Presenilin 1 regulates β-catenin-mediated transcription in a Glycogen Synthase Kinase-3-independent Fashion*

Received for publication, June 11, 2001, and in revised form, August 9, 2001
Published, JBC Papers in Press, August 14, 2001, DOI 10.1074/jbc.M105376200

James J. Palacino‡, M. Paul Murphy§, Ohoshi Murayama¶, Katsunori Iwasaki‡, Michihiro Fujisawa‡, Akihiko Takashima¶, Todd E. Golde§, and Benjamin Wolozin‡**

From the ‡Department of Pharmacology and Neuroscience Program, Loyola University Medical Center, Maywood, Illinois 60153, the §Department of Pharmacology, Mayo Clinic, Jacksonville, Florida 32224, the ¶Laboratory of Alzheimer's Disease, RIKEN, Japan, and the¶Fukuoka University, Fukuoka, Japan

Presenilin 1 (PS1) is linked with Alzheimer’s disease but exhibits functional roles regulating growth and development. For instance, PS1 binds to β-catenin and modulates β-catenin signaling. In the current study, we observed that knockout of PS1 inhibited β-catenin-mediated transcription by 35%, as shown by a luciferase reporter driven by the hTcf-4 promoter. Overexpressing wild-type PS1 increased β-catenin-mediated transcription by 57.5%, and overexpressing PS1 with mutations associated with Alzheimer’s disease decreased β-catenin-mediated transcription by 66%. To examine whether regulation of β-catenin by PS1 requires phosphorylation by glycogen synthase kinase 3β (GSK 3β), we examined whether inhibiting GSK 3β activity overcomes the inhibition of β-catenin transcription induced by mutant PS1 constructs. Cells expressing wild-type or mutant PS1 were treated with LiCl, which inhibits GSK 3β, or transfected with β-catenin constructs that lack the GSK 3β phosphorylation sites. Neither treatment overcame PS1-mediated inhibition of β-catenin signaling, suggesting that regulation of β-catenin by PS1 was not affected by the activity of GSK 3β. To investigate how PS1 might regulate β-catenin signaling, we determined whether PS1 interacts with other elements of the β-catenin signaling cascade, such as the Tcf-4 transcription factor. Coimmunoprecipitation studies showed binding of PS1 and hTcf-4, and examining nuclear isolates indicated that nuclear hTcf-4 was decreased in cells expressing mutant PS1. These data show that PS1 interacts with multiple components of the β-catenin signaling cascade and suggest that PS1 regulates β-catenin in a manner independent of GSK 3β activity.

* This work was supported by an Alzheimer Association grant (to B. W.), by an NIA, National Institutes of Health (NIH) Grant AG17485 (to B. W.), and by NIH Grant 1F31MH12479 (to J. J. P.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1754 solely to indicate this fact.

** To whom correspondence should be addressed: Dept. of Pharmacology, Molecular Pharmacology Laboratory, Loyola University Medical Ctr., 2160 S. First St., Bldg. 102, Rm. 3634, Maywood, IL 60153. Tel.: 708-216-6195; Fax: 708-216-6596; E-mail: bwolozi@wpo.it.luc.edu.

† The abbreviations used are: PS1, presenilin 1; GSK 3β, glycogen synthase kinase 3β; AD, Alzheimer’s disease; HEK, human embryonic kidney; LEF, lymphocyte enhancer factor; hTcf-4, human T-cell factor 4; APP, amyloid precursor protein; CMV, cytomegalovirus; DTT, dithiothreitol.

