI2 transfected cells is below the detection limit.

**Figure 3.** BRI2 effects on Aβ and C99 are independent of the BRI peptide. HEK293-APPwt cells were transfected with BRI2 or BRI2Δ, a construct that expresses BRI2 lacking the C-terminal peptide (aa 244-266). Both BRI2 and BRI2Δ lead to a decrease in secreted Aβ compared to controls (co). Western Blot detection with the antibody A8717 shows an increase in C99 but no change in AICD or APP levels. Expression of the BRI2 proteins was confirmed by western blot using the ITM2B antibody. Detection of GAPDH served as a loading control.

**Figure 4.** BRI2 increases levels of secreted IDE. HEK293-APPwt cells were transfected with BRI2, ADanPP or BRI2Δ expression plasmids, or empty vector as a control (co). (A) Levels of neprilysin in the membrane fraction of cells remained unchanged by BRI2. APP in the membrane fraction is shown as a loading control. (B) Secreted IDE was increased in the conditioned cell media from all cells transfected with one of the BRI2 constructs. In contrast, intracellular IDE detected in cell lysates remained unchanged by BRI2 expression. (C) Quantification of secreted IDE by densitometric analysis of band intensities. The 3–fold increase in secreted IDE relative to
control (co) from three independent experiments is shown (**p<0.0001 relative to control; BRI2 vs ADanPP, BRI2 vs BRI2Δ, ADanPP vs BRI2Δ, p=0.05).

**Figure 5.** Insulin treatment inhibits BRI2 mediated Aβ decrease. BRI2 or vector control (co) transfected HEK293-APPwt cells were supplemented with fresh medium 1d after transfection and incubated for 24h to generate conditioned cell medium. Where indicated, 10µM insulin was added. (A,B) Without addition of insulin BRI2 expression prominently decreases levels of secreted Aβ. Inhibition of IDE activity with insulin prevents this Aβ decrease almost completely. (A) Levels of secreted Aβ are shown in two exposures of the same blot. Secreted IDE is increased with BRI2 expression and remains unchanged by the addition of insulin. In addition, APP expression remains unaltered by insulin. GAPDH serves as a loading control. (B) Aβ40 and Aβ42 from conditioned cell media were analyzed by immunoassay from five independent experiments. Graphs show % remaining Aβ relative to controls in BRI2 transfected cells and BRI2 transfected cells treated with insulin. Insulin significantly blocked the BRI2-mediated reduction in Aβ40 and Aβ42 (**p<0.0001). (C) Addition of insulin does not alter levels of APP-C-terminal fragments.

**Figure 6.** Transfer of conditioned cell medium from BRI2 transfected HEK293 cells to untransfected HEK293-APPwt cells causes a decrease in secreted Aβ. (A) Schematic overview of the experiment: HEK293 cells were transfected with BRI2 or empty control vector (co). Conditioned cell medium from these cells was transfected to untransfected HEK293-APPwt cells with or without addition of 10µM insulin as indicated and incubated for 24h. (B) Parallel to BRI2 expression increased levels of IDE were detected in conditioned cell media from BRI2 transfected HEK293 cells compared to controls. (C,D) HEK293-APPwt cells treated with conditioned cell medium from BRI2 transfected cells (BRI2-CM) showed decreased levels of secreted Aβ compared to cells treated with control medium (co-CM). This effect is inhibited by the addition of insulin (+Ins). (C) Detection of secreted Aβ by western blot. (D) Analysis of secreted Aβ40 and Aβ42 by immunoassay from four independent experiments. Graphs show % remaining Aβ relative to control for cells incubated with BRI2-CM compared to BRI2-CM incubated cells additionally treated with insulin. BRI2-CM significantly reduced secreted Aβ40 and Aβ42 relative to controls (p<0.001), while insulin blocked this reduction completely (**p<0.0001). (E) The IDE in conditioned cell medium from BRI2 transfected HEK293 cells was immunodepleted using an IDE-specific antibody (IDE-IP) or only beads as a control (co-IP). IDE levels in immunodepleted or control cell media as well as the precipitated IDE from control and IDE-specific immunoprecipitation is shown (precip.). (F, G) Conditioned cell media were then transferred to HEK-APPwt cells, incubated for 24h and secreted Aβ analyzed by western blot. Immunodepletion of IDE reverts the BRI2-mediated decrease in Aβ. (G) Quantification of total Aβ by immunoassay from three independent experiments shows that immunodepletion of IDE from the cell media of BRI2 transfected cells blocks its effect on Aβ decrease. (IDE-IP-CM vs co-IP-CM **p=0.0098; BRI2-CM vs co-CM ***p<0.0001). (H) Treatment with BRI2-CM did not lead to an increase in levels of the C99 fragment in HEK293-APPwt cells. The addition of insulin does not alter levels of APP, APP-C-terminal fragments, or AICD. GAPDH is shown as a loading control.

**Figure 7.** Enhanced IDE mediated degradation of Aβ1-40 in cell media of BRI2 transfected cells. (A,B) Degradation of Aβ1-40 by recombinant IDE. Synthetic Aβ1-40 (9µM) was incubated with purified recombinant IDE (0.6ng/µl) for 1 h at 37 °C. Samples obtained before (A) and after (B) the incubation period were analyzed by MALDI-TOF MS. Representative spectra indicate peaks at m/z value of 4332.8 and half m/z value of 2165.5 corresponding to single and doubly ionized Aβ1-40 in samples prior to incubation (A). Following incubation with IDE, several degradation products of Aβ1-40 are detected (B). The annotation of peaks to the corresponding
peptides is given in Table 1. (C-F) HEK-APPwt cells were transfected with control vector (pcDNA3.1) or BRI2 expression plasmid. Conditioned cell media were collected and incubated with Aβ1-40 (10µM) for 1h. The Aβ levels in control and BRI2-transfected cell medium before (C and E) and after (D and F) incubation was analyzed by MALDI-TOF MS. Enhanced IDE-mediated degradation of Aβ1-40 is evident in cell medium of BRI2 transfected cells (F) as compared to control cells (D). The Aβ degradation products in the conditioned cell medium are similar to that from recombinant IDE (B), further confirming the IDE-mediated degradation in the conditioned medium.

