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An Alzheimer's Disease-relevant Presenilin-1 Mutation Augments Amyloid-beta-induced Oligodendrocyte Dysfunction

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Abstract

White matter pathology has been documented in the brains of familial Alzheimer's disease (FAD)-afflicted individuals during pre-symptomatic and pre-clinical stages of AD. How these defects in myelination integrity arise and what roles they may play in AD pathophysiology have yet to be fully elucidated. We previously demonstrated that triple-transgenic AD (3xTg-AD) mice, which harbor the human amyloid precursor Swedish mutation, presenilin-1 M146V (PS1^{M146V}) knock-in mutation, and tau^{P301L} mutation, exhibit myelin abnormalities analogous to FAD patients and that A β ₁₋₄₂ contributes to these white matter deficits. Herein, we demonstrate that the PS1^{M146V} mutation predisposes mouse oligodendrocyte precursor (mOP) cells to A β ₁₋₄₂-induced alterations in cell differentiation *in vitro*. Furthermore, PS1^{M146V} expression compromised mOP cell function and MBP protein distribution, a process that is further aggravated with exposure to A β ₁₋₄₂. We found that the myelination defect and MBP subcellular mislocalization triggered by PS1^{M146V} and A β ₁₋₄₂ can be effectively prevented by treatment with the GSK-3 β inhibitor, TWS119, thereby implicating GSK-3 β kinase activity in this pathogenic cascade. Overall, this work provides further mechanistic insights into PS1^{M146V} and A β ₁₋₄₂-driven oligodendrocyte dysfunction and myelin damage during early pre-symptomatic stages of AD, and provides a new target in oligodendrocytes for developing therapies designed to avert AD-related white matter pathology.

Keywords

Alzheimer's disease; white matter; myelin; oligodendrocyte; MBP; amyloid beta; presenilin-1; 3xTg-AD

Introduction

White matter lesions and pathology have been extensively documented in the brains of incipient and mildly afflicted AD patients (Dickerson and Sperling 2008; Roth et al. 2005).

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More specifically, white matter aberrations have been reported in late-myelinating brain regions of presymptomatic and preclinical carriers of FAD-associated presenilin 1 (PS1) mutations (Ringman et al. 2007). Similarly, triple-transgenic AD (3xTg-AD) mice (Oddo et al. 2003a), which express the human presenilin-1 M146V mutation (hPS1^{M146V}), human amyloid precursor protein Swedish mutation (hAPP^{Swe}), and the human tau P301L mutation (htau^{P301L}), exhibit white matter deficits in comparable brain regions at ages prior to the appearance of overt amyloid and tau-related pathology (Desai et al. 2009). Of note, the 3xTg-AD mouse harbored hPS1^{M146V} knock-in mutation can be expressed in cell types supportive of murine PS1 promoter-driven transcription, including oligodendrocytes, whereas the hAPP^{Swe} and htau^{P301L} mutant transgenes are expressed exclusively by neurons.

PS1 is the catalytic component of the multi-subunit gamma secretase complex, arguably best known for its role in amyloidogenic processing of APP to yield pathogenic A β peptide species (Scheuner et al. 1996). Previous studies have also revealed a role for gamma-secretase in oligodendrocyte maturation and myelinating function (Lai and Feng 2004; Watkins et al. 2008). Other reports have drawn a more direct link between PS1 and myelin by showing high co-expression between PS1 and canonical myelin genes in the CA1 hippocampal region of both aging and AD brains (Miller et al. 2008). Studies have demonstrated myelin deterioration in the spinal cords of APP/PS1 adult mice (Wirths et al. 2006), while Pak et al. reported that PS1^{M146V}-expressing oligodendrocytes show increased vulnerability to various toxic and nutritional insults (Pak et al. 2003a; Pak et al. 2003b). The myelin aberrations detected in the brains of 3xTg-AD mice further support this argument (Desai et al. 2010; Desai et al. 2009), while corroborating studies revealed increased sensitivity of hPS1^{M146V}-expressing oligodendrocytes to A β -induced toxicity, exacerbated white matter damage, and cognitive deficits in the brains of transgenic mice (Pak et al. 2003a). This collective evidence implicates mutant hPS1^{M146V} and insults incited by A β ₁₋₄₂ exposure in collectively influencing the fate/function of oligodendrocytes in the brains of AD patients.

In the current study, we show that oligodendrocyte cell differentiation and function are indeed affected by the co-presence of hPS1^{M146V} and A β ₁₋₄₂ using mouse oligodendrocyte precursor (mOP) cells. These perturbations lead to abnormalities in myelin basic protein (MBP) distribution patterns in cells expressing hPS1^{M146V} and these defects are exacerbated by ectopic A β ₁₋₄₂ peptide exposure. We found that glycogen synthase-3-beta (GSK-3 β) activity at least partially underlies the hPS1^{M146V} and A β ₁₋₄₂-induced alterations on oligodendrocyte homeostasis, as these effects are rescued upon GSK-3 β inhibition. Finally, we demonstrate that MBP distribution patterns are significantly altered in mature oligodendrocytes in the brains of 3xTg-AD mice using a newly created compound 3xTg-AD/CNP-EGFP mouse model. In aggregate, this study reveals a novel pathogenic role of hPS1^{M146V} and early A β ₁₋₄₂ exposure in disrupting oligodendrocyte homeostasis and provides a foundation for the development of future therapeutic interventions to maintain, rescue, and/or restore myelin integrity in the brains of AD-afflicted individuals.

Materials and Methods

Mouse oligodendrocyte precursor (mOP) cell line

The mOP cell line was developed and kindly provided by Dr. Steven A. Reeves (Massachusetts General Hospital, Charlestown, MA) (Lin et al. 2006). The cell line was maintained in the mOP proliferation medium (PM) as previously described (Lin et al. 2006). The mOP cells were plated at a density of 1×10^4 in 12-well plates for flow cytometric analyses. For myelination studies, 1×10^4 mOP cells were plated in PDL-coated 12-well

plates with and without coverslips for western blotting and immunocytochemistry, respectively.