This paper is available online at http://www.jbc.org
Presenilin Mutations Inhibit β-Catenin Signaling

RESULTS

Presenilin 1 Knockout Inhibits β-Catenin Transcription

Reports in the literature suggest that PS1 regulates the activity of β-catenin by regulating its stability via a GSK 3β-dependent mechanism. Based on this, we hypothesized that knockout of PS1 would remove the interaction between PS1, β-catenin, and GSK 3β. Loss of this interaction might decrease phosphorylation of β-catenin by GSK 3β, leading to higher levels of free cytosolic β-catenin and increased transcriptional activity. To monitor β-catenin signaling, we measured activation of the β-catenin/hTcf-4 transcription complex. Cells were transfected with a luciferase reporter construct, termed pTopFlash. This construct is a β-catenin-sensitive promoter that contains four tandem repeats of the hTcf-4 DNA binding domains, which is a transcription factor thought to mediate β-catenin transcriptional signaling (24, 25). Previous studies show that binding of β-catenin to hTcf-4 activates transcription of the pTopFlash reporter (24, 25). The data was standardized by cotransfecting with a constitutively active Renilla luciferase vector, which controls for variations in transfection efficiency.

Using the pTopFlash construct, we transiently transfected immortalized fibroblasts isolated from PS1+/− mice and their wild-type littermates (13). In initial experiments, we analyzed the signal at 0.5, 1, 2, and 3 days and determined that the level of signal was maximal 2 days following transfection (data not shown), hence the 2-day time point was chosen for all subsequent experiments. The data represent the mean of two experiments (n = 8).

Cells lacking PS1 exhibited impaired β-catenin-mediated signaling. PS1−/− cells showed a 35 ± 4% decrease in luciferase signal (Fig. 1). The decrease in signal suggested a role for PS1 in the regulation of β-catenin phosphorylated transcription. Alternatively, PS1 may be active as an inhibitor of GSK 3β-mediated degradation. PS1 has been shown to bind both β-catenin and GSK 3β, but not APC, which is thought to be essential for the regulation of the phosphorylation. It is possible that PS1, by binding GSK 3β and β-catenin, but not APC, may protect free β-catenin for a short period. This protection could
allow the “protected” free β-catenin additional time to activate transcription.

Presenilin 1 Mutations Inhibit β-Catenin-mediated Transcriptional Activity—To investigate the regulation of β-catenin signaling by PS1, we generated stably transfected N2a neuroblastoma lines overexpressing different PS1 constructs, including wild-type, A246E, Δ9, D257E/D385E, and D257A/D385A mutants. The latter two constructs each contained mutation of both aspartyl groups thought to be essential for PS1 γ-secretase function. Immunoblots of lysates from these cells showed that the aspartyl mutants were not normally cleaved and existed as a single 45-kDa holoprotein, which is consistent with observations by others (Fig. 2A).

Following confirmation of PS1 overexpression, we examined the regulation of β-catenin-mediated transcription by PS1. Samples were analyzed after 48 h following transient transfection. Overexpression of wild-type PS1 showed no significant effect on the level of β-catenin-mediated transcription. Conversely, expression of the familial AD variants or aspartyl mutations of PS1 decreased the transcription by an average of 35 ± 4% (p < 0.01), depending on the construct (Fig. 2A). Data represent the mean of five individual experiments (n = 15).

To exclude the possibility of clonal effects of the stable N2a lines, we repeated the experiment using a transient overexpression of PS1. For this experiment, cells were cotransfected with 1 μg of the PS1 plasmids described above and 1 μg of pTopFlash plasmid and assayed after 2 days. Analysis of β-catenin-mediated transcriptional activity showed that the mutant forms of PS1 also inhibited β-catenin activity in the transient system (Fig. 2B). In fact, the inhibition was more robust in the transient overexpression paradigm producing an average inhibition of 44.8 ± 2.2% (p < 0.01) for all the mutant isoforms. The efficacy and rapidity of response in this transient experiment indicates that PS1 mutations can rapidly inhibit β-catenin-mediated transcription.

The changes in β-catenin-mediated transcription could not be explained by reduction of β-catenin levels or by increased proteolysis, because immunoblotting of cell extracts from the N2a PS1 lines shows that steady-state levels of β-catenin were consistent between cell lines (data not shown). Consistent levels of total β-catenin suggest that the cellular β-catenin pool remained consistent. Reports have indicated changes in β-catenin stability following overexpression of or mutations in PS1, but the data are contradictory (15, 20). It is possible that catenin turnover is altered, but the maintenance of a consistent steady-state protein concentration indicates that similar amounts of catenin should be available for signal transduction-related events.

