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Presenilins Mediate Phosphatidylinositol 3-Kinase/AKT and ERK Activation via Select Signaling Receptors

SELECTIVITY OF PS2 IN PLATELET-DERIVED GROWTH FACTOR SIGNALING*[S]

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The Alzheimer's disease-linked genes, *PS1* and *PS2*, are required for intramembrane proteolysis of multiple type I proteins, including Notch and amyloid precursor protein. In addition, it has been documented that PS1 positively regulates, whereas PS1 familial Alzheimer disease mutations suppress, phosphatidylinositol 3-kinase (PI3K)/Akt activation, a pathway known to inactivate glycogen synthase kinase-3 and reduce tau phosphorylation. In this study, we show that the loss of presenilins not only inhibits PI3K/Akt signaling and increases tau phosphorylation but also suppresses the MEK/ERK pathway. The deficits in Akt and ERK activation in cells deficient in both *PS1* and *PS2* (*PS−/−*) are evident after serum withdrawal and stimulation with fetal bovine serum or ligands of select receptor tyrosine kinases, platelet-derived growth factor receptor β (PDGFRβ) and PDGFRα, but not insulin-like growth factor-1R and epidermal growth factor receptor. The defects in PDGF signaling in *PS−/−* cells are due to reduced expression of PDGF receptors. Whereas fetal bovine serum-induced Akt activation is reconstituted by both PS1 and PS2 in *PS−/−* cells, PDGF signaling is selectively restored by PS2 but not PS1 and is dependent on the N-terminal fragment of PS2 but not γ-secretase activity or the hydrophilic loop of PS2. The rescue of PDGF receptor expression and activation by PS2 is facilitated by FHL2, a PS2-interacting transcriptional co-activator. Finally, we present evidence that PS1 mutations interfere with this PS2-mediated activity by reducing PS2 fragments. These findings highlight important roles of both presenilins in Akt and ERK signaling via select signaling receptors.

Mutations in two homologous presenilin genes, *PS1* and *PS2*, account for the vast majority of early onset familial Alzheimer disease (FAD[1]) (1, 2), whose pathology is virtually identical to sporadic AD characterized by deposition of Aβ and hyperphosphorylated tau inclusion in neurofibrillary tangles. The presenilins are polytopic proteins with 6–8 transmembrane domains that are found in high molecular weight complexes together with nicastrin, *pen-2*, and *aph-1*. These four proteins are required for the regulated intramembrane proteolysis (RIP or γ-secretase activity) of various substrates, including the amyloid precursor protein (APP) and Notch (3). Accordingly, null mice for any one of these components display embryonic lethality associated with severe malformations of the axial skeleton and cerebral hemorrhage, resembling that of Notch deficiency (3). As expected, this activity is conserved in *Caenorhabditis elegans* and *Drosophila*, where the presenilin complex functions to facilitate Notch signaling (4, 5).

All presenilin mutations associated with FAD favor the enhanced production of the pathogenic Aβ42 peptide, suggesting that this is the primary cause of familial cases of AD. Although the amyloid hypothesis is the leading model for AD pathogenesis, a number of concerns with this hypothesis have led many to suggest that non-amyloid-related cellular perturbations of presenilin mutations contribute to the disease phenotype. This is especially plausible in light of the recent observations that three separate PS1 mutations are associated with frontotemporal dementia, a neurodegenerative disorder characterized by tauopathy and without Aβ/amyloid pathology (6–8). The tau pathology in these cases may be explained in part by the role of presenilin in tau phosphorylation. For example, conditional ablation of both *PS1* and *PS2*, but not either gene alone, in adult brain resulted in prominent hyperphosphorylation of tau that was associated with neuronal degeneration and severe learning and memory deficits (9, 10). In sum, these observations strongly suggest that presenilin dysfunctions can contribute to tau pathology and neurodegeneration independent of its effects on Aβ/amyloid pathology.

The manner in which presenilins could directly contribute to tauopathy and neurodegeneration is unknown. However, presenilins are multifunctional proteins. In addition to their roles in Aβ production and Notch proteolysis, presenilins have been shown to mediate other physiological activities *in vitro* and

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[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. 1–3.

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[1] The abbreviations used are: FAD, familial Alzheimer disease; AD, Alzheimer disease; APP, amyloid precursor protein; RTK, receptor tyrosine kinase; MEK, mitogen-activated protein kinase/extracellular signal-regulated kinase kinase; ERK, extracellular signal-regulated kinase; GSK-3, glycogen synthase kinase-3; PI3K, phosphatidylinositol 3-kinase; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonic acid; IGF, insulin-like growth factor; EGF, epidermal growth factor; PDGF, platelet-derived growth factor; PDGFR, PDGF receptor; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; siRNA, small interfering RNA; RT, reverse transcription; CTF, C-terminal fragment; NTF, N-terminal fragment; CREB, cAMP-response element-binding protein; CBP, CREB-binding protein.

in vivo. First, a role for presenilins in the trafficking and maturation of select membrane proteins and/or intracellular vesicles has been shown, although the mechanisms of this activity are unclear (11–14). For example, Naruse *et al.* (13) demonstrated that the glycosylation, trafficking, and signaling of TrkB, a receptor tyrosine kinase (RTK) that signals through the phosphatidylinositol 3-kinase (PI3K)/Akt/glycogen synthase kinase-3 (GSK-3) and MEK/ERK pathways, are altered in *PS1*-deficient neurons. Second, the turnover of other membrane proteins, such as α-synuclein and telencephalin, is delayed by *PS1* deficiency, and the molecules accumulate in large vacuolar structures resembling autophagosomes (12, 14). Third, we previously demonstrated that presenilin negatively regulates the Wnt/β-catenin signaling pathway through facilitating the paired phosphorylation and degradation of β-catenin by acting as a scaffold upon which a priming kinase, GSK-3, and β-catenin are assembled (15, 16). In a different modality, several studies reported that PS1 and PS1 FAD mutations can also differentially modulate GSK-3 activity via PI3K/Akt signaling, a pathway that leads to GSK-3 inactivation and reduced tau phosphorylation (17–19). In this study, we investigated the mechanistic framework by which presenilins could modulate the phosphorylation of tau via the PI3K/Akt pathway. Specifically, we tested the well established model in which the PI3K/Akt pathway is typically activated by extracellular signaling ligands (20). In this model, we hypothesized that select upstream cell surface signaling receptors are affected by presenilin activity rather than downstream signaling components.

The RTK family of cell surface receptors activates not only PI3K/Akt but also the MEK/ERK mitogen-activated protein kinase signaling pathway. This broad family includes the Trk neurotrophin receptors, fibroblast growth factor receptors, IGF-1 receptor, EGF receptor, and PDGF receptors, which are well known for their neurotrophic and/or neuroprotective properties (20, 21). Upon binding to their respective ligands, RTKs undergo dimerization and autophosphorylation of their cytoplasmic tails at multiple tyrosine residues. This leads to the recruitment and activation of a number of signaling molecules, including PI3K and Grb2/Sos. In one arm of RTK signaling, PI3K activates Akt, which then inactivates the tau kinase GSK-3, but also influences a number of important cellular pathways, including pro- and antiapoptotic pathways such as BAD, caspase 9, the FOXO family of transcription factors, and NF-κB. In another arm of RTK signaling, Grb3/Sos activates Ras/Raf-1, which then activates the MEK/ERK mitogen-activated protein kinase pathway. Among many downstream events, ERK is known to mediate molecular processes important for learning and memory, in particular in the activation of CREB (22). In this report, we show that the loss of *presenilins* results in elevated tau phosphorylation and defects in not only PI3K/Akt signaling but also MEK/ERK activation via select cell surface signaling receptors. We further detail the role of both PS1 and PS2 in Akt/ERK signaling induced by serum components and known ligands that activate their corresponding receptors.

MATERIALS AND METHODS

Antibodies and Reagents—The monoclonal antibody PSN2 (against residues 31–56 of human PS1) and polyclonal APP antibody CT15 (against C-terminal 15 residues of APP) have previously been described (23). Monoclonal antibodies against phosphotyrosine (Zymed Laboratories Inc.), phospho-ERK1/2 (Cell Signaling), and FHL2 (MBL) were obtained from commercial sources. Monoclonal antibodies against tau, PHF1 (Peter Davies), Tau-1 (Gloria Lee), and Tau46 (Gloria Lee) were generous gifts. Polyclonal antibody against phospho-202 tau was purchased from BIOSOURCE. Polyclonal antibodies against phospho-GSK3α/β, phospho-Akt, Akt, ERK1/2, PDGFRβ, IGF1-R, PS2 CTF, and phospho-33/37/41 β-catenin were purchased from Cell Signaling. Other reagents (PDGF-AA (Upstate Biotechnology, Inc.), PDGF-BB (Upstate Biotechnology), ^{125}I-PDGF-BB (Amersham Biosciences), MEK inhibitor PD98059 (Calbiochem), PI3K inhibitor Wortmannin (Cell Signaling), puromycin (Calbiochem), bleocin (Calbiochem), and polyclonal anti-PS2 N-terminal fragment (NTF) antibody (Calbiochem) were purchased from the indicated vendors.

cDNA Constructs—cDNAs encoding wild type PS1, wild type PS2, and PS2 M239V were subcloned into the retroviral vector pBabe-puro. cDNAs encoding PS2 D366A, PS2Δloop, wild type PS1, PS1 E280G, PS1 M146L, PS1 ΔX9, PS1N-PS2C, and tau383 (gift from Gloria Lee) were also subcloned into pBabe-bleo. The PS2Δloop construct contains an in-frame deletion of PS2 hydrophilic loop residues 309–352. The PS1N-PS2C construct fuses the NTF of PS1 (residues 1–280) with CTF of PS2 (residues 287–448), obtained from Dr. Gopal Thinakaran (24). All constructs were transfected together with a plasmid encoding the VSV-G envelope into 293GP packaging cells, and the resulting supernatants were prepared for retroviral transduction as previously described (25).