Plasmid Construction and Verification

Plasmids harboring human VRSQ-deleted variants of PS1^{WT} and PS1^{M146V} were kindly provided by D. W. Van Nostrand (SUNY-Stony Brook, NY), respectively. The hPS1^{WT} and hPS1^{M146V} expression cassettes were excised from the original pcDNA3 constructs and ligated into the multiple cloning site of the pHSVPrPUC/CMVeGFP dual promoter vector using the *Xba*I and *Hind*III restriction sites to generate recombinant HSVhPS1^{WT}/CMVeGFP (hPS1^{WT}) and HSVhPS1^{M146V}/CMVeGFP (hPS1^{M146V}) plasmid constructs. The plasmid constructs contained two promoters; the CMV promoter driving enhanced green fluorescent protein (eGFP) expression and the Herpes simplex virus immediate-early 4/5 (IE4/5) gene promoter driving the expression of the gene of interest, namely hPS1^{WT} or hPS1^{M146V}. GFP expression facilitated the detection and analyses of transfected cells, also expressing the specific gene of interest. The original pHSVPrPUC/CMVeGFP (GFP) was used as a non-PS1-expressing vector control for all experiments. To confirm that the plasmid vectors expressed the gene of interest, the GFP, hPS1^{WT}, and hPS1^{M146V} constructs were transiently transfected into baby hamster kidney (BHK-21) cells and cultures were analyzed 48 h later. Expression of each hPS1 open reading frame was verified at the transcript level using quantitative real-time RT-PCR and protein level using immunocytochemistry to detect hPS1 protein expression in GFP-positive BHK-21 cells.

Quantitative Real-time RT-PCR Analysis

Forty-eight hours post-transfection, total RNA was purified from the BHK-21 cells using the TRIzol (Invitrogen, Carlsbad, CA) phenol–chloroform method according to manufacturer's instructions. Two micrograms of RNA was converted into cDNA using a high-capacity cDNA archiving kit (Applied Biosystems, Foster City, CA) and the cDNA was utilized to quantify the transcript levels with an Assay-on-Demand primer probe set (Applied Biosystems) specific to the hPS1 transcript. An 18S rRNA-specific primer/probe set was used as an internal control.

Transfection and Treatments of mOP cells

The recombinant plasmid vectors, GFP, hPS1^{WT}, and hPS1^{M146V} were transiently transfected into mOP cells using the LipofectamineTM 2000 reagent (Invitrogen) according to manufacturer's instructions. Synthetic A β _{1–42} or A β _{42–1} peptides (American Peptide, Sunnyvale, CA) stock solutions were prepared to 110.7 μ M in double-distilled sterile H₂O and stored at –20°C until use. The mOP cells were treated with 0.5 μ M A β _{1–42} or A β _{42–1} peptides 24 h post-transfection. The cells were incubated with A β peptides for an additional 72 h and processed for various assays.

In experiments using the GSK-3 β inhibitor, TWS119 (Tocris Bioscience, Ellisville, MI), the mOP cells were transfected as described above and 5 h post-transfection 50nM TWS119 or DMSO vehicle control was added to the cells. The cells were then treated with 0.5 μ M A β peptides 24 h post-transfection and were processed for immunocytochemistry 72 h after initiation of A β peptide exposure.

Flow Cytometry Analysis

Post-transfection and treatment, the mOP cell suspensions were fixed using 4% (w/v) paraformaldehyde (PFA), followed by permeabilization and blocking in 10% normal goat serum (NGS). Cell suspensions were incubated with the appropriate primary antibodies for GFP (1:1000, Invitrogen), CC-1 (1:1000, EMD Chemicals Inc., San Diego, CA) and MBP

(1:500, Millipore, Billerica, MA). The cells were washed in phosphate buffered saline (PBS) and incubated with the appropriate Alexa Fluor 488, 647, and PE-680 secondary antibodies (1:2000, Molecular Probes, Carlsbad, CA). The cells were further washed and then subjected to flow cytometry.

Cells were analyzed for light forward- and side-scatter using a BD-LSR II instrument (Becton Dickinson, San Jose, CA). No-primary negative controls (non-transfected) were used to set the fluorescence background. Cells singly stained for GFP, CC-1, or MBP were used to set the compensation measurements. The sorting speed was approximately 1000–2000 cells/sec. A total of 30,000 events were recorded for each condition (n=4). A total of 4 independent experiments were performed. The data were analyzed using the FlowJo Analysis Software (Tree Star Inc., Ashland, OR). The CC-1 and MBP-positive cells were gated on GFP-positive cells. This allowed us to determine the total number of transfected (GFP-positive) cells that expressed the signature CC-1 and MBP markers for oligodendrocytes.

In Vitro Immunocytochemistry and Hoechst Staining

The transfected/treated BHK-21 and mOP cells were fixed using 4% (w/v) PFA and washed using PBS. Fixed mOP cells were stained for GFP (1:1000, Invitrogen), hPS1 (1:1000, Abnova, Taipei City, Taiwan), or MBP (1:1000, Chemicon, Temecula, CA), and subsequently Hoechst 33342 dye (Sigma) using the previously described method{Desai, #962}. Stained cells were analyzed using an Olympus DP71 microscope (Olympus, Melville, NY) and images were captured under 100X magnification. Representative MBP images were captured using an Olympus BX61WI microscope under 60X magnification using sequential fluorescence scanning.

Cell Death Analysis

Images of Hoechst 33342 stained GFP-positive cells for all conditions were captured under 40X magnification using an Olympus DP71 microscope (Olympus). An average of 50–70 GFP-positive cells were randomly sampled per coverslip (n=4) per condition. The data were obtained from 3 independent experiments. The number of pyknotic cells with condensed or fragmented nuclei was summated for the sampled regions and the percentage of pyknotic cells per coverslip was subsequently calculated. The investigator was blinded to the identity of each experimental group throughout the analyses.

MBP Localization and Myelination Analysis

The mOP cells were stained for MBP and analyzed at 100X magnification using an Olympus DP71 microscope (Olympus) as described earlier. Several images of sequential focal planes of MBP-stained cells were captured specifically for GFP-positive cells and scored based on MBP localization patterns and presence of myelin sheets. The sequential focal planes were captured at a 2- μ m step size to ensure a comprehensive assessment of MBP expression through the entire cell. MBP localization differences in the cells were scored based on two criteria: cell body-restricted MBP expression and mOP cells exhibiting cell body and process MBP expression. Myelination was determined by the presence of sheets adjoined to the processes and sheets extending from the cytoplasm of the cells. Next, the percentage of cells undergoing myelination was enumerated. A total of 300–400 cells were randomly analyzed per condition (n=4 per condition). The data were obtained from 3 independent experiments for assessment of PS1 and A β peptide effects. To study the effects of GSK-3 β using the TWS119 inhibitor, a total of 100–300 cells were analyzed per condition (n=4 per condition) and 2 independent experiments were performed. The investigator was blinded to the identity of each experimental group during the imaging and scoring.