We wanted to examine the effect of activation of the Wnt/β-catenin cascade. To do this, we examined β-catenin-mediated transcription in HEK293 cells stably transfected with wild-type PS1, the M139V familial AD mutant, and the D385E aspartate mutant. This cell line has previously been shown to be very responsive to chemical activation of the Wnt cascade.
through inhibition of GSK 3β (19). Using 10 mM LiCl, we mimicked the activation of the Wnt cascade by inhibiting GSK 3β activity. This results in an increase in cytosolic levels of β-catenin. Increasing free β-catenin facilitates the association of β-catenin with hTcf-4 and the subsequent transcription by the complex. Cells were again transfected with the pTopFlash plasmid and, following transfection, treated with 10 mM LiCl or aqueous vehicle for 24 h. Treatment with LiCl resulted in an average 7-fold increase in signal, consistent with an increase in free β-catenin available for transcriptional activity (Fig. 3A). As with the N2a cells, the HEK293 cells expressing the M139V familial AD mutation and the D385E aspartate mutation both showed a highly significant inhibition of β-catenin-mediated transcription, with a 59.0 ± 3.0% and 66.5 ± 2.3% decrease in signal, respectively (Fig. 3B). The degree of inhibition was consistent as compared with the vector both basally and following LiCl treatment. The HEK293 cell line overexpressing wild-type PS1 showed an average 23.5 ± 3.5% increase in β-catenin-mediated transcription as compared with the vector (Fig. 3B). The results were consistent both basally and following LiCl treatment. It should be noted that there is variation between the results for the wild-type PS1 transfected in the N2a and HEK293 cells. This is most likely related to cell type variability. We have previously shown that the wild-type PS1 transfected in PC12 cells shows a significant decrease in β-catenin-mediated signaling, which is further inhibited by mutations in PS1 (14).

As an independent method of inhibiting GSK 3β activity, we repeated the experiments using a cotransfection protocol with a mutant β-catenin construct. The S33V substitution in β-catenin removes one of the two critical GSK 3β phosphorylation sites, resulting in a decreased rate of degradation for β-catenin (25). Cotransfection of this construct with the pTopFlash plasmid increased the luciferase signal approximately 3-fold over the control cotransfection of pcDNA3/TopFlash (Fig. 3C). We also examined pTopFlash transcription following treatment with LiCl (10 mM) or vehicle for 24 h. The M139V and the D385E mutants caused a significant decrease in transcription both basally and following LiCl treatment (60.7 ± 1.3 and 77.4 ± 1.6, respectively), which is consistent with the data from the initial experiment (Fig. 3C). The wild-type continued to show a similar degree of enhancement, with an increase of 51.6 ± 3.5% (Fig. 3B). The data represents the mean of three experiments (n = 9).

Inhibiting GSK 3β activated β-catenin-mediated transcription in each cell line; however, we were interested in determining whether inhibiting GSK 3β overcame the inhibition of β-catenin transcription induced by the presence of mutant PS1 transcripts. To explore this question, we compared the relative level of TopFlash transcription under each condition. For each condition (LiCl, S33V β-catenin, or LiCl + S33V β-catenin), the signal for the cell line containing only endogenous PS1 was set at 100 and then compared with signal in the wild-type PS1, M139V PS1, or D385E PS1 cell lines under the same conditions (Fig. 3D). Data from each mutant cell line was then expressed as a percentage of the vector values for the appropriate treatment paradigm. Cells expressing wild-type PS1 showed an increase of 17.8–63.9% over vector for each condition; the mean increase was 37.5 ± 4.5% (Fig. 3D). Conversely, the mutants decreased the Topflash signal by 51.5–63.2% for M139V (average 59.9 ± 2.3%) and 75.1–75.3% for D385E (average 72.0 ± 2.2%) (Fig. 3D). Statistical analysis showed that the percent changes were not different among the four treatment paradigms within each cell line.