Cell Lines and Transfections—All cell lines were grown in DMEM containing 10% fetal bovine serum (FBS) unless explicitly stated otherwise. *PS*+/+ and *PS*−/− (genetically deficient in *PS1* and *PS2*) have been described previously (16). To introduce PS1, PS2, or PS2MV into *PS*−/− cells, corresponding retroviral supernatants from 293GP cells were added in the presence of 10 μg/ml polybrene and selected with 3 μg/ml puromycin. Resistant cells were pooled and maintained in the presence of 3 μg/ml puromycin without clonal selection. To transfect PS2 D366A, PS2Δloop, PS1, PS1 E280G, PS1 M146L, PS1ΔX9, PS1N-PS2C, or Tau383, corresponding retroviral supernatants were added to *PS*−/−, *PS*−/− (hPS2), or *PS*+/+ cells in the presence of 10 μg/ml polybrene and selected with 100 μg/ml bleocin. Resistant cells were pooled and maintained in the presence of appropriate antibiotic(s). siRNA targeted for exon 3 of mouse FHL2 (sense, 5′-GGAUCGGCACUGGCAUGAAtt-3′) was purchased from Ambion (catalog number 16708). For RNA interference experiments, 25% confluent *PS*+/+ cells were transfected with a final concentration of 60 nM FHL2 siRNA twice using Lipofectamine 2000 (Invitrogen) over a 56-h period.

Cell Lysis and Signaling Assays—Cells were lysed in buffer containing 1% CHAPS, 0.1% SDS, 50 mM Tris (pH 8.0), 150 mM NaCl, 0.002% sodium azide, 400 nM Microcystin-LR, 0.5 mM sodium vanadate, and 1× protease complete inhibitor mixture (Roche Applied Science). For FHL2 detection, cells were gently lysed in 1% Triton X-100 buffer (identical to CHAPS buffer with the exception of detergent) on ice for 10 min without scraping cells off of culture dishes. Triton X-100-insoluble materials (mostly nuclei) still remaining on culture plates were then solubilized with radioimmune precipitation buffer (containing protease and phosphatase inhibitors) and sonicated to shear genomic DNA. For signaling assays, overnight confluent cultures were serum-starved in DMEM for 4 h, and ligands (PDGF-AA (25 ng/ml), PDGF-BB (25 ng/ml), EGF (25 ng/ml), and IGF-1 (50 nM)) were added for the indicated times, or 20% FBS was added for 2 h. Protein quantitations from cell lysates were performed by the micro-BCA method (Pierce), and equal amounts of protein were then subjected to either direct immunoblotting with the indicated antibodies or immunoprecipitated with PDGFRβ antibody and immunoblotted with phosphotyrosine antibody. All experiments were performed at least three times, and representative experiments and/or quantitations are shown.

Reverse Transcriptase PCR Analysis—*PS*+/+, *PS*−/−, *PS*−/− (hPS1), and *PS*−/− (hPS2) cells were grown to confluence, and total RNA was isolated using the RNeasy mini kit from Qiagen. Two micrograms of total RNA were subjected to reverse transcriptase (RT) first strand synthesis using the Superscript kit (Invitrogen) according to the manufacturer's instructions. Equal amounts of the RT product were then used for PCR of PDGFRα or PDGFRβ within a linear range of amplification, empirically determined to be between 16 and 22 cycles. Quantitations were performed using a digital camera-based imaging system. All experiments were performed at least three times, and results were normalized to *PS*+/+ cells.

Cell Surface ^{125}I-PDGF-BB Binding Assays—Overnight confluent cultures of *PS*+/+, *PS*−/−, *PS*−/− (hPS1), and *PS*−/− (hPS2) were placed on ice and washed four times with ice-cold phosphate-buffered saline. ^{125}I-PDGF (0.625 nM) in 3% bovine serum albumin/phosphate-buffered saline was added to cells in the presence or absence of a 100-fold excess of unlabeled PDGF-BB for 30 min. Unbound material was washed five times with 3% bovine serum albumin/phosphate-buffered saline on ice, cells were lysed in 0.2 N NaOH, and bound radioactivity was then quantitated by scintillation counting. Specific binding was calculated by subtracting the values from internal controls subjected to a 100-fold excess of unlabeled PDGF-BB. Experiments were conducted three times, and results normalized to *PS*+/+ cells are shown (means + S.E.).

RESULTS

Loss of Presenilins Impairs PI3K/Akt and ERK Signaling, Elevates GSK-3 Activity, and Increases Tau Phosphorylation—We previously demonstrated that presenilin plays an important role in promoting the degradation of β-catenin, a key signaling intermediate in the Wnt signaling pathway, by acting as a scaffold upon which GSK-3, β-catenin, and a priming kinase are brought together to facilitate the stepwise phosphorylation of β-catenin (16). A primary mechanism of modulating GSK-3 activity is through its phosphorylation by the PI3K/Akt pathway, resulting in GSK-3 inactivation (26). Thus, we examined the phosphorylation status of GSK-3, indicative of general nonprimed GSK-3 activity, in *PS*−/− (lacking both *PS1* and *PS2*) and *PS*+/+ control fibroblasts. Under basal cell culture conditions with 10% FBS, the phosphorylation of both GSK-3α (*upper band*) and GSK-3β (*lower band*), representing the inactive kinase, was substantially reduced in *PS*−/− fibroblasts (Fig. 1*A*). Because GSK-3 is a major tau kinase, we next expressed human four-repeat tau in *PS*+/+ and *PS*−/− fibroblasts to assess whether tau phosphorylation is differentially affected by this alteration in GSK phosphorylation. Indeed, phosphorylated tau on residues 202 and 396/404 (PHF1), two GSK-3 sites, was substantially elevated in *PS*−/− cells under basal conditions (Fig. 1*A*), consistent with recent observations in *PS1*-deficient cells and in forebrains of mice lacking both *PS1* and *PS2* (9, 10). Since GSK-3 can be inactivated by PI3K/Akt-mediated phosphorylation (26), we assessed the phosphorylation status of Akt in *PS*+/+ and *PS*−/− cells. As expected, the active phosphorylated species of Akt (*P-Akt*) was substantially reduced in presenilin-deficient cells (Fig. 1*B*) under basal conditions, consistent with the reduced phosphorylation of GSK-3 species. Further, because PI3K/Akt activation is principally generated by cell surface RTKs that also signal through the MEK/ERK mitogen-activated protein kinase pathway, we next examined whether the activation of ERK is affected by the loss of presenilins. Indeed, the active phosphorylated ERK1 and -2 (*P-ERK1/2*) species were also substantially reduced in *PS*−/− cells under basal conditions (Fig. 1*B*), indicating that the perturbations in ERK and Akt phosphorylation may both be regulated by upstream RTK activity.

Severe Impairment in Akt and ERK Activation via Select Receptor Tyrosine Kinases in Presenilin-deficient Cells and Effects on Tau Phosphorylation—The novel observation that both Akt and ERK signaling pathways were impaired by *presenilin* deficiency led us to hypothesize that select cell surface receptors themselves rather than downstream signaling factors are primarily affected by presenilins. Thus, as a first step, we examined the signaling events activated by several prototypic growth factors (PDGF-BB, PDGF-AA, IGF-1, and EGF). The addition of IGF-1 or EGF to serum-starved *PS*+/+ or *PS*−/− cells for varying time intervals demonstrated robust Akt and ERK phosphorylation in both genotypes, which did not markedly differ from each other (supplemental Fig. 1*A*). In contrast, PDGF-BB (25 ng/ml) or PDGF-AA (25 ng/ml) induced activation of PI3K/Akt/GSK-3 and ERK1/2 phosphorylation after serum deprivation was severely reduced in *PS*−/− cells (Fig. 1*C*, supplemental Fig. 1*B*). In the same experiments, activation of Akt by PDGF had no effect on the GSK-3-mediated phosphorylation of β-catenin (*P-33/37/41*) in either *PS*+/+ or *PS*−/− cells (Fig. 1*C*), indicating that GSK-3 activity on β-catenin through priming and scaffolding mechanisms is independent of Akt-mediated control of GSK-3 activity. In dose-response experiments, we found that the response to PDGF-BB was saturated at 25 ng/ml and did not increase further at 50 ng/ml in both *PS*+/+ and *PS*−/− cells, although the magnitude of the response was much weaker in *PS*−/− at all concentrations