Western Blot Analysis

For western blots, the mOP cells were washed with PBS and homogenized in cold lysis buffer (50mM Tris-HCl pH 7.5, 5mM EDTA, 1% TX-100) with protease inhibitors. All samples were subjected to the DC protein assay (Bio-Rad, Hercules, CA). Protein lysates (40µg) were mixed with Laemmli sample buffer, then loaded on 14% sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis and subsequently transferred to polyvinylidene difluoride membrane (Bio-Rad). Membranes were incubated with the one of the following primary antibodies: MBP (1:500; Millipore), phosphorylated GSK-3β (1:1000, Cell Signaling, Boston, MA), or GSK-3α/β (1:1000, Santa Cruz Biotechnology, Santa Cruz, CA) using the previously described method (Desai et al. 2009). To ensure equal protein loading, the MBP immunoblots were stripped and re-probed for GAPDH (1:5000, Sigma). The films were scanned with Imageworks Image Acquisition and Analysis Software (UVP, Upland, CA) and quantitation was performed on the appropriate bands with ImageJ software (National Institutes of Health, Bethesda, MD). The data were obtained from 2 independent experiments for each set of studies.

Mouse Strains

Previously described, 3xTg-AD mice were kindly provided by Dr. Frank LaFerla (Oddo et al. 2003b). The CNP-EGFP mice were generated on a FVB/N × C57BL/6 background as previously described and kindly provided by Dr. Vittorio Gallo (George Washington University, Washington, DC) (Yuan et al. 2002). The 3xTg-AD/CNP-EGFP mice were generated using a monogamous breeding strategy of CNP-EGFP and 3xTg-AD mice until homozygous transfer of all AD-related transgenes to the offspring was achieved. Briefly, the parental CNP-EGFP and 3xTg-AD mice were bred to generate (F1) offspring composed of heterozygous 3xTg-AD and CNP-EGFP genes. Subsequently, the F1 generation mice were backcrossed with 3xTg-AD mice to generate (F2) mice with the CNP-EGFP transgene and homozygous copies of all three 3xTg-AD mutations (hAPP^{sw}, htau^{P301L}, and hPS1^{M146V}). 3xTg-AD/CNP-EGFP mice were identified by polymerase chain reaction (PCR) screening for all related genes using previously described strategies (Guo et al. 1999; Oddo et al. 2003b). Non-Tg/CNP-EGFP control mice were generated by breeding the CNP-EGFP mice and C57BL/6 mice. Control mice were PCR screened for eGFP expression. All animal housing and procedures were performed in compliance with guidelines established by the University Committee of Animal Resources at the University of Rochester.

Immunocytochemical Detection in Mouse Brain Tissue

Age-matched 9 month-old female Non-Tg/CNP-EGFP and 3xTg-AD/CNP-EGFP mice were perfused transcardially, subsequently their brains were removed and sequentially stored in 4% PFA, 20% sucrose, and 30% sucrose. The brains were sectioned coronally (30µM) and stored in cryoprotectant at −20°C until use. Immunocytochemistry with primary antibody specific for NeuN (1:1000, Millipore), GFAP (1:1000, Dako, Carpinteria, CA), Iba1 (1:750, Wako, Richmond, VA), or MBP (1:1000, Chemicon) was performed as using as previously described (Desai et al. 2009). The stained tissue was mounted on glass slides, allowed to dry, sealed with glass coverslips using Mowiol, and visualized using a using Olympus BX50WI microscope (Olympus). The images were captured at 100X magnification using sequential fluorescent scanning. Three consecutive sections from both hemispheres for each mouse for different regions of the cortex were analyzed. The images were analyzed for cell body-associated GFP and MBP staining pixel intensity using the FluoView Software Version 2.1 (Olympus). Investigator-blinded scoring was performed to assess the fraction of total GFP-positive mature oligodendrocytes with MBP staining in both the cell body and processes compared to oligodendrocytes with MBP staining restricted exclusively to the processes.

Statistical Analysis

Statistical analysis was performed by means of 2-way ANOVA followed by the Bonferroni post-test using GraphPad Prism version 5.0 for Macintosh (GraphPad Software, San Diego, CA).

Results

Development and verification of PS1 vectors

The 3xTg-AD mice express the hAPP^{swe} and htau^{P301L} transgenes exclusively in neurons, whereas the hPS1^{M146V} knock-in mutation is expressed in neurons and glia, including oligodendrocytes (Oddo et al. 2003b). To study the role of mutant PS1 in oligodendrocytes *in vitro*, we generated plasmid vectors containing dual promoters that drive the expression of hPS1^{WT} or hPS1^{M146V} transgenes together with eGFP (Figure 1A). A GFP-only plasmid served as a negative control. To confirm that the vectors express the genes of interest, we transiently transfected BHK-21 cells with the plasmids for 48 h and assessed hPS1 transcript and protein expression. Quantitative real-time RT-PCR results revealed comparable expression of hPS1 transcripts with both the hPS1^{WT}- and the hPS1^{M146V}-encoding plasmids compared to the GFP-only vector or non-transfected controls (Figure 1B). Furthermore, immunocytochemical detection revealed hPS1 and GFP co-expression in both hPS1^{WT} and hPS1^{M146V} transfected cells (Figure 1, C–K). No hPS1 expression was detected in cells transfected with the control GFP plasmid. These validated expression vectors were subsequently utilized for selective analysis of transfected cells to assess hPS1^{M146V} effects on mouse oligodendrocyte precursor (mOP) cells.

hPS1^{M146V} and A β _{1–42} Effects on mOP Cell Death

We designed the subsequent *in vitro* experiments to closely mimic the temporal relationship between PS1^{M146V} expression and A β _{1–42} exposure encountered by the oligodendrocyte population in 3xTg-AD mice and in individuals that may harbor FAD-related PS1 mutations. The myelination changes in adult 3xTg-AD mice are first observed at 6 months of age (Desai et al. 2010; Desai et al. 2009). Since the PS1^{M146V} mutation engineered into the 3xTg-AD mouse model is a “knock-in” mutation, its gene product is expressed in many cell types, including oligodendrocytes, from embryonic stages of development (Oddo et al. 2003b). hAPP^{swe} transgene expression in 3xTg-AD mice is specific to neurons, leading to the generation of detectable intraneuronal A β _{1–42} starting at 3 months of age. Extracellular A β _{1–42} peptide levels at this age and times prior, although undetectable, could impact oligodendrocyte function, but likely not before PS1^{M146V}. Hence, the inherent design of the 3xTg-AD mouse speaks to PS1^{M146V}-mediated predisposition of oligodendrocytes to subsequent A β -induced damage.