The effects of the PS1 isoforms on β-catenin-mediated signaling were proportional for all treatment paradigms. We have shown, using two discrete methods, that the changes in transcription are consistent for the PS1 variants. If PS1 were regulating β-catenin through a GSK 3β-dependent mechanism, blockade of GSK 3β should reverse the effects of the mutants. This was not the case, because the mutants showed no differential response to antagonism of GSK 3β activity. Therefore, PS1 apparently regulates β-catenin-mediated transcription through a mechanism not dependent on GSK 3β activity. Because the inhibition of GSK 3β does not normalize responses, we hypothesized that the effect of PS1 was downstream of GSK 3β. The main downstream event in the Wnt cascade is the association of β-catenin with the leucine zipper transcription factor family.

Presenilin 1 Interacts with Tcf4—PS1 is known to have multiple binding partners, including β-catenin (reviewed in Ref. 26). Because the mutations in PS1 apparently inhibit transcriptional activity of β-catenin downstream of GSK 3β, we hypothesized that PS1 may be interacting with the binding partner of β-catenin, namely hTcf-4. To assess this possibility, we performed immunoprecipitations with an hTcf-4 antibody and probed with PS1 to determine whether there is a physical interaction between the two proteins. Analysis of the immunoprecipitates on Western blot using the PS1 loop antibody revealed the presence of a specific band at ~46 kDa, which comigrated with the band for full-length PS1 (Fig. 4A). There was no evidence of hTcf-4 immunoprecipitating the endogenous PS1 C-terminal fragment, suggesting that the interaction was specific for the full-length PS1. Examination of the levels of PS1/hTcf-4 immune complexes indicates that there was no difference in affinity based on the total levels of PS1 present in the lysates (Fig. 4B).

Presenilin 1 Inhibits Tcf-4 Nuclear Translocation—We next wanted to determine by what mechanism PS1 was exerting its influence in regulating the β-catenin cascade. Previous reports have indicated that mutant PS1 inhibits nuclear translocation of β-catenin (19). However, there is also evidence that the β-catenin analog Armadillo is capable of translocation to the nucleus without activating transcription (27). A recent report by Honda et al. (28) indicates that wild-type PS1 normally resides in the nuclear envelope as a holoprotein and migrates to the endoplasmic reticulum following its constitutive endoproteolysis. They further reported that mutant isoforms of PS1 do not take up residence in the nuclear envelope, rather, they remain in the endoplasmic reticulum as both holoproteins and cleaved products. The authors suggest a possible role for the holoprotein in regulating a novel function within the nuclear membrane. Based on our immunoprecipitation data, and the findings of Nishimura et al. (19), we hypothesized that the mutants could be affecting the translocation of proteins bound to the holo-PS1.

We chose to examine the nuclear localization of hTcf-4 in HEK293 cells. Cells were grown to ~90% confluency (to enhance total nuclear levels of hTcf-4) and then were treated for 3 h with 10 mM LiCl. Cells were collected, nuclei were isolated, and hTcf4 was immunoblotted. Similarly to described reports on β-catenin, we observed a decrease in nuclear levels of hTcf-4 protein in the cells overexpressing the mutant PS1 constructs as compared with vector, and we saw an enhancement in hTcf-4 translocation in the cell line overexpressing wild-type PS1 (Fig. 5A). Semiquantitative densitometry analysis revealed that the M139V and D385E mutants significantly decreased nuclear hTcf-4 levels by 34.6 and 34.4%, respectively. The wild-type cells showed a significant increase in Tcf-4 levels of 34.0% (Fig. 5B). These results are qualitatively consistent with our findings on transcription by the TopFlash reporter. Additionally, they are similar to findings reported by Nish-
imura et al. (19) indicating an inhibition of β-catenin translocation in PS1 mutant cells following LiCl stimulation. Our data on the binding of PS1 to hTcf-4 and the inhibition of hTcf-4 nuclear translocation by the mutant PS1 isoforms support the hypothesis that PS1 is coupling β-catenin to hTcf-4 and might be involved in the transport of this transcriptional complex to the nucleus.