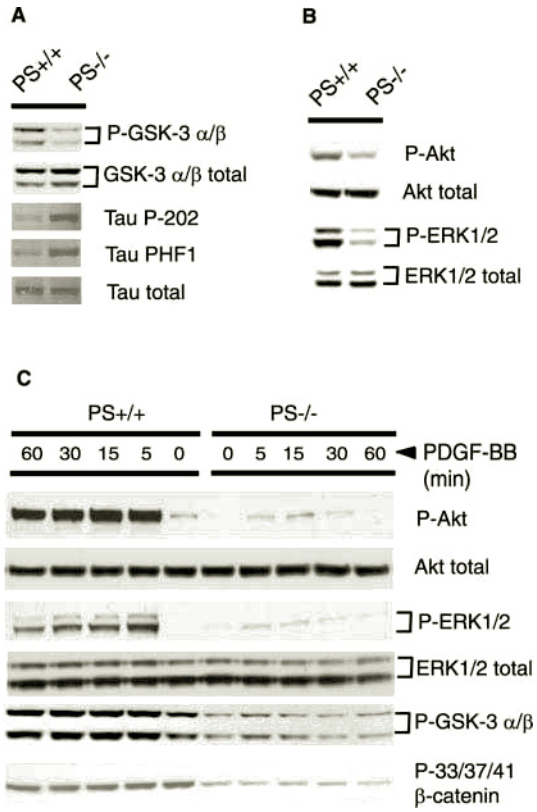

FIG. 1. *A*, enhanced GSK-3 activity correlates with increased tau phosphorylation. Equal protein amounts from confluent overnight cultures of *PS*+/+ and *PS*−/− (*PS1*−/−;*PS2*−/−) cells stably transfected with human Tau383 and maintained in DMEM with 10% FBS were immunoblotted for phospho-GSK-3α/β, total GSK-3α/β, phospho-202 tau, PHF-1 tau (phospho-306/404), and total tau (Tau46). The *upper* and *lower* GSK-3 bands correspond to GSK-3α and GSK-3β, respectively. Note that phosphorylated GSK-3α/β represent the inactivated kinases. *B*, loss of *presenilins* not only reduces Akt but also ERK activation. Equal protein amounts from overnight confluent cultures of *PS*+/+ and *PS*−/− cells maintained in DMEM containing 10% FBS were immunoblotted for phospho-Akt, total Akt, phospho-ERK1/2, and total ERK1/2. The *upper* and *lower* ERK bands represent ERK1 and ERK2, respectively. Note that phosphorylated Akt and ERK1/2 represent active states. *C*, presenilin deficiency reduces Akt and ERK activation via select receptor tyrosine kinases, PDGFRα and PDGFRβ. Overnight confluent cultures of *PS*+/+ and *PS*−/− cells were serum-starved for 4 h in DMEM and PDGF-BB (25 ng/ml), PDGF-AA (supplemental Fig. 1*B*), IGF-1, or EGF (supplemental Fig. 1*A*) were added for the indicated time periods. Equal protein amounts were immunoblotted for phospho-Akt, total Akt, phospho-ERK1/2, total ERK1/2, phospho-GSK-3α/β, total GSK-3α/β, and phospho-33/37/41 β-catenin. Note that phospho-33/37/41 β-catenin does not correlate with the activation of either Akt or GSK-3, whereas *PS*−/− cells exhibit lower phospho-33/37/41 β-catenin compared with *PS*+/+ cells (16).

tested (supplemental Fig. 1*C*). This suggested abnormalities in receptor activity and/or expression in *PS*−/− cells. Indeed, the level of PDGFRβ protein, especially the mature glycosylated product, was reduced by ~3-fold in *PS*−/− cells (Fig. 2*A*). Quantitative RT-PCR analysis also consistently showed that both PDGFRβ and PDGFRα mRNAs were reduced by more than 2-fold in *PS*−/− cells (Fig. 2, *B* and *C*), closely mirroring the protein levels. Moreover, the number of specific ^{125}I-PDGF-BB binding sites on the cell surface of *PS*−/− cells was similarly reduced by >2-fold (Fig. 2*D*). Finally, there was also a strong decrease in PDGF-induced activation and autophosphorylation of PDGFRβ in *PS*−/− cells (Fig. 2*A*), the magnitude of which closely reflected the level of Akt and ERK phosphorylation (Fig. 1*C*). Upon ligand exposure and autophosphorylation, PDGFR is normally rapidly internalized and degraded in lysosomes (27). In serum-starved *PS*+/+ cells,

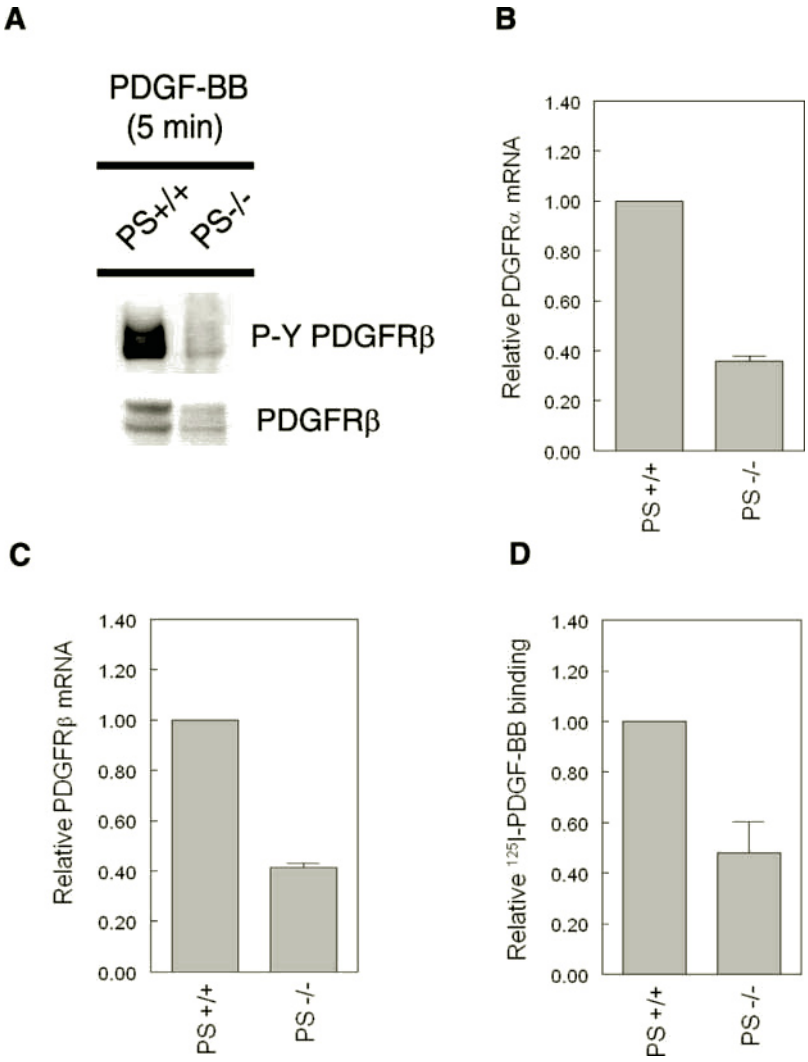

FIG. 2. *A*, reduced PDGFRβ protein and PDGF-BB induced tyrosine autophosphorylation by the loss of presenilins. In the *first panel*, confluent cultures of *PS*+/+ and *PS*−/− cells serum-starved for 4 h in DMEM were treated with PDGF-BB (25 ng/ml) for 5 min, and equal protein amounts were immunoprecipitated for PDGFRβ and immunoblotted for phosphotyrosine (*P-Y*). In the *second panel*, equal protein amounts from confluent cultures of *PS*+/+ and *PS*−/− cells were directly immunoblotted for PDGFRβ without serum starvation or PDGF-BB treatment. *B* and *C*, presenilin deficiency lowers PDGFRα and PDGFRβ mRNA levels. Total RNA was isolated from overnight confluent cultures of *PS*+/+ and *PS*−/− cells, and equal amounts of RNA were subjected to quantitative RT-PCR within a linear range of amplification. Experiments were conducted three times, and graphs show means and S.E. values normalized to *PS*+/+ cells. *D*, reduced PDGF-BB cell surface binding in PS−/− cells. ^{125}I-PDGF-BB (0.625 nM) binding to the cell surface of confluent *PS*+/+ and *PS*−/− cells was quantitated by scintillation counting. Specific binding was calculated by subtracting the values from samples subjected to a 100-fold excess of unlabeled PDGF-BB. Experiments were conducted three times, and graphs show means and S.E. values normalized to *PS*+/+ cells and protein concentrations.

PDGF reliably induced the rapid loss of PDGFR (supplemental Fig. 2*A*). In contrast, ligand-induced degradation of PDGFR was completely absent in *PS*−/− cells up to 1 h, consistent with the considerable reduction in PDGFR activation and autophosphorylation from the cell surface (supplemental Fig. 2*A*).

Since GSK-3 inactivation was markedly reduced upon PDGF signaling in presenilin-deficient cells, we reexamined *PS*+/+ and *PS*−/− cells stably transfected with four-repeat tau for potential PDGF-induced changes in tau phosphorylation. Treatment of PDGF-BB suppressed the phosphorylation of tau in serum-deprived *PS*+/+ but not *PS*−/− cells, as evidenced by two well characterized antibodies, the dephospho-tau antibody Tau-1 (*dephospho-202/205*, *lower band*) and phospho-tau antibody PHF1 (*phospho-396/404*) (Fig. 3). The magnitude of reduction in PHF1 and increase in Tau-1 immunoreactivity upon PDGF treatment in *PS*+/+ cells was ~40 and ~250%, respectively. This reduction in tau phosphorylation was blocked by the specific PI3K inhibitor wortmannin but not the mitogen-activated protein kinase/MEK1 inhibitor PD98059 (Fig. 3), indicating that the PI3K/Akt/GSK-3 pathway plays the major role in this reduction of tau phosphorylation.