We applied an analogous *in vitro* paradigm to assess the gross effects of hPS1^{M146V} and A β _{1–42} treatment on mOP cells (Lin et al. 2006). We initially transfected differentiating mOP cell cultures with the GFP, hPS1^{WT}, and hPS1^{M146V} plasmids, treated the cells with A β peptides 24 h later and assessed for various parameters 72 h post-treatment. The mOP cultures did not reveal gross morphological divergence amongst the three transfection conditions when assessed by phase contrast microscopy (Figure 2A–D). Furthermore, immunocytochemistry results confirmed that expression of hPS1 and GFP was maintained in the differentiated mOP cells 96 h post-transfection (Figure 2E–M). Previously, we showed that mOP sub-populations (~10%) exhibit increased sensitivity to A β _{1–42} peptide toxicity at 4 h post-exposure (Desai et al. 2010). We sought to study the fate of the viable mOP cell populations at later time points under the influence of hPS1^{M146V} and A β _{1–42} insults. To this end, mOP cells were transfected with the GFP, hPS1^{WT}, and hPS1^{M146V}-expressing vectors and 24 h later treated with A β peptides for a period of 72 h as described

above. We assessed cell death in the transfected mOP cultures under the different conditions using Hoechst staining, which facilitates the detection of fragmented or condensed nuclei, for signs reminiscent of apoptotic cell death (Figure 2, N–P). Quantification was selectively performed on transfected GFP-positive cells to assess cell death (Figure 2Q). The data revealed no statistically significant differences between the different treatment groups. We also performed western blot analysis to verify the status of A β _{1–42} peptide species in the mOP media at the point of addition (0 h) and following incubation (72 h) (Figure 2R), as A β _{1–42} peptide aggregation is determined by factors that include pH and ionic strength of a solution (Harper et al. 1999; Nerelius et al. 2009). Our results revealed the presence of primarily A β _{1–42} monomers and low levels of oligomers at both time points, a pattern that we have reported previously for this relatively short time of A β _{1–42} peptide incubation in culture (Ryan et al. 2010).

Effects of A β _{1–42} Exposure on Differentiation Marker Expression in mOP Cells Transfected with hPS1^{M146V}

We previously demonstrated elevated numbers of mature CC-1-positive oligodendrocytes in the brains of 6 month-old 3xTg-AD mice, which concurrently exhibit declining normal MBP marker staining patterns (Desai et al. 2010). Those studies further demonstrated the restoration of mature oligodendrocyte cell marker expression upon selectively lowering parenchymal A β _{1–42} levels by delivery of an A β _{1–42}-specific intrabody to 3xTg-AD neurons, thus establishing a strong link between A β _{1–42} and altered oligodendrocyte differentiation in these mice. We sought to assess the possible influence of hPS1^{M146V} on oligodendrocyte differentiation patterns *in vitro* in the presence and absence of A β _{1–42} peptides. For these studies, we performed flow cytometry on mOP cells that were transfected with the GFP, hPS1^{WT}, or hPS1^{M146V} plasmids and subsequently treated with A β peptides for 72 h. The gating strategy was applied to specifically select GFP-expressing transfected cells (Figure 3A–B). CC-1 and MBP-positive cell populations were analyzed on the GFP gate (Figure 3C–D). Quantification of GFP-positive mOP cells indicated comparable transfection efficiencies amongst all experimental groups (Figure 3E). Further analysis of the mOP cells revealed significant elevation in the frequency of CC-1 expressing populations in hPS1^{M146V}-transfected cells treated with A β _{1–42} peptides compared to the A β _{42–1} treated control condition (Figure 3F). Numbers of CC-1 expressing cells were not altered by the other treatment conditions. This observation indicates predisposition of hPS1^{M146V}-expressing mOP cells to an A β _{1–42}-induced shift in differentiation pattern. The quantification of MBP-expressing cell population revealed comparable numbers of MBP-positive cells between all transfection groups (Figure 4G) with or without exposure to A β _{1–42}.

hPS1^{M146V} and A β _{1–42} Influence Myelination and MBP Distribution

Although prior evidence demonstrated significant compromise in myelin integrity and abnormal MBP marker staining patterns within sub-regions of 3xTg-AD mouse brain (Desai et al. 2009), our *in vitro* data described above revealed no marked differences in total MBP-expressing mOP cell numbers between all transfection groups, in the presence or absence of A β _{1–42}. Collectively, these data point to a possible aberration in myelination function by hPS1^{M146V}-expressing mature oligodendrocytes upon A β _{1–42} insult. Consequently, we assessed MBP expression levels using western blot analysis on mOP whole cell lysates under the influence of the hPS1^{M146V} expression and A β peptide exposure (Figure 4A). There were significant alterations in MBP levels between hPS1^{WT}- and hPS1^{M146V}-expressing mOP cells that were treated with A β _{1–42} (Figure 4B). No intra-transfection group differences were observed between A β _{42–1} and A β _{1–42} treatments. These observations could be attributed to an overall reduction in MBP protein levels or altered *in vitro* myelination status. We further investigated the ability of mOP cells to form myelin sheets *in vitro*

following GFP, hPS1^{WT}, or hPS1^{M146V} transfection and A β peptide incubation. Treated mOP cells were immunocytochemically stained for MBP and morphometric analysis was performed to assess myelinating cells. Myelinating oligodendrocytes were classified as mOP cells with MBP-expressing membranous sheets adjoined to the processes or emerging from the cytoplasm of the cell body (Figure 4D and G). The enumeration of myelinating cells revealed a significant reduction in A β ₁₋₄₂-treated, hPS1^{M146V}-expressing mOP cells compared to A β ₁₋₄₂-treated, hPS1^{WT}-expressing and GFP control cells (Figure 4I). Furthermore, a marked reduction in myelinating cell numbers was detected in hPS1^{M146V}-expressing mOP cells with A β ₁₋₄₂ exposure compared to A β ₄₂₋₁ group. No differences were observed between the A β ₁₋₄₂ and A β ₄₂₋₁ treatment in the hPS1^{WT}- or GFP-transfected mOP cells.