**DISCUSSION**

We have investigated the interaction between PS1 and β-catenin in several cell lines by monitoring β-catenin transcriptional activation. Our findings indicate that deletion of PS1 inhibits the level of β-catenin-mediated transcription. Overexpression of wild-type PS1 did not alter β-catenin-mediated transcription in one cell line (N2a) and increased β-catenin-mediated signaling in a second cell line (HEK293). Conversely, overexpressing familial AD mutant PS1 or aspartyl mutant PS1 strongly inhibited β-catenin-mediated transcription. This was true in the both N2a neuroblastoma and HEK293 cells. The aspartyl mutant PS1 constructs, which have been previously described to inhibit Aβ processing, also...

**FIG. 3.** PS1 alters β-catenin-mediated signal transduction independently of the action of GSK 3β. A, immunoblot of PS1 in HEK293 cells stably expressing PS1 constructs. Lane 1, untransfected; lane 2, wild-type PS1; lane 3, M139V; lane 4, D385E PS1. Arrows point to the holo-protein and cleaved protein. B, HEK293 cell lines stably overexpressing wild-type, M139V, and D385E PS1 constructs were transiently transfected with the pTopFlash construct, as above, samples were cotransfected with the pRL-CMV Renilla luciferase construct to control for variations in transfection efficiency. Following transfection, half the samples for each cell line were treated with 10 μM LiCl to inhibit GSK 3β activity; the remaining samples were treated with vehicle (distilled water). Following 24 h to allow for stimulation, samples were lysed and analyzed as described. Similarly to the data in the N2a cell lines, overexpression of the mutant isoforms of PS1 resulted in a significant decrease in transcription, both basally and following LiCl treatment. Overexpression of the wild-type PS1 construct resulted in a significant increase in transcription over vector. All cell lines showed an average 7-fold increase in signal following treatment with LiCl. C, to inhibit the action of GSK 3β on β-catenin turnover independent of pharmacological treatment, we cotransfected with a β-catenin construct that is partially insensitive to GSK 3β phosphorylation. The cell lines described above were cotransfected with the pTopFlash, pRL-CMV, and the S33V β-catenin expression vector driven by a CMV promoter. This mutation is a naturally occurring mutant in tumors, and has a mutation in one of the two key GSK 3β phosphorylation sites. As a result, the turnover of this protein is much slower than the wild-type β-catenin. The presence of this protein alone was enough to result in an almost 3-fold increase in TopFlash signal over the vector-transfected samples. The samples were treated with LiCl or vehicle for 24 h and analyzed. Treatment with LiCl resulted in an average 5.7-fold increase in signal, consistent with inhibition of GSK 3β action on the other phosphorylation site. D, the same data from C are shown without normalization to the Renilla luciferase signal. E, to examine the effects of the overexpression of the PS1 isoforms and their effects on β-catenin-mediated signal transduction we replotted the data as a factor of the vector signal. For each treatment paradigm (control, LiCl, S33V β-catenin, and S33V β-catenin with LiCl), data from the vector-transfected cells were set to an arbitrary value of 100. Data from each of the overexpressing cell lines were then analyzed against the corresponding vector value and plotted as a percent change from vector. Overexpression of the wild-type PS1 construct resulted in an average increase of 37.5% above vector. Conversely, overexpression of the M139V and D385E mutant isoforms resulted in average decreases of 59.9 and 70.3%, respectively. Analysis of each cell line across treatment paradigms revealed no significant difference in the degree of change, indicating that inhibition of GSK 3β function had no effect on the regulation of β-catenin-mediated transcription by PS1. Data were analyzed by ANOVA with Fisher's post hoc analysis. *, p < 0.01.
that, in PS1-overexpressing cells, substitution occurs for both PS1 and PS2, resulting in decreased levels of PS2.