Differential Effects of PS1 and PS2 in Akt/ERK Activation via PDGFR—Because the alterations in Akt and ERK activation were seen in fibroblasts deficient in both *PS1* and *PS2* (*PS*−/−), it is unclear whether the defects are secondary to PS1, PS2, or both species. Therefore, wild type human PS1 or PS2 was expressed in *PS*−/− cells to assess whether either or both presenilins can rescue this phenotype. Similar with results from a previous study (17), we found that reconstitution of PS1 expression in *PS*−/− cells facilitated PI3K/Akt signaling induced by FBS after serum deprivation compared with parental *PS*−/− cells (Fig. 4*A* and supplemental Fig. 3*A*). However, this activity was not confined to PS1, as PS2 also similarly increased Akt activation (Fig. 4*A* and supplemental Fig. 3*A*). In addition, both PS1 and PS2 increased FBS-induced ERK1/2 activation, with PS2 showing an overall stronger degree of rescue (Fig. 4*A* and supplemental Fig. 3*A*). Surprisingly, Akt and ERK signaling induced by treatment of PDGF-BB or PDGF-AA was restored by PS2 but not by PS1 (Fig. 4, *B* and *C*). This rescue of phenotype by PS2 was accompanied by increased PDGFR expression, maturation (*upper band*, mature; *lower band*, immature), and PDGF-induced receptor tyrosine phosphorylation, in contrast to that seen with PS1 (Fig. 4*A*). Moreover, quantitative RT-PCR analysis also showed that *PS*−/− cells transfected with PS2 selectively restore the levels of both PDGFRα and PDGFRβ mRNA to levels comparable with those in *PS*+/+ cells (see Fig. 6, *D* and *E*). Similarly, PS2 preferentially reconstituted cell surface ^{125}I-PDGF-BB binding to levels seen in *PS*+/+ cells (Fig. 4*F*). Unlike its wild type counterpart, the PS2 M239V FAD mutation exhibited little to no activity in restoring PDGF-AA- or PDGF-BB-induced Akt and ERK activation or PDGFR expression (Figs. 4*C* and 6*A* and supplemental Fig. 3), although the level of PS2 CTF was identical to wild type PS2 (Fig. 4*C*). Taken together, these results demonstrate that whereas both PS2 and PS1 can facilitate Akt and ERK activation induced by serum components,

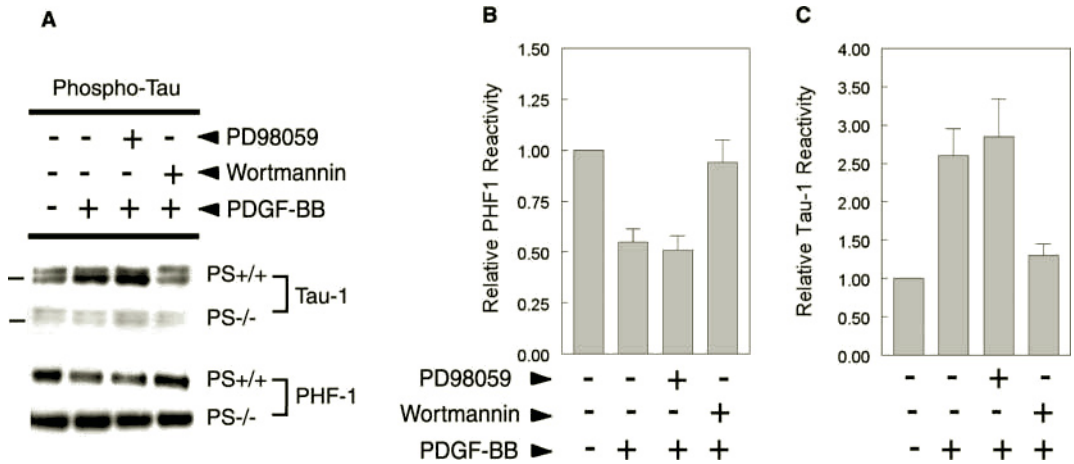

FIG. 3. **Dephosphorylation of tau by PDGF is reversed by PI3K/Akt inhibition in *PS*+/+ but not *PS*−/− cells.** *A*, confluent overnight cultures of *PS*+/+ and *PS*−/− cells were serum-starved for 4 h in DMEM and then treated with or without PDGF-BB (25 ng/ml) together with or without the PI3K inhibitor wortmannin or MEK/ERK inhibitor PD98059 for 90 min. Equal protein amounts from lysates were then subjected to immunoblotting for dephospho-202/205 tau (Tau-1) or phospho-396/404 tau (PHF1). Note the reduction of tau phosphorylation by PDGF-BB in *PS*+/+ but not *PS*−/− cells as well as the complete reversal of tau dephosphorylation in *PS*+/+ cells by wortmannin but not PD98059. A representative experiment is shown. *B* and *C*, graphs show quantitations from three experiments representing the relative levels of Tau-1 (dephospho-202/205) and PHF1 (phospho-396/404) reactivity from *PS*+/+ cells normalized to no-treatment control. The *error bars* represent S.E.

wild type PS2 but not PS2 M239V mutation or PS1 facilitates PDGFR-mediated signaling by regulating the expression of PDGFRs.

Role of PS2 N-terminal Fragment but Not γ-Secretase Activity or Large Hydrophilic Loop of PS2 in PDGF-induced Akt/ERK Activation—Since the major function of the presenilin complex is to mediate the proteolysis of intramembrane peptide bonds as an intramembrane cleaving protease (I-CLiP), we next asked whether the γ-secretase activity of PS2 might be involved in PDGF-induced Akt/ERK activation. We initially utilized pharmacological γ-secretase inhibition with the compound L685,458 for varying time intervals in *PS*+/+ cells. Serum-starved *PS*+/+ cells were pretreated with L685,458 for 4 h before and during the addition of PDGF-BB for 0, 15, 30, and 60 min. Under these conditions, pharmacological γ-secretase inhibition had no effect on PDGFR levels, ligand-induced degradation of PDGFR, or Akt phosphorylation but, as expected, elevated APP C-terminal fragment (CTF) levels (supplemental Fig. 2*B*). This indicated that active γ-secretase inhibition does not interfere with the rapid ligand binding and intracellular PDGF-induced signaling events. We next tested whether potential changes due to prolonged or chronic γ-secretase inhibition could affect PDGF signaling. For these experiments, *PS*+/+ cells were treated with L685,458 for 48 h before and during serum withdrawal and the addition of PDGF. In contrast to the short term treatment, prolonged treatment with γ-secretase inhibitor resulted in marked reduction in PDGFR levels and corresponding diminution in PDGF-induced Akt/ERK activation (supplemental Fig. 2*C*). This suggested that indirect changes resulting from prolonged catalytic γ-secretase inhibition and/or the physical binding of the inhibitor to presenilins underlie this effect.

To determine the direct role of PS2 catalytic γ-secretase activity, we tested whether the critical aspartate on position 366 of PS2, required for γ-secretase activity (28), is also required for PDGF-induced Akt/ERK activation (Fig. 5*A*). As expected in *PS*−/− cells stably transfected with the PS2 D366A mutation, no endoproteolytic fragment of PS2 was detected, and the full-length PS2 D366A was expressed at levels similar to wild type PS2 CTF (Fig. 5*B*). Under these conditions, treatment of PDGF-BB to *PS*−/− (hPS2 D366A) cells after serum deprivation consistently resulted in restoration of Akt/ERK activation comparable with wild type PS2, conclusively demonstrating that the proteolytic γ-secretase activity of PS2 is dispensable for this effect. Thus, the reduction in PDGFR expression secondary to prolonged exposure to γ-secretase inhibitor is not attributable to the inhibition of catalytic γ-secretase activity *per se* but is probably due to functional changes in conformation of the PS2 protein while bound to the γ-secretase inhibitor.

Since PS2 but not PS1 was capable of facilitating PDGF signaling, we next set out to outline the region of PS2 involved in this activity. Two major regions in PS2 are highly divergent in sequence from PS1: the large hydrophilic loop and NTF excluding transmembrane domains. However, it is also notable that a single FAD PS2 M239V mutation is sufficient to abrogate activity with regard to PDGF signaling (Figs. 4*C* and 6*A* and supplemental Fig. 3*B*), indicating high sensitivity to changes in protein structure caused by select mutations. The hydrophilic loop regions of PS1 and PS2 are devoid of FAD mutations and appear not to change the conformation/structure of presenilin proteins. For example, the large hydrophilic loops of PS1 or PS2 are not required for γ-secretase activity and are neutral to changes in Aβ42 caused by PS FAD mutations (29). Thus, a PS2Δloop construct lacking residues 309–352 of PS2 (divergent sequences in the PS2 loop) was stably expressed in *PS*−/− cells. Immunoblot analysis showed that the PS2 NTF of PS2Δloop was expressed at levels similar to that of the wild type PS2 counterpart (Fig. 5*C*). Likewise, serum starvation and the addition of PDGF-BB demonstrated that the PS2Δloop restores Akt/ERK activation to levels comparable with wild type PS2 (Fig. 5*C*). Thus, the divergent sequences 309–352 in the large hydrophilic loop of PS2 are not required for this activity. To assess the contribution of PS2 NTF in PDGF signaling, we next expressed a fusion chimeric protein (PS1N-PS2C) in which PS1 NTF (residues 1–280) was fused to PS2 (residues 287–448; Fig. 5*A*). This protein was previously shown to undergo normal endoproteolytic cleavage, assemble into stable complexes, and mediate γ-secretase activity (24). Stable transfection of PS1N-PS2C in *PS*−/− cells resulted in strong expression of PS2 CTF (Fig. 5*B*). In contrast to PS2Δloop, however, PS1N-PS2C completely failed to restore PDGF-induced Akt and ERK activation (Fig. 5*B*), demonstrating the critical role of the PS2 NTF in this activity. These results taken together clearly demonstrate that whereas the hydrophilic loop and γ-secretase activity are dispensable, the NTF of PS2 is