Previously, heterogeneous MBP protein distribution patterns have been reported in the oligodendrocytes of adult mouse brains, where mature oligodendrocytes express MBP exclusively in myelin sheaths (Hardy et al. 1996; Sternberger et al. 1978a; Sternberger et al. 1978b). This led us to investigate as to whether the expression of the hPS1^{M146V} mutant and/or A β ₁₋₄₂ exposure would alter MBP distribution patterns within mOP cells. MBP is typically detected in cell bodies and processes in cultured oligodendrocytes, contrasting the mature cell population in adult mouse brain where MBP is primarily myelin sheath-localized (Sternberger et al. 1978a). Taking this into account, we classified treated mOP cells into two categories based on apparent MBP distribution: 1) mOP cells expressing MBP in the processes as well as cell body, and 2) mOP cells that retained MBP expression exclusively within the cell body (Figure 4K and N, respectively). Quantitative analysis was performed based on numbers of transfected cells depicting cell body-retained MBP expression. The results revealed a significant elevation in cell body-only counts for mOP cells expressing hPS1^{M146V} as compared to hPS1^{WT} (Figure 4P). These differences were further augmented in hPS1^{M146V}-expressing cells following A β ₁₋₄₂ exposure. Collectively, these results indicate that both hPS1^{M146V} and A β ₁₋₄₂ contribute to abnormalities in MBP distribution patterns with mature mOP cells.

GSK-3 β Involvement in PS1^{M146V} and A β ₁₋₄₂ Effects on mOP Cells

Prior reports have described increased glycogen synthase kinase-3 beta (GSK-3 β) kinase activity in the presence of hPS1^{M146V}, as well as A β ₁₋₄₂ in neurons (Lazarov et al. 2007; Resende et al. 2008). Given this earlier evidence, we assessed GSK-3 β activation status using a surrogate GSK-3 β phospho-epitope in hPS1-expressing mOP cells with/without A β peptide exposure. We employed western blot analysis to assess the levels of the inactive serine-9 phosphorylated GSK-3 β (pGSK3 β) and total GSK-3 β protein levels (Figure 5A). Quantification of inactive pGSK-3 β levels to total GSK-3 β protein revealed a significant decline in hPS1^{M146V}-transfected mOP cells with A β ₄₂₋₁ and A β ₁₋₄₂ peptides compared to control conditions (Figure 5B). The data also revealed a significant increase in this ratio in hPS1^{WT}-expressing mOP cells, which had demonstrated fewer myelination and MBP localization abnormalities (Figure 4). Together, these data suggest that increased GSK-3 β kinase activity (as measured by decreased ratio of pGSK-3 β to GSK-3 β) contribute towards abnormalities in hPS1^{M146V}-expressing mOP cells.

Given the role of GSK-3 β kinase activity in disruption of microtubule-mediated transport in neurons (LoPresti et al. 1995), we next sought to determine the integrity of the transport machinery. Others have shown significant sequence homology in the phosphorylation and tubulin-binding sites of MBP and tau protein (Karthigasan et al. 1995). Therefore, evaluating the distribution patterns of tau might provide insight into the functional integrity of microtubule-mediated transport in mOP cells. Given this, we assessed Tau-5 expression patterns in the mOP cells under all different conditions. Tau-5 staining was found in the cell body as well as processes of all transfected cells with A β ₄₂₋₁ or A β ₁₋₄₂ treatments.

Qualitative assessment revealed comparable patterns of Tau-5 distribution amongst all experimental conditions (Figure 5C–O), indicating that the abnormalities observed in MBP distribution are not due to a general defect in intracellular protein trafficking. We further confirmed GSK-3 β kinase involvement in myelination and MBP localization deficits by concurrently treating mOP cultures with the GSK-3 β inhibitor, TWS119. This inhibitor restored numbers of myelinating mOP cells expressing hPS1^{M146V} and exposed to A β _{1–42} to those detected in hPS1^{WT} and GFP control conditions (Figure 5P). Similarly, TWS119 treatment corrected the MBP mislocalization phenotype in hPS1^{M146V}-expressing mOP cells treated with A β _{1–42} (Figure 5Q). The IE4/5 promoter of the pHSVPrUC/CMVeGFP plasmid drives the expression of the PS1 genes. Thus far, GSK-3 β -driven effects on the IE4/5 promoter-driven gene expression have not been reported. We assessed the possibility that GSK-3 β inhibition may interfere with PS1 expression using immunocytochemistry. No detectable alterations were exhibited in hPS1 expression levels in transfected mOP cells, with or without GSK-3 β inhibitor treatment (data not shown). Therefore, we infer that the observed effects on myelination are in fact a result of PS1 function.