PS1 has also been hypothesized to regulate β-catenin catenolism, and it is possible that changes in β-catenin turnover could impact on β-catenin signaling. Reports by Takashima and colleagues (14, 30) indicate that PS1 binds both β-catenin and GSK 3β proximally to each other, placing them in a common milieu. A recent report by Kang et al. (15) indicates that mutations in PS1 increase the half-life of β-catenin, although steady-state levels of catenin do not change. Studies of the association of PS1 with GSK 3β, by Gantier and colleagues (31), support the hypothesis that mutation in PS1 alters this interaction. They have identified a mutation in PS1 that decreases the binding affinity of PS1 for GSK 3β. In contrast, Zhang et al. (20) observed that overexpression of wild-type PS1, but not FAD mutant PS1, stabilizes β-catenin. The sensitivity of GSK 3β binding to the particular mutation in PS1 might help explain the disparity of the results seen in the Zhang and Kang papers. A report by Kirschenbaum et al. (32) indicates that mutations in a GSK 3β phosphorylation site in PS1 can block the binding of β-catenin to PS1. Additionally, they show that this mutation in PS1 does not affect the rate of turnover of β-catenin. These findings suggest that the effect of PS1 on regulation of β-catenin may be linked to something distinct from regulation of its turnover.

Our data on the regulation of β-catenin signaling suggests that the mechanism of regulation of β-catenin by PS1 might be downstream of GSK 3β. Treatment of the mutant PS1 cell lines with lithium chloride or transfection with a GSK 3β-insensitive mutant isoform of β-catenin showed no alterations in the relative transcription. If the effect of the mutants is to alter β-catenin levels through a differential affinity for GSK 3β, then inhibition of GSK 3β activity would be expected to rescue the mutant phenotype. Our data show that this is not the case. All PS1-overexpressing cell lines showed a consistent percent change from vector for each treatment paradigm. This suggests that PS1 was exerting its influence downstream of the β-catenin/GSK 3β regulatory point.

The downstream event following accumulation of cytosolic β-catenin involves binding of β-catenin to transcription factors from the Tcf/LEF family and activation of transcription. It has been shown that β-catenin translocation to the nucleus is impaired by mutations in PS1 (19). There is also evidence in the literature for nuclear translocation of Armadillo, a Drosophila catenin analog, in the absence of transcription, suggesting β-catenin can translocate to the nucleus without binding to transcription factors, despite the absence of a nuclear localization signal sequence (27). We now report that PS1 is capable of binding to at least one of the transcription factors that binds to β-catenin, namely hTcf-4. We have additionally found that PS1 mutations inhibit the nuclear localization of hTcf-4 following treatment with lithium. These data strongly suggest that PS1 is involved in the regulation of the association of β-catenin with at least one of the family of Tcf/LEF transcription factors.

Several groups have indicated that PS1 expression is found within the nucleus. Recently, Honda et al. (28) reported that full-length PS1 has been associated with the nuclear membrane. Following the endogenous endoproteolysis, wild-type PS1 migrates to the membrane of the endoplasmic reticulum, consistent with several other published reports on PS1 localization. They also show that the mutants impair this nuclear localization, causing the holo-PS1 to remain in the membrane of the endoplasmic reticulum. We have shown that hTcf-4 binds to holo-PS1, but there is no evidence of binding of the PS1 C-terminal fragment. We also see an inhibition of the transcriptional activity of a β-catenin/hTcf-4 reporter in the pres-

Inhibiting β-catenin transcriptional activity. Our data indicate that these mutations in PS1 impact on multiple cellular processes rather than selectively inhibiting APP processing.