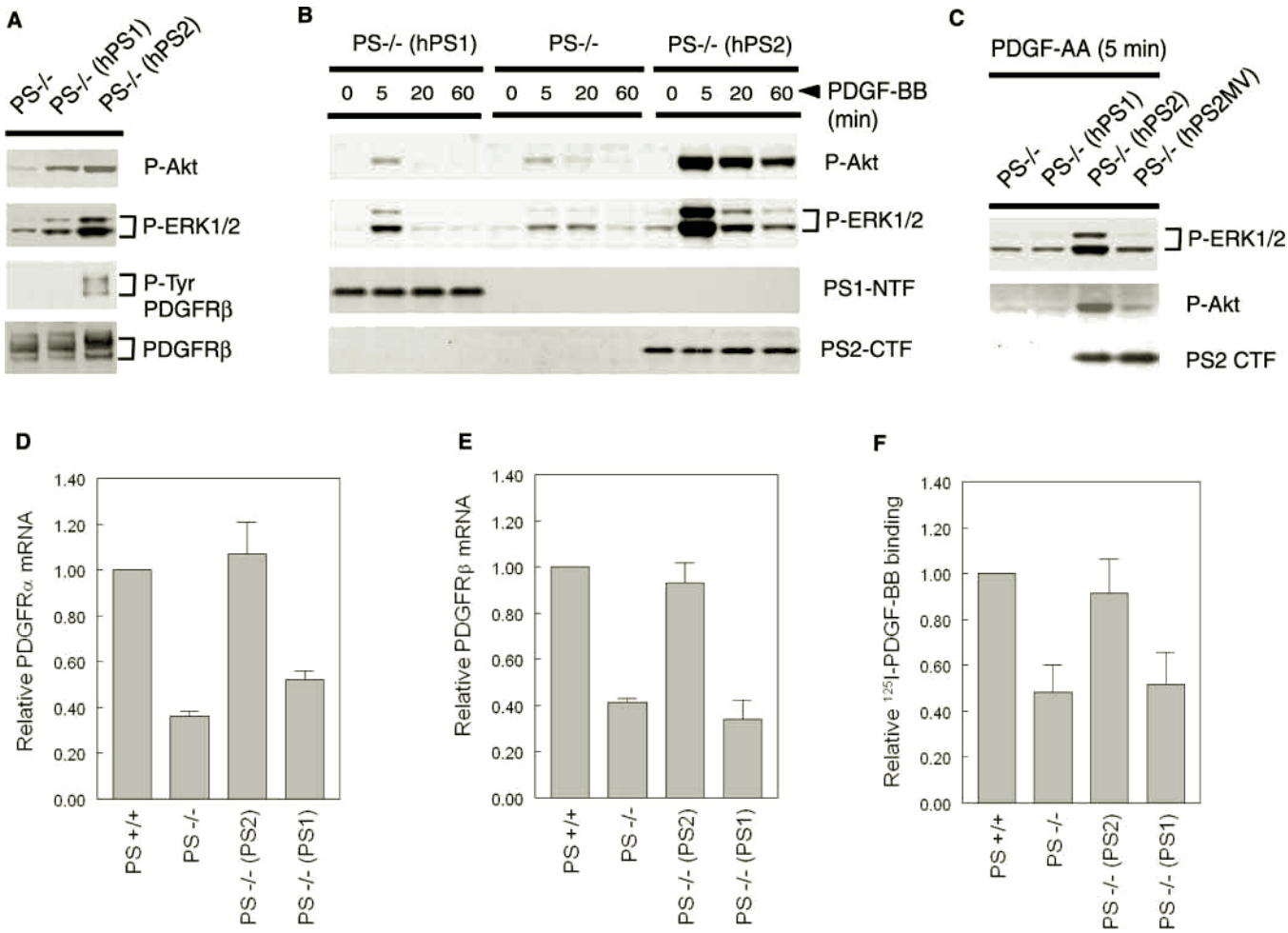

FIG. 4. *A*, both PS1 and PS2 restore serum-induced Akt and ERK phosphorylation, but PS2 selectively restores PDGFR expression and PDGF-induced PDGFR tyrosine phosphorylation. Confluent cultures of *PS*−/− cells and those stably reconstituted with human PS1 (*PS*−/− (hPS1)) or PS2 (*PS*−/− (hPS2)) were serum-starved for 4 h in DMEM and FBS at a final concentration of 20% (*upper two panels*) or PDGF-BB (25 ng/ml; *third panel*) was added to the medium for 2 h or 5 min, respectively. In the *upper two panels*, representing treatment with FBS, equal protein amounts from CHAPS lysates were subjected to immunoblotting for phospho-Akt and phospho-ERK1/2. In the *third panel*, representing serum withdrawal and treatment with PDGF-BB, PDGFR immunoprecipates from equal protein amounts were immunoblotted for phosphotyrosine (*P-Y*). In the *bottom panel*, untreated confluent cultures of *PS*−/−, *PS*−/− (hPS1), and *PS*−/− (hPS2) cells were lysed with CHAPS, and equal protein amounts were immunoblotted for PDGFR*β*. Note the increase in PDGFR expression and maturation by PS2. *B* and *C*, PS2 selectively rescues PDGF-induced Akt and ERK activation. Confluent cultures of *PS*−/−, *PS*−/− (hPS1), *PS*−/− (hPS2), and *PS*−/− (PS2 M239V) cells were serum-starved for 4 h in DMEM and treated with PDGF-BB (25 ng/ml) or PDGF-AA (25 ng/ml) for the indicated times. CHAPS lysates were then subjected to immunoblotting for phospho-Akt, phospho-ERK1/2, PS1 NTF, and PS2 CTF. Note that the PS2 M239V FAD mutation does not restore PDGF-induced signaling. *D* and *E*, PS2 selectively restores the levels of PDGFR*β* and PDGFR*α* mRNAs. Total RNA was isolated from confluent cultures of *PS*+/+, *PS*−/−, *PS*−/− (hPS1), and *PS*−/− (hPS2) cells, and equal amounts of RNA were subjected to quantitative RT-PCR within a linear range of amplification. Experiments were conducted three times, and graphs show means and S.E. values normalized to *PS*+/+ controls. *F*, PS2 selectively restores the level of PDGF-BB cell surface binding. ^{125}I-PDGF-BB (0.625 nM) binding to the cell surface of confluent *PS*+/+, *PS*−/−, *PS*−/− (hPS1), and *PS*−/− (hPS2) cells was quantitated by scintillation counting. Specific binding was calculated by subtracting the values from internal controls subjected to a 100-fold excess of unlabeled PDGF-BB. Experiments were conducted three times, and graphs show means and S.E. values normalized to *PS*+/+ cells and protein concentrations.

required for PDGF-induced activation of Akt/ERK and cannot be substituted by PS1.

Role of FHL2 in PS2-mediated Control of PDGFR Expression—Three proteins are known to selectively interact with PS2 but not PS1: calmyrin, sorcin, and FHL2 (30–32). Among these, FHL2 (also called DRAL) is a LIM domain protein that interacts with PS2 NTF and functions as a transcriptional co-activator for a number of different transcriptional complexes (33–35). Because PDGFR mRNA and protein levels were significantly reduced in *PS*−/− cells and restored by PS2, we hypothesized that PS2 might alter the biology of FHL2 and, in turn, affect PDGFR levels. FHL2 is a soluble protein that normally shuttles between the cytoplasm and the nucleus. To test whether FHL2 levels and/or localization are altered by PS2 variants or PS1, we separated Triton X-100-soluble and -insoluble materials from *PS*+/+, *PS*−/−, *PS*−/− (hPS2), *PS*−/− (hPS2 M239V), and *PS*−/− (hPS1) cells on culture dishes without using a cell scraper. Microscopic examination of the insoluble material remaining on culture dishes after Triton extraction revealed a nearly complete absence of membranes/cytoplasm and the salient presence of almost exclusively nuclei and few cytoskeletal elements. The Triton X-100-insoluble material was then solubilized in radioimmune precipitation buffer with sonication to shear the genomic DNA. By immunoblotting, the levels of Triton X-100-insoluble and presumably nuclear FHL2 protein were significantly diminished in *PS*−/− cells compared with *PS*+/+ cells and restored by wild type PS2 but not PS2 M239V or PS1 (Fig. 6*A*). This tightly correlated with PDGFR levels in these cells (*i.e.* the capacity to signal via PDGF) (Fig. 6*A*), suggesting that PS2 might regulate PDGFR levels through FHL2. A similar but much weaker correlation between FHL2 and PDGFR level was seen in the Triton X-100-

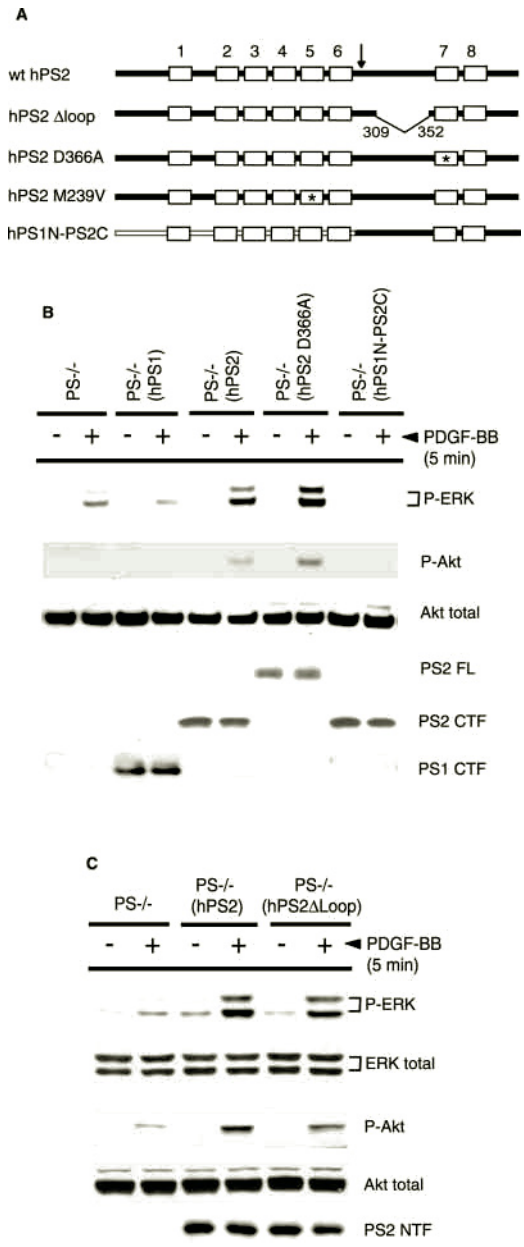

FIG. 5. *A*, schematic of wild type PS2 and different PS2 mutant constructs. The *open boxes* represent the transmembrane domains 1–8, and the *asterisks* indicate positions of D366A and M239V PS2 mutations. The *arrow* indicates the position of endoproteolytic cleavage. PS2Δloop represents a deletion of residues 309–352 within the hydrophilic loop of PS2, whereas PS1N-PS2C represents a fusion of PS1 NTF (residues 1–280) with PS2 CTF (residues 287–448). *wt*, willd type. *B*, the N-terminal fragment but not the catalytic γ-secretase activity of PS2 is required for PDGF-induced Akt and ERK activation. Overnight confluent cultures of parental *PS*−/− cells and *PS*−/− cells stably transfected with PS1, PS2, or PS2 D366A constructs were serum-starved for 4 h in DMEM and treated with PDGF-BB (25 ng/ml) for 5 min. Equal protein amounts of cell lysates were then immunoblotted for phospho-ERK, phospho-Akt, total Akt, PS2, and PS1. *C*, the hydrophilic loop region of PS2 is not required for PDGF-induced Akt and ERK activation. Overnight confluent cultures of parental *PS*−/− cells and *PS*−/− cells stably transfected with wild type PS2 or PS2Δloop were serum-starved for 4 h in DMEM and treated with PDGF-BB (25 ng/ml) for 5 min. Equal protein amounts of cell lysates were then immunoblotted for phospho-ERK, total ERK, phospho-Akt, total Akt, and PS2 NTF.