Alterations in MBP Distribution in the Brains of 3xTg-AD/CNP-EGFP Mice

Our *in vitro* data described above speak to a potential role for PS1^{M146V} and A β _{1–42} in the mislocalization of MBP and myelination activity. To study MBP distribution in mature oligodendrocytes in relation to AD pathogenesis *in vivo*, we generated 3xTg-AD/CNP-EGFP mice, which selectively express the eGFP reporter transgene under the transcriptional control of the 2', 3'-cyclic nucleotide 3'-phosphodiesterase (CNPase) promoter within the oligodendrocyte lineage. Non-Tg/CNP-EGFP mice were generated as controls. Transgene-positive mice were identified by PCR-based screening for the specific transgenes. As expected, the Non-Tg/CNP-EGFP mice harbored only the eGFP transgene, whereas the 3xTg-AD/CNP-EGFP mice carried all transgenes (eGFP, hAPP^{Swe}, hTau^{P301L}, hPS1^{M146V}) (Figure 6A–D). The brains of 9 month-old Non-Tg/CNP-EGFP and 3xTg-AD/CNP-EGFP mice were then subjected to co-immunocytochemical analyses for GFP and either NeuN (Figure 5F and I), GFAP (Figure 5L and O), or Iba1 (Figure 5R and U) markers specific to neurons, astrocytes, or microglia, respectively. GFP co-expression with NeuN (Figure 6G and J), GFAP (Figure 6L and O), or Iba1 (Figure 6R and U) was absent in both groups of mice. The brain sections were then stained for MBP protein and GFP to confirm oligodendrocyte-specific GFP expression. GFP expression was localized throughout the oligodendrocyte cell body and processes, thus allowing us to selectively compare MBP subcellular distribution profiles within mature multipolar oligodendrocytes *in vivo*. Representative mature oligodendrocytes from the superficial layers of the entorhinal cortex region with process-restricted MBP staining in oligodendrocytes from control Non-Tg/CNP-EGFP mice are depicted in Figure 6Y. The oligodendrocyte cell bodies were selected (Figure 6Ac) and assessed for the distribution pattern and staining intensities of both GFP and MBP (Figure 6Ad–CAe, respectively). Non-Tg/CNP-EGFP oligodendrocytes exhibited low MBP staining within the cell body, as illustrated by the 3-dimensional histogram depicting pixel intensity throughout the cell body (Figure 6Ad). Conversely, a large proportion of oligodendrocytes from the same brain region in 3xTg-AD/CNP-EGFP mice demonstrated prominent cell body-associated MBP expression in addition to process staining (Figure 6Ab). The histogram corresponding to MBP staining in the cell bodies of 3xTg-AD/CNP-EGFP oligodendrocytes demonstrated high intensities throughout the cell body (Figure 6Ah). GFP expression was maintained throughout the cell bodies of mature oligodendrocytes in both Non-Tg/CNP-EGFP and 3xTg-AD/CNP-EGFP mice (Figure 6W and Z, respectively) and corresponding histograms (Figure 6Ad and Ag, respectively). Enumeration of oligodendrocytes exhibiting either expression pattern revealed Non-Tg/CNP-EGFP oligodendrocytes overwhelmingly harbor process-specific MBP stain and are devoid of cell-body associated expression (Figure 6Ai), while 3xTg-AD/CNP-EGFP mice

have a substantial number of mature oligodendrocytes with cell body-restricted MBP staining patterns. These results corroborate our *in vitro* observations on alterations in MBP expression patterns in the presence of hPS1^{M146V} and A β ₁₋₄₂. Given this observation, we believe the 3xTg-AD/CNP-EGFP mouse model provides a valuable tool for further evaluating how oligodendrocyte-specific alterations drive myelin abnormalities during early AD pathogenesis.

Discussion

White matter degeneration has been extensively reported in the brains of AD patients (Firbank et al. 2007; Hanyu et al. 1998; Roher et al. 2002). Ringman and colleagues demonstrated myelin disintegrity and white matter track atrophy in late-myelinating regions specifically within the brains of pre-symptomatic PS1 FAD mutation carriers compared to non-carrier family members (Ringman et al. 2007). Several studies have recorded myelin degeneration in the brains of PS1 mutation carriers that exhibit non-AD related symptomatic dementia, thus incriminating PS1 mutations in white matter pathology (Dermaut et al. 2004; Marrosu et al. 2006). Moreover, white matter abnormalities have been reported in the 3xTg-AD and APP/PS1 transgenic mice correlating with elevated levels of intracellular A β ₁₋₄₂ prior to the manifestation of overt plaque and tangle pathology (Desai et al. 2010; Wirths et al. 2006).

Myelin breakdown is not exclusive to PS1 mutation carriers, as white matter alterations have also been noted in the brains of individuals with late-onset AD (Firbank et al. 2007), and hAPP^{Swe} and PDAPP transgenic mice, coinciding with stages of advanced amyloid plaque pathology (Harms et al. 2006; Song et al. 2004). This evidence suggests that A β -related insults also impact oligodendrocyte and/or myelin integrity independent of PS1 mutant expression. However, the early onset of white matter pathology in the PS1 knock-in mouse models, implicates PS1 dysfunction as a predisposing condition that can be exacerbated by coincident A β accumulation. Supporting this scenario, oligodendrocytes expressing hPS1^{M146V} in a transgenic mouse model exhibit increased vulnerability to A β peptide species *in vitro* and enhanced white matter pathology *in vivo* (Pak et al. 2003a). In the current study, we employed mOP cells as a model system to examine the influence of PS1 on oligodendrocyte cell fate in the presence and absence of A β ₁₋₄₂ exposure. We had previously reported that a subpopulation (~10%) of A β -treated immature and mature mOP cells are sensitive to A β ₁₋₄₂ toxicity (Desai et al. 2010). However, a majority of differentiated mOP cells remain viable, and here we have shown that these latter cells, when simultaneously expressing hPS1^{M146V} and exposed to A β ₁₋₄₂, are impaired in their abilities to properly traffic MBP to their distal processes and elaborate myelin sheaths *in vitro*.

The roles of hPS1^{M146V} and A β ₁₋₄₂ on cell differentiation patterns have been primarily examined in the context of the neuronal lineage, although those findings remain relatively controversial. PS1 has been shown to regulate neuronal differentiation, while PS1 mutations lead to premature differentiation (Handler et al. 2000; Hong et al. 1999). Another study supports these observations by indirectly correlating hPS1^{M146V} status to neuronal cell differentiation (Hatchett et al. 2007). Contrary to these observations, hPS1^{M146V}-regulated retardation of cell differentiation has also been noted (Tokuhiro et al. 1998). Studies describing the effects of A β peptide species have demonstrated induction of progenitor cell differentiation into neuronal cells (Chen and Dong 2009) whereas other evidence suggests impaired neuronal differentiation (Liu et al. 1998). How these AD-associated factors impact oligodendrocyte cell differentiation has been even less clear. Oligodendrocytes undergo sequential steps in maturation that is accompanied by a coordinated change in the expression of specific antigenic signatures prior to fully differentiating into mature myelinating oligodendrocytes (Baumann and Pham-Dinh 2001). We assessed the numbers of mature

non-myelinating (CC-1) and myelinating (MBP) mOP cells arising from each treatment condition, and observed an increase in numbers of CC-1 positive mature oligodendrocytes with A β ₁₋₄₂ treatment in hPS1^{M146V}-expressing mOP cells. These results are analogous to the increase in CC-1 positive cells previously observed in the CA1 region of 6 month-old 3xTg-AD mouse brains (Desai et al. 2010). Others have described that a functional gamma secretase complex is required for maturation of oligodendrocytes at later stages of differentiation (Lai and Feng 2004). Thus, it is possible that altered gamma secretase activity due to inclusion of the hPS1^{M146V} mutant subunit that is expressed in 3xTg-AD mice or hPS1^{M146V} plasmid-transfected mOP cells may impair further maturation of CC-1 positive cell subsets into MBP-positive myelinating cells. Future studies will be designed to determine the significance of this hPS1^{M146V}/A β ₁₋₄₂-induced CC-1 sub-population and to elucidate the mechanism underlying this possible blockade.