Table 1. MALDI-TOF MS analysis of the cleavage products of Aβ1-40 peptide by recombinant insulin degrading enzyme.

Expected proteolytic products and the molecular mass of the respective peptide fragments upon cleavage by IDE. The calculated mass (Da) and observed mass (Da) are indicated. 1-40* represents doubly ionized full length peptide (m/2z instead of normal m/z).

Figure 8. Increased IDE levels in CSF of wtBriPP mice. (A) IDE expression in total brain homogenates of 1.8-2 month old wtBriPP mice and non-tg littermates was analyzed. GAPDH serves as a loading control. (B) Densitometric analysis of IDE band intensities normalized to GAPDH reveals no significant differences between tg and non-tg mice. (C) Western blot analysis of IDE levels in CSF collected from mice analyzed in (A). (D) Densitometric analysis of IDE band intensities reveals 4.5-fold higher levels IDE in the CSF of tg mice compared to control mice (*p=0.0196; all females, n=3 tg, n=3 non-tg).
Figure 1

(A) 4 month old mice

(B) 1.4 month old mice

(C) wtBriPP- / APPPS1+ wtBriPP+ / APPPS1+

(D) 4 month old mice

(E) 1.4 month old mice

(F) wtBriPP- / APPPS1+ wtBriPP+ / APPPS1+

(G) wtBriPP- / APPPS1+ wtBriPP+ / APPPS1+

(H) wtBriPP- / APPPS1+ wtBriPP+ / APPPS1+

(I) Aβ40, Aβ42, Aβ38
Figure 2

A) Western blots showing ITM2B, GAPDH, APP, and secreted Aβ.

B) Bar graphs showing secreted Aβ40 and Aβ42 levels as a percentage of control.

C) Western blots showing APP, C99, C83, and AICD.

D) Graphs showing the levels of Aβ40, Aβ42, and Aβ38 with and without BR12 and ADanPP.
Figure 3

Co  BRI2  BRI2Δ

50
ITM2B

36
GAPDH

98
APP

secreted Aβ

3
C99

17
C83

AICD

6

AICD
Figure 4

A  
co  BRI2

98  neprilysin
50  APP
98  ITM2B

B  
co  BRI2  ADanPP  BRI2Δ

50  ITM2B
36  GAPDH
98  secreted IDE
98  intracellular IDE

C  
secreted IDE

fold change relative to control

0.0  0.5  1.0  1.5  2.0  2.5  3.0  3.5  4.0

co  BRI2  ADanPP  BRI2Δ

*** ***
**Figure 5**

(A) Western blot analysis showing the effects of BRI2 co-expression with and without 10 μM insulin control on secreted ITM2B, APP, GAPDH, IDE, and Aβ. Long exposure is indicated.

(B) Bar graphs representing the secretion of Aβ40 and Aβ42 as a percentage of control under the conditions of BRI2 co-expression with and without 10 μM insulin control. *** indicates statistical significance.

(C) Western blot analysis showing the expression of C99, C83, and AICD under control and 10 μM insulin conditions.
Figure 6

A  HEK293 cells
  control vector  BRI2
  2d post transfection
  conditioned cell medium (24h)
  Transfer to HEK293-APPwt cells:
  conditioned cell medium from control
  conditioned cell medium from BRI2
  conditioned cell medium from control + insulin
  conditioned cell medium from BRI2 + insulin

B  APP  APP
  Aβ  Aβ
  co  co
  BRI2  BRI2
  secreted IDE
  50%
  98%

C  co-CM  BRI2-CM
  36%
  98%

D  Aβ40  Aβ42
  secreted Aβ (% of control)
  co-CM  BRI2-CM +Ins
  co-CM  BRI2-CM +Ins
  0  50  100

E  precip.
  IP: IDE-IP-co-IP
  BRI2-CM  BRI2-CM
  98%

F  secreted Aβ
  IP: IDE-IP-co-IP
  BRI2-CM  BRI2-CM
  0  50  100

G  secreted Aβ
  IP: IDE-IP-co-IP
  co-CM  BRI2-CM
  0  50  100

H  APP  GAPDH
  C99  C83
  AICD
  98%
  36%
  17%
  6%

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Figure 7
Table 1

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Figure 8

A

wtBriPP - + - + +
98 -
36 -

IDE

GAPDH

B

wtBriPP - wtBriPP +
0.0 2.0 4.0 6.0 8.0

IDE relative to control

wtBriPP - wtBriPP +
0.0 0.5 1.0 1.5 2.0

IDE relative to control

C

wtBriPP - + - + +
98 -

IDE

D

wtBriPP - wtBriPP +
6.0 4.0 2.0 0.0

wtBriPP - wtBriPP +
8.0 6.0 4.0 2.0

*
BRI2 regulates β-amyloid degradation by increasing levels of secreted insulin degrading enzyme (IDE)

Ellen Kilger, Anika Buehler, Heidrun Woelfing, Sathish Kumar, Stephan A. Kaeser, Amudha Nagarathinam, Jochen Walter, Mathias Jucker and Janaky Coomaraswamy

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