soluble cytoplasmic fraction (not shown). Since FHL2 is a transcriptional co-activator, we hypothesized that the reduced expression of PDGFR in *PS*−/− cells might at least in part result from reduced FHL2 level or activity. Thus, we sought to mimic this *PS*−/− condition by experimentally lowering FHL2 levels in *PS*+/+ cells by RNA-mediated interference. By transient transfection of siRNA targeted toward FHL2 for 56 h, we were able to achieve an average 61% reduction in FHL2 protein from three experiments. As predicted from our hypothesis, this reduced FHL2 expression led to a corresponding but more modest ~45% decrease in PDGFR (Fig. 6, *B* and *C*). No significant changes were seen in total Akt levels (Fig. 6*B*). After serum withdrawal and stimulation with PDGF-BB, we also observed a modest reduction in Akt activation upon FHL2 siRNA transfection, presumably resulting from the decrease in PDGFR expression (Fig. 6*B*, *lower panel*). Thus, FHL2 positively regulates PDGFR expression directly or indirectly. This at least partially explains the decrease in PDGFR in *PS*−/− cells. Because PS2 restored nuclear FHL2 levels in *PS*−/− cells and knockdown of FHL2 reduced PDGFR expression in *PS*+/+ cells, we interpret these data to indicate that PS2 facilitates PDGF signaling through an FHL2-mediated increase in PDGFR expression.

PS1 Mutations Inhibit Serum- and PDGF-induced Akt/ERK Activation—The inability of PS1 to restore PDGF-induced Akt/ERK activation in *PS*−/− cells can be explained by the failure to restore FHL2 and PDGFR levels. However, we found that PS1 could facilitate Akt activation induced by FBS (Fig. 4*A*). This observation is analogous to a previous study in which PS1 enhanced Akt phosphorylation in E-cadherin-transfected *PS1*−/− cells in FBS-containing culture conditions (17). Thus, the mechanisms by which presenilins augment Akt/ERK signaling via PDGF and serum are at least partially distinct. In previous studies, it was also reported that FAD PS1 mutations suppress the basal level of PI3K/Akt activation compared with wild type PS1 in cells where PS2 was not experimentally altered (17, 19). To investigate whether similar results could be observed in our experimental setting, wild type PS1 or two PS1 FAD mutations (E280G and M146L) were stably expressed in *PS*−/− cells previously transfected with human PS2 (PS−/− (hPS2)). Immunoblot analysis showed that PS1 NTF expression was comparable between wild type and mutant proteins, with little to no detectable full-length PS1 (Fig. 7*A*). Upon serum withdrawal and treatment with 20% FBS for 2 h, we found that FAD mutations noticeably suppressed Akt activation compared with wild type PS1 (Fig. 7*A*), results that are similar to those seen in previous studies in different cellular models (17, 19). In addition, serum-induced ERK1/2 phosphorylation was also reduced by FAD mutations compared with wild type PS1 (Fig. 7*A*), suggesting that both Akt and ERK pathways are altered by PS1 via a common mechanism. Surprisingly, although the level of PS1 expression was comparable among wild type and mutant PS1 proteins, there was >2-fold reduction of PS2 CTFs in cells expressing FAD PS1 mutations compared with wild type PS1 (Fig. 7*A*). Accordingly, serum withdrawal and PDGF-AA treatment showed that FAD mutations also reduced PDGF-dependent Akt/ERK activation compared with wild type PS1 (Fig. 7, *B–D*). Specifically, whereas both mutations inhibited Akt and ERK phosphorylation, the PS1 E280G mutation showed a stronger effect, with an average reduction in PDGF-induced Akt and ERK phosphorylation by 62 and 70%, respectively (Fig. 7, *B–D*). Similar results were seen after serum withdrawal and treatment with PDGF-BB (not shown). These results taken together indicate that FAD PS1 mutations suppress not only Akt and ERK activation induced by serum components but also that induced by PDGF, the latter of which is dependent on PS2 levels. Moreover, these data raise the intriguing possibility that multiple PS1 activities may in part function in coordination with PS2.

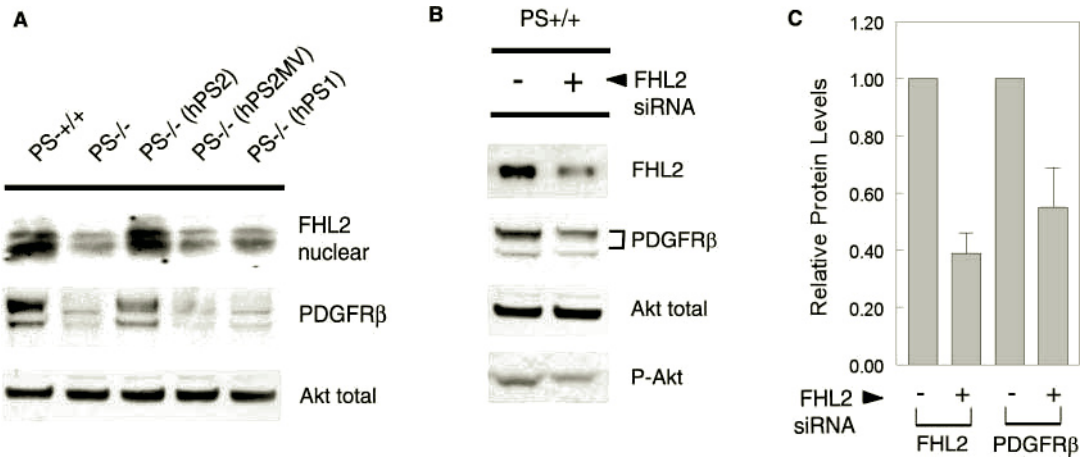

FIG. 6. *A*, nuclear FHL2 levels correlate with PDGFR expression and the capacity to signal via PDGF. Overnight confluent cultures of *PS*+/+, *PS*−/−, *PS*−/− (hPS2), *PS*−/− (hPS2 M239V), and *PS*−/− (hPS1) cells were solubilized with 1% Triton X-100 buffer on ice. The Triton X-100-insoluble nuclei remaining on culture dishes were then solubilized with radioimmune precipitation buffer and subjected to sonication. In the *upper panel*, equal protein amounts of Triton X-100-insoluble nuclear fraction were immunoblotted for FHL2. In the *lower two panels*, equal protein amounts of Triton X-100-soluble material were immunoblotted for PDGFRβ and total Akt. *B*, experimental reduction in FHL2 results in lower PDGFR levels in *PS*+/+ cells. Twenty-five percent confluent *PS*+/+ cells were transfected with Lipofectamine 2000 plus FHL2 siRNA or Lipofectamine alone for 56 h. In the *upper three panels*, cells were lysed in 1% Triton X-100 buffer, and equal protein amounts were subjected to immunoblotting for FHL2, PDGFRβ, and total Akt. In the *bottom panel*, cells were serum-starved for 4 h and treated with PDGF-BB (25 ng/ml) for 30 min, and equal protein amounts were immunoblotted for phospho-Akt. A representative experiment is shown. *C*, the *graph* represents the average level of FHL2 and PDGFRβ protein in *PS*+/+ cells after FHL2 siRNA transfection normalized to mock transfection from three experiments. The *error bars* represent S.E.