The functional fate of oligodendrocytes in the presence of hPS1^{M146V} expression and A β ₁₋₄₂ exposure occurs via a process that also remains relatively understudied. Extant data suggest gamma-secretase complex activation is required for oligodendrocyte-mediated myelination (Lai and Feng 2004). Consistent with these observations, our results reveal that the *in vitro* myelination activity of mOP cells is enhanced by overexpression of hPS1^{WT}. However, hPS1^{M146V} expression perturbed the formation of myelin sheets in a significant fraction of the cells, and this effect was further exacerbated with A β ₁₋₄₂ exposure. This observation confirmed our previous reports incriminating A β ₁₋₄₂ peptide species in oligodendrocyte and myelin disruptions in the brains of 3xTg-AD mice (Desai et al. 2010). During the course of our myelination analyses, we observed distinct MBP distribution patterns by mOP cells exposed to hPS1^{M146V} and A β ₁₋₄₂. MBP distribution in oligodendrocytes *in vitro* extends from the perikaryon and processes to the peripheral membranes of the cell (Ainger et al. 1993). The expression of hPS1^{M146V} led to significant retention of MBP within the cell body and this phenotype was augmented with addition of A β ₁₋₄₂. Analogous observations were made in the mature multi-polar oligodendrocytes of 3xTg-AD/CNP-EGFP mice at an age coincident with the appearance of myelin abnormalities (Desai et al. 2010). Process-localized MBP was detected in oligodendrocytes of 3xTg-AD/CNP-EGFP and Non-Tg/CNP-EGFP mice, but cell body-restricted MBP was detected exclusively in oligodendrocyte populations of 3xTg-AD/CNP-EGFP mouse brains.

There are several possible explanations as to why MBP subcellular distribution within oligodendrocytes is altered in the presence of hPS1^{M146V} and A β ₁₋₄₂. MBP mRNA, rather than the encoded protein, is transported and targeted to processes, thereby enabling “on site” protein synthesis (Barbarese et al. 1999). Translocation of MBP mRNA along processes requires intact microtubules and kinesin-based transport machinery (Carson et al. 1997). The retention of MBP within the cell bodies is suggestive of a disrupted transport mechanism. It is also plausible that premature translation and/or MBP post-translational modifications prevent the trafficking of the protein from the cell body to distal sites. In a normally functioning oligodendrocyte, MBP mRNA is trafficked to the processes, and upon translation, the polypeptide avidly associates with cellular membranes and is directly incorporated into the developing myelin sheet (Brophy et al. 1993). MBP has been described as the only myelin-specific protein known to be vital and indispensable for myelin biogenesis (Boggs 2006). We posit that the absence of MBP at process termini, observed in the presence of hPS1^{M146V} and A β ₁₋₄₂, renders oligodendrocytes incapable of myelin sheet formation. Reports have also suggested the role of exon 2-containing MBP in differentiation of oligodendrocytes (Gould et al. 1999). Gould and colleagues observed that exon 2-containing isoforms decrease during maturation, while exon 2-deficient isoforms increasingly localize to the processes. This raises the possibility that the presence of hPS1^{M146V} and A β ₁₋₄₂ inhibits exon 2 splicing. Elevated exon 2-containing MBP levels

could impair further differentiation of CC-1 positive oligodendrocytes and diminish MBP levels in cellular processes.

GSK-3 β has been implicated in a number of AD-related pathogenic processes (Takashima 2006). In the current study, we found that GSK-3 β is a promising mechanistic link between PS1 and A β peptides and oligodendrocyte dysfunction. PS1 has been shown to regulate GSK-3 β kinase activity, which can be altered upon introduction of the hPS1^{M146V} mutation (Baki et al. 2004; Pigino et al. 2001). Others have reported that AD-related pathology is exacerbated by A β ₁₋₄₂-associated activation of GSK-3 β (Resende et al. 2008; Terwel et al. 2008). Further supporting these observations are the studies performed in 3xTg-AD mice demonstrating reduced pathology with GSK-3 β inhibition (Caccamo et al. 2007). Our present study correlates oligodendrocyte-specific activation of GSK-3 β with the presence of hPS1^{M146V} and A β ₁₋₄₂ peptide species. These results were further confirmed by restoring MBP distribution and myelin sheet formation in hPS1^{M146V} expressing, A β ₁₋₄₂-treated mOP cultures with TWS119 treatment. Impaired kinesin-based axonal transport resulting from hPS1^{M146V} expression and enhanced GSK-3 β activity has been reported in neurons (Lazarov et al. 2007; Morfini et al. 2002; Pigino et al. 2001). GSK-3 β -mediated phosphorylation of MBP has also been reported *in vitro* (Kawakami et al. 2008), and it is possible that such a modification leads to retention of MBP within the cell body. It is reasonable to propose analogous mechanisms are at play within oligodendrocytes under assault from AD-related processes. An *in vivo* approach of oligodendrocyte-specific GSK-3 β inhibition may serve rescue brain myelination in AD mice similar to our *in vitro* observations. Subsequently, axonal impulse propagation may be restored, thus abolishing the early disturbances observed in electrophysiological functioning in AD mouse models. Future studies will explore the effects of oligodendrocyte-specific GSK-3 β inhibition on myelination employing various molecular, biochemical, and electrophysiological assays.