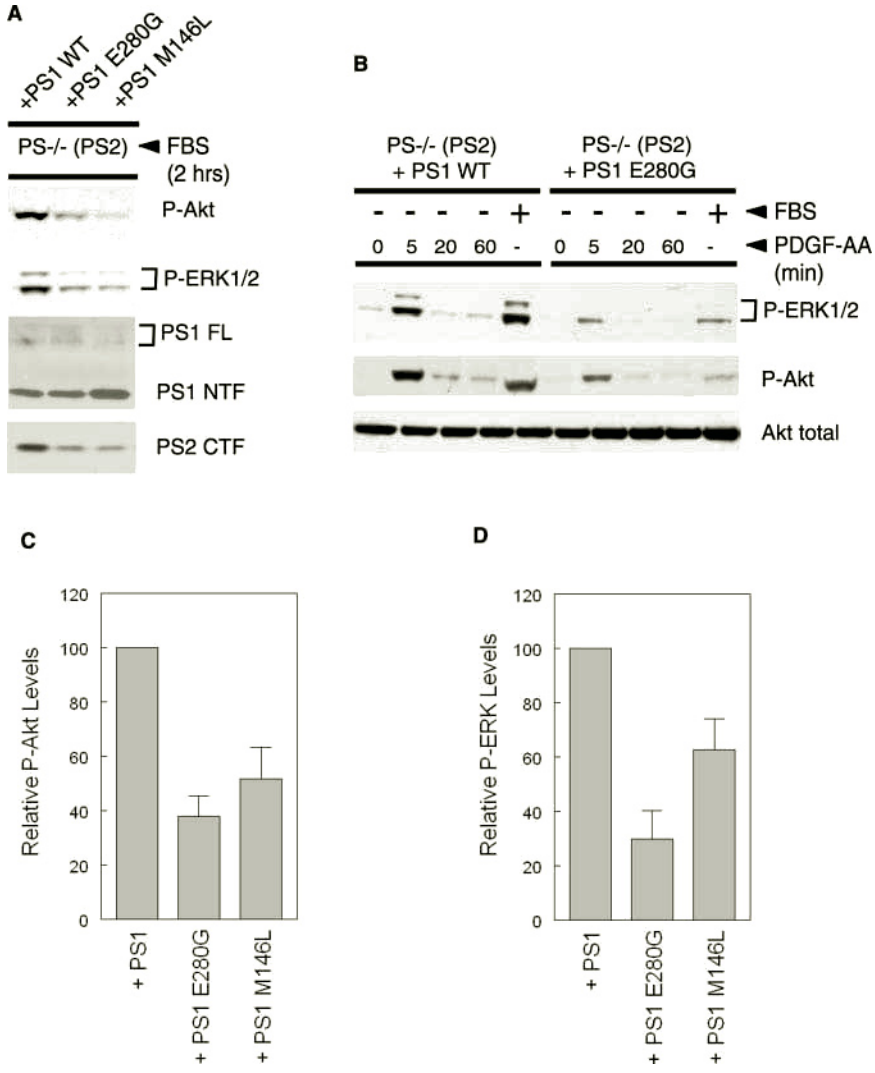

FIG. 7. *A*, FAD PS1 mutations inhibit serum-induced Akt and ERK activation and lower PS2 fragment levels. Confluent cultures of *PS*−/− (hPS2) cells stably transfected with wild type PS1, PS1 E280G, or PS1 M146L were serum-starved for 4 h in DMEM and treated with FBS to a final concentration of 20% for 2 h. Cell lysates were then subjected to immunoblotting for phospho-ERK1/2, phospho-Akt, total Akt, PS1 NTF, and PS2 CTF. A representative experiment is shown. *B–D*, PS1 FAD mutations suppress PDGF-induced Akt and ERK activation. Confluent cultures of *PS*−/− (hPS2) cells stably transfected with wild type PS1, PS1 E280G, or PS1 M146L were serum-starved for 4 h in DMEM and treated with PDGF-AA for the indicated times or FBS (20% final) for 2 h. Cell lysates were then subjected to immunoblotting for phospho-Akt, total Akt, and phospho-ERK1/2. A representative experiment is shown in *B*. The *graphs* in *C* and *D* show quantitations from three experiments representing the levels of phospho-Akt and phospho-ERK after 5 min of PDGF-AA treatment normalized to wild type PS1 controls. The *error bars* represent S.E.

DISCUSSION

Presenilins in Akt/ERK Signaling and Tau Phosphorylation via Select Cell Surface Signaling Receptors—Accumulating evidence suggests that presenilins play important roles in Akt/GSK signaling and tau phosphorylation that may impinge on the pathology of neurodegenerative diseases. It has been reported that that FAD PS1 mutations reduce Akt activity, increase GSK-3 activity, and render PC12 cells more vulnerable to apoptosis (19). Moreover, the loss of *PS1* and FAD PS1 mutations were reported to enhance tau phosphorylation and reduce kinesin-based transport by increasing GSK-3 activity in primary neurons (18). These earlier studies, however, did not provide a mechanistic framework by which PS1 or FAD mutations could potentially account for these effects. In a recent study using E-cadherin-transfected *PS1*-deficient and control cells, it was proposed that one mechanism by which PS1 could activate Akt and reduce tau phosphorylation is by bridging the p85 regulatory subunit of PI3K to cadherins, the loss of which abrogated cadherin-mediated activation of Akt in *PS1*-deficient cells (17). In this cadherin-dependent model, wild type PS1 facilitates the recruitment and activation of PI3K on cadherins upon cell-cell interactions, whereas FAD PS1 mutations inhibit this process.

In this study, we used *PS1/PS2* double deficient cells (*PS*−/−) without E-cadherin overexpression. Thus, our results are contextually distinct from the study by Baki *et al.* (17) and need to be interpreted accordingly. However, it is clear from this study that that the cadherin-based mechanism of Akt activation is but one of multiple routes by which presenilins facilitate signaling from the cell surface. Indeed, the loss of presenilins markedly diminished not only Akt but also ERK activation induced by PDGF-AA, PDGF-BB, or FBS. The last ERK pathway is not known to be activated by cadherins. In contrast, signals activated by EGF or IGF-1 were comparatively intact in terms of the loss of presenilins, indicating that the capacity to signal through Akt/ERK is essentially intact in *PS*−/− cells. These data taken together indicated that deficits in Akt/ERK activation originated from select cell surface signaling receptors rather than downstream signal transduction mechanisms. Indeed, the specific defects in PDGF signaling in *PS*−/− cells were due to a marked reduction in the expression and activation of PDGF receptors. Based on the current and previous studies, signaling receptors affected by presenilins now include TrkB (13), cadherins (17), PDGFRα, and PDGFRβ.

Selectivity of PS2 in PDGF-induced Signaling Independent of γ-Secretase Activity—The observation that PS1 restored Akt signaling induced by FBS in *PS1/PS2* double deficient cells is analogous with previous results in E-cadherin-transfected *PS1* single deficient cells in which PS1 restored Akt activation (17). However, this was not unique to PS1, since we found that PS2 also rescued this phenotype. Thus, the degree of rescue by PS1 and PS2 is probably additive and dependent on the overall expression of both presenilins. This may explain the observed deficit in Akt activation by the loss of *PS1* alone in the previous study. Moreover, it is notable that E-cadherin binds to PS1 through a region that is not conserved in PS2 (36); thus, E-cadherin overexpression in the previous study (17) probably amplified the PS1-dependent cadherin-based route of Akt activation. The manner in which presenilins facilitated FBS-induced Akt and ERK activation in the current study is unknown. In addition to the deficits in cadherin-mediated Akt activation, we hypothesize that other signaling receptors activated by serum components are also compromised by loss of presenilins. Such receptors probably resemble receptor tyrosine kinases, since both Akt and ERK pathways are concomitantly affected.

Unlike signaling induced by FBS, Akt/ERK activation induced by PDGF treatment was selectively reconstituted by PS2 but not PS1, indicating at least partially distinct mechanisms. The NTF of PS2 (residues 1–286) was required for restoring PDGF signaling in *PS*−/− cells and could not be substituted by the same region in PS1. It has been documented that the highly divergent hydrophilic loops of PS1 and PS2 are neither required for γ-secretase activity nor sensitive to changes in Aβ42 caused by FAD mutations (29). Likewise, the large hydrophilic loop (residues 309–352) of PS2 was dispensable for PDGF-induced Akt/ERK phosphorylation. Furthermore, the catalytic γ-secretase activity of PS2 was completely dispensable for this activity, since PS2 D366A, a catalytic γ-secretase-defective mutant, was fully capable of restoring PDGF-induced Akt/ERK activation. This biological property of PS2 therefore adds to a growing list of presenilin-mediated functions that are not dependent on catalytic γ-secretase activity (12, 14, 16).

Despite the functional rescue of PDGF signaling by the catalytically dead PS2 D366A mutation, prolonged treatment of γ-secretase inhibitor in *PS*+/+ cells reduced PDGFR levels and signaling, at least partially mimicking the *PS*−/− state. This apparent discrepancy therefore emphasizes caution in the interpretation of results secondary to the use of pharmacologic γ-secretase inhibitors. For example, γ-secretase inhibitors not only inhibit the catalytic protease activity of the γ-secretase complex but also alter the trafficking of presenilin fragments and Pen-2 (37). Thus, the binding of γ-secretase inhibitors to the presenilin complex can alter the biology of presenilins that are dependent and independent of γ-secretase activity, a latter phenotype seen by the reduction in PDGFR expression and PDGF signaling. In dose response experiments, certain γ-secretase inhibitors, such as L685,458, elevate the Aβ42/Aβ40 ratio at low concentrations prior to complete inhibition of γ-secretase activity at higher concentrations (38). Thus, γ-secretase inhibitors may also functionally alter the conformation of PS2 to resemble a mutant state, much like the PS2 M239V mutation that failed to restore PDGF signaling and PDGFR expression in *PS*−/− cells.

FHL2 in PS2-mediated PDGFR Expression—The observation that PDGF receptors were reduced at the level of protein and mRNA by the loss of presenilins and selectively rescued by PS2 but not PS1 suggested indirect transcriptional control of PDGFR by PS2. Among three proteins that are known to interact specifically with PS2 but not PS1, we first selected FHL2 for further study, because it binds to the NTF of PS2 and fulfills the criteria as a potential transcriptional candidate. The direct physical association between FHL2 (also called DRAL) and PS2 was previously discovered in a yeast two-hybrid screen and confirmed in co-immunoprecipitation experiments (32). FHL2 was first identified as a co-activator of the androgen receptor (39) and was later found to function as either a co-activator or co-repressor of multiple transcriptional assemblies. For example, FHL2 co-activates AP-1 (35), CREB/cAMP-response element modulator (33), and CBP/β-catenin (34), whereas it co-represses FOXO1 (40). Our initial observation that Triton X-100-insoluble nuclear FHL2 levels were significantly diminished in *PS*−/− cells and restored by PS2 but not PS1 suggested a positive role of FHL2 in PS2-mediated PDGFR expression and signaling. Fulfilling this prediction, experimental reduction of FHL2 in *PS*+/+ cells by RNAi indeed led to a significant decrease in PDGFR, indicating that PDGFR is either a direct or indirect target of FHL2-mediated transcriptional co-activation. Thus, we interpret these results to indicate that PS2 facilitates PDGFR expression, at least in part, through FHL2-mediated co-activation of PDGFR.

The manner by which FHL2 co-activates PDGFR expression

or PS2 enhances nuclear FHL2 levels is unknown. One potential mechanism by which PS2 can increase nuclear FHL2 levels is by facilitating its nuclear translocation. FHL2 normally translocates into the nucleus in the presence of serum (35). Given that a major fraction of PS2 is found in membranes of the outer nuclear envelope and endoplasmic reticulum, the juxtaposition of PS2 to the nucleus may serve to facilitate the normal serum-induced route of FHL2 nuclear translocation. In the nucleus, FHL2 directly interacts with CBP/p300 and synergistically co-activates target genes (34). Since CBP/p300 facilitates NF-Y-induced stimulation of the PDGFRβ promoter (41), it is conceivable that FHL2 synergistically enhances this transcriptional route of PDGFR expression.

The M239V mutation, unlike its wild type counterpart, failed to rescue PDGFR expression and signaling when expressed in *PS*−/− cells. Likewise, it also failed to restore nuclear FHL2 levels, consistent with the involvement of FHL2 in PDGFR expression. However, the PS2 M239V mutation is not contained within a putative FHL2 binding site (residues 269–298 of PS2) (32). We interpret this to suggest that conformational changes caused by this mutation also abrogate functional interactions with FHL2. The PS1N-PS2C protein, which failed to reconstitute PDGF signaling in *PS*−/− cells, lacks the entire NTF of PS2, including the putative FHL2 binding site, consistent with the role of FHL2 in PS2-mediated PDGF signaling.

The possibility that additional mechanisms unrelated to FHL2 might be involved in PS2-mediated PDGF signaling cannot be fully precluded by this study. Presenilins are known to mediate the trafficking and maturation of select membrane proteins and/or intracellular vesicles. For example, glycosylation, trafficking, and signaling of TrkB are altered in *PS1*-deficient neurons (13), and α-synuclein and telencephalin accumulate in large vacuolar structures resembling autophagosomes by *PS1* deficiency (12, 14). Thus, it is conceivable that PS2 is also involved in mediating the proper trafficking of vesicles containing PDGFRs in addition to its effects on PDGFR expression. The observation that PDGF-induced degradation of PDGFR was absent in *PS*−/− cells may be evidence of such a trafficking defect.

FAD PS1 Mutations in Serum and PS2-mediated PDGF Signaling—Previous studies have reported that FAD PS1 mutations suppress the basal level of Akt activation in two different cell culture models in which PS2 was not experimentally altered (17, 19). Indeed, the current results confirmed this prediction in our own cell culture system using *PS*−/− cells stably transfected with PS2. One surprising finding was that PS1 FAD mutations led to a substantial reduction in PS2 fragment levels compared with wild type PS1. This in turn resulted in a corresponding decrease in PDGF-induced Akt and ERK activation by FAD PS1 mutations (E280G and M146L) compared with wild type PS1. The generation of PS1 and PS2 fragments are coordinately controlled by competition for limiting cellular factors. Therefore, extreme overexpression of either PS1 or PS2 can cross-interfere with the generation of both PS1 and PS2 fragments (28, 42). However, the expressions of wild type and mutant PS1 proteins in this study were similar, with little to no detectable full-length molecule. Therefore, it is unlikely that the reduction in PS2 CTF by PS1 mutations is due to differences in the expression of PS1 variants. Instead, it has been demonstrated that whereas both wild type and FAD mutant PS1 fragment levels reach a saturation point regardless of excess full-length precursor, fragments derived from FAD PS1 mutations (A246E and M146L) tend to accumulate more readily than those of wild type PS1 prior to the saturation point (43). Therefore, the reduction of PS2 CTF by FAD PS1 mutations compared with wild type PS1 may reflect the tendency of FAD mutants to more effectively compete for limiting cellular factors needed for generating PS1 fragments at the expense of PS2. In this way, FAD PS1 mutations would not only impinge on PS1 but also on PS2 activities, as observed with PDGF signaling in this study. These results taken together demonstrate that the coordinated actions of both PS1 and PS2 are important in Akt and ERK signaling via select cell surface signaling receptors and suggest the possibility that FAD presenilin mutations might compromise neuroprotective Akt and ERK signaling through both PS1- and PS2-dependent pathways in brain.

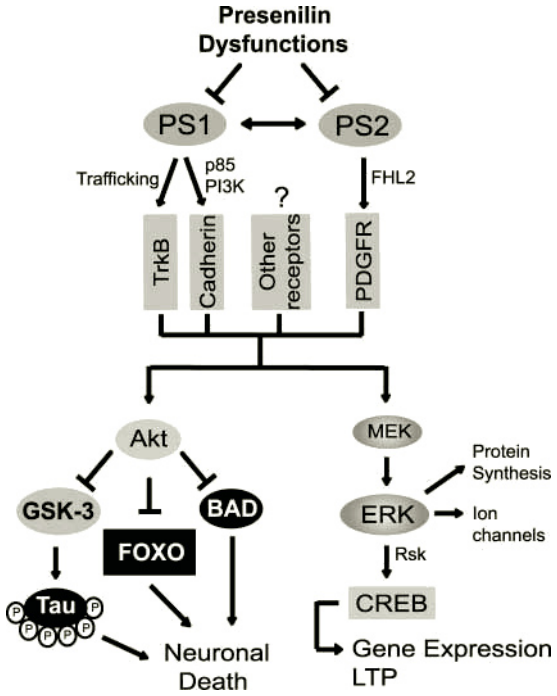

FIG. 8. **Proposed model of presenilins in neurodegeneration and tau phosphorylation via cell surface signaling receptors.** This model proposes that presenilins normally function to promote Akt and/or ERK signaling via PDGFRs, TrkB (13), cadherins (17), and as yet unidentified signaling receptors. However, such signaling functions are compromised by mutations or dysfunctions in either PS1 or PS2. Mutations in PS1 may also lead to interference of PS2 function and *vice versa*, such as the inhibition of PS2-mediated PDGF signaling by FAD PS1 mutations. Through select signaling receptors, activated PI3K/Akt inhibits multiple proapoptotic pathways (*i.e.* BAD and FOXO), activates prosurvival NF-κB, and inhibits GSK-3, all leading to enhanced neuronal survival and reduced tau phosphorylation. Simultaneously, the activation of the MEK/ERK pathway stimulates protein synthesis, changes ion channel properties, and stimulates CREB-mediated transcription, pathways important for gene expression and consolidation of memory. Thus, deficits in Akt and ERK activation by presenilin dysfunctions are predicted to increase the phosphorylation of tau, render neurons more vulnerable to degeneration, and impair learning and memory.

In Vivo Correlations to Neurodegeneration Associated with Loss of Presenilins—A recent study documented that the G183V PS1 mutation co-segregates with pathologically confirmed familial frontotemporal dementia (6), a tauopathy lacking Aβ/amyloid. This finding, together with two recent studies showing striking neurodegeneration, hyperphosphorylation of tau, and memory deficits by the conditional loss of both *PS1* and *PS2*, strongly indicates that perturbations in presenilin functions can directly impinge on tangle pathology and neurodegeneration (9, 10). However, the mechanisms by which the loss of presenilins results in these phenotypes are unknown. We hypothesize that defects in Akt and ERK activation by loss of presenilins seen in this study, at least in part, account for such a neurodegenerative phenotype seen in the *PS* dKO mice.

PI3K/Akt signaling is known to inactivate the tau kinase GSK-3, inhibit several proapoptotic pathways, and activate the prosurvival NF-κB pathway (21, 44). Activation of ERK mediates many molecular processes important for learning and memory as well as confers neuroprotective effects (22). Thus, deficits in Akt and ERK activation are predicted to increase the phosphorylation of tau, render neurons more vulnerable to degeneration, and impair learning and memory, precisely as that seen in the *PS* dKO mice (Fig. 8).

It has been demonstrated that PDGFRα/β-mediated signaling exerts neuroprotective effects (45–47). However, it is unlikely that these receptors alone can account for the degenerative phenotype seen in the *PS* dKO mice. The deficits in Akt/ERK activation induced by serum components seen in *PS*−/− cells lead us to hypothesize that multiple cell surface receptors are also affected by PS1 and PS2 (Fig. 8). This prediction is supported by the involvement of PS1 in cadherin-mediated Akt activation (17) and severe reduction in TrkB-mediated signaling in *PS1*-deficient neurons (13). In the latter study, the maturation and brain-derived neurotrophic factor-mediated autophosphorylation of TrkB were severely compromised by the loss of *PS1* in a manner analogous to that seen with PDGFR in this study. Moreover, it has also been reported that PS1 is also involved in the proper maturation and trafficking of N-cadherin to the cell surface (48). Although the precise mechanisms underlying the perturbations in each of these signaling receptors appear to be distinct, they all signal through the common PI3K/Akt and/or MEK/ERK pathways. Based on the current data and previous studies, we propose a model in which mutations or dysfunctions in PS1 or PS2 partially interfere with select cell surface signaling receptors and that such inhibition may constitute a pathogenic mechanism for accelerated neurodegeneration and tau pathology (Fig. 8). The accumulation of the pathogenic Aβ42 species probably further compromises this neuroprotective process. Recent studies have also documented that the PI3K/Akt signaling pathway is also inhibited by dysfunctions in APP and apoE receptors (49–53). These findings therefore raise the intriguing possibility that perturbations in PI3K/Akt and/or MEK/ERK signaling may constitute common pathway(s) for neurodegeneration in both early and late onset AD.

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