In conclusion, the present study identifies a novel role for mutant hPS1 and A β ₁₋₄₂ in the development of white matter pathology during early AD. Future studies will focus on further explicating the signaling pathways by which hPS1^{M146V} alters oligodendrocyte and myelin homeostasis. Understanding how the signaling pathways that control the complex stages of oligodendrocyte differentiation and myelin development are affected by AD-related pathogenic factors will help in devising strategies to promote the maintenance, repair, and restoration of myelin in AD-afflicted individuals.

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Figure 1. Construction and verification of the hPS1-expressing plasmid constructs

Illustration of dual-promoter constructs encoding hPS1^{WT} and hPS1^{M146V} under the transcriptional control of Herpes simplex virus (HSV) immediate-early 4/5 gene (IE4/5) promoter and eGFP under the control of cytomegalovirus (CMV) immediate-early promoter (A). Human PS1 mRNA (B) and protein (C) expression was verified in BHK-21 cells transfected separately with each construct. Scale bar indicates 30 μ m. Error bars indicate standard deviation. “***” indicates $P < 0.001$.

Figure 2. Viability of mOP cells following exposure to hPS1 and A β ₁₋₄₂

Representative phase contrast photomicrographs depict the gross morphology of mOP cells transfected with the GFP, hPS1^{WT}, and hPS1^{M146V} plasmid constructs (**A–D**).

Representative images confirm eGFP and hPS1 co-expression in mOP cells 96 h post-transfection (**E–M**). Hoechst 33342 staining was performed to assess cell death of transfected eGFP-positive cells (**O–P**). Quantitative cell death analysis in transfected cells amongst the different experimental conditions is depicted (**Q**). A total of 400–500 cells were enumerated per condition (n=4 per condition) per experiment. Scale bars indicate 30 μ m.

Error bars indicate standard deviation. Western blot analysis reveals A β ₁₋₄₂ peptide conformer composition prior to treatment (0 h) and post-treatment of mOP cells (72 h) (**R**). Asterisk (*) depicts monomeric form of A β ₁₋₄₂ while arrowheads demarcate higher molecular weight A β ₁₋₄₂ aggregates.

Figure 3. hPS1^{M146V} and Aβ₁₋₄₂ effects on mOP cell differentiation patterns

Dot plots illustrating the gating strategy employed to isolate the eGFP-positive population within mOP cultures transfected with the GFP plasmid (**B**) compared to no-transfection controls (**A**) are shown. The gating strategy for CC-1 (**C**) and MBP-stained (**D**) mOP populations is also depicted. The frequencies of eGFP-positive cells demonstrating transfection efficiency for all experimental conditions are illustrated (**E**). Similarly, quantification for GFP/CC-1 (**F**) and GFP/MBP (**G**) double-positive mOP cell populations is shown. A total of 30,000 events for each group (n=4 per group) were collected for each experiment. Error bars indicate standard deviation. “*” indicates P<0.05.

Figure 4. Myelination and MBP distribution patterns following PS1 expression and Aβ₁₋₄₂ treatment

Western blots for MBP and GAPDH protein expression in GFP, hPS1^{WT}, and hPS1^{M146V}-transfected and Aβ peptide-treated mOP cells are depicted (A). Quantitative analysis of MBP levels normalized to GAPDH expression is illustrated (B). Western blot analysis was performed on 40μg of total cell protein lysates collected for each condition (n=4 for each condition) per experiment. Representative images for transfected mOP cells with myelin sheets, as indicated by arrowheads are demonstrated (C–H). Quantification of percentage of myelinating mOP cells is also depicted (I). Additionally, representative images for profiling of MBP distribution in mOP cells are shown (J–O). Quantification of the percentages of

transfected mOP cells with cell body-retained MBP localization is demonstrated (**P**). A total of 300–400 cells were analyzed for each condition (n=4 for each condition) per experiment. Scale bar indicates 10 μ m. Error bars represent standard deviation. “***” indicates P<0.001, “**” indicates P<0.01, and “*” indicates P<0.05.

Figure 5. GSK-3 β contribution to PS1^{M146V} and A β ₁₋₄₂-associated alterations in mOP cells
 Detection of serine 9 phosphorylated GSK-3 β (pGSK-3 β) and total GSK-3 β expression for different groups of mOP cells using western blotting is depicted (A) and quantification of pGSK-3 β to total GSK-3 β protein levels is illustrated (B). Western blot analysis was performed on 40 μ g of total cell lysate protein per condition (n=4 for each condition) for each experiment. Representative images for Tau-5 staining in mOP cells for each experimental condition are represented (C–O). Enumeration of the percentages of myelinating mOP cells (P) and oligodendrocytes with cell body retained MBP distribution (Q) following TWS119 treatment for all groups are depicted. A total of 100–300 cells were analyzed for each condition (n=4 for each condition) per experiment. Scale bar indicates

30 μ m. Error bars represent standard deviation. “***” indicates $P < 0.001$, “**” indicates $P < 0.01$, and “*” indicates $P < 0.05$.

Figure 6. MBP distribution patterns in the oligodendrocytes of 3xTg-AD/CNP-EGFP mice
 Genotypic analysis for GFP (A), hAPP^{Swe} (B), htau^{P301L} (C), and hPS1^{M146V} (D) transgene products are shown for Non-Tg/CNP-EGFP (Lane 1) and 3xTg-AD/CNP-EGFP (Lane 2). Confocal images representing NeuN, GFAP, and Iba1 staining patterns in relation to GFP expressing oligodendrocytes in the cortex of Non-Tg/CNP-EGFP mice (E–G, K–M, and Q–S, respectively) and 3xTg-AD/CNP-EGFP (H–J, N–P, and T–V, respectively) mice are illustrated. Representative confocal images for localization of MBP in mature oligodendrocytes in the brains of Non-Tg/CNP-EGFP (W–Y) and 3xTg-AD/CNP-EGFP (Z–Ab) mice are depicted. Insets represent 2.5X magnification of the original selection and depict the cell bodies of the representative oligodendrocytes from the cortex of Non-Tg/CNP-EGFP (Ac) and 3xTg-AD/CNP-EGFP mice (Af). Histograms reflecting a 3-D representation of the staining intensities of eGFP (Ad and Ag) and MBP (Ae and Ah) of the marked circled region of the magnified cell bodies are also illustrated. The graph depicts the quantification of fractions of mature oligodendrocyte populations within each MBP distribution pattern category (Ai). Scale bars indicate 20 μ m.