The fact that loss of PS1 also inhibits β-catenin function suggests that the deficits in β-catenin signaling seen with the mutant PS1 constructs are caused by a loss of function due to the mutations. Our data show that the mutations inhibit both nuclear translocation and transcription. Based on this, the simplest explanation is that normal PS1 function is needed to facilitate nuclear translocation of β-catenin in a complex with hTcf-4. The residual activity in all cell lines may be partially linked to the presence of PS2, which has also been described to bind to β-catenin (29). Differences between the knockout cells and the cells overexpressing the mutants may also be linked to the expression levels of PS2. We have seen that the knockout cells increase PS2 expression, in an apparent attempt to compensate for the absence of PS1. Conversely, there is evidence
ence of the mutant PS1 alleles. Our data may hint at a possible role for holo-PS1 within the nuclear milieu. Several groups, including ours, have proposed a shuttling or trafficking function for PS1 (13, 33). Georgakopoulos et al. (7) also describe PS1 incorporation into cadherin/catenin complexes at the cytoplasmic membrane in confluent cells. This incorporation is also present in synaptic adhesions in brain. This suggests that PS1 is an important regulator of β-catenin function within the cadherin complex. Our finding may represent an additional aspect of PS1-mediated regulation of β-catenin function, by incorporating full-length PS1 into a mechanism for regulation of the nuclear migration of β-catenin/hTcf-4 complexes.

REFERENCES

1. Hardy, J. (1997) Trends Neurosci. 4, R154–R159
2. Doan, A., Thinakaran, G., Borghelt, D. R., Slunt, H. H., Ratovitski, T., Podlisny, M., Selkoe, D. J., Seeger, M., Gandy, S. E., Price, D. L., and Sisodia, S. S. (1996) Neuron 17, 1023–1030
3. Beher, D., Elle, C., Underwood, J., Davis, J. B., Ward, R., Karran, E., Masters, C. L., Beyreuther, K., and Multhaup, G. (1999) J. Neurochem. 72, 1564–1573
4. Zhang, J., Kang, D. E., Xia, W., Okochi, M., Mori, H., Selkoe, D. J., and Koo, E. H. (1998) J. Biol. Chem. 273, 12436–12442
5. Kovacs, D. M., Fassett, H. J., Page, K. J., Kim, T. W., Moor, R. D., Merriam, D. E., Hollister, R. D., Hallmark, O. G., Mancini, R., Felsenstein, K. M., Hyman, B. T., Tanzi, R. E., and Wusko, W. (1996) Nat. Med. 2, 224–229
6. Busiglio, J., Hartmann, H., Lorenzo, A., Wong, C., Baumann, K., Sommer, B., Street, R., and St. George-Hyslop, P. (1999) Nat. Med. 5, 164–169
7. Georgakopoulos, A., Marambaud, P., Efthimiopoulos, S., Shioi, J., Cui, W., Li, H. C., Schutte, M., Gordon, R., Holstein, G. R., Martinelli, G., Mehta, P., Friedrich, V. L., Jr., and Robakis, N. K. (1999) Mol. Cell. 4, 893–902
8. Thinakaran, G., Borghelt, D. R., Lee, M. K., Klit, H. H., Spitzer, L., Kim, G., Ratovitski, T., Davenport, F., Nordstedt, C., Seeger, M., Hardy, J., Leve, A. I., Gandy, S. E., Jenkins, N. A., Copeland, N. G., Price, D. L., and Sisodia, S. S. (1996) Neuron 17, 181–190
9. Borghelt, D. R., Thinakaran, G., Eckman, C. B., Lee, M. K., Davenport, F., Ratovitski, T., Prada, C. M., Kim, G., Seekins, S., Yager, D., Slunt, H. H., Wang, R., Seeger, M., Levey, A. I., Gandy, S. E., Copeland, N. G., Jenkins, N. A., Price, D. L., Younkin, S. G., and Sisodia, S. S. (1996) Neuron 17, 1095–1113
10. Levitan, D., and Greenwald, I. (1998) Development 125, 3599–3606
11. De Strooper, B., Annanet, W., Cupers, P., Saftig, P., Craessaerts, K., Mummi, J. S., Schroeter, E. H., Schrijvers, W., Wolle, M. S., Ray, W. J., Goate, A., and Kopan, R. (1999) Nature 398, 518–522
12. De Strooper, B., Saftig, P., Craessaerts, K., Vanderstichele, H., Guhde, G., Annaert, W., Von Figura, K., and Van Leuven, F. (1998) Nature 391, 387–390
13. Palacino, J. J., Berechid, B. E., Alexander, P., Eckman, C., Younkin, S., Nye, J. S., and Wolszyn, B. (2000) J. Biol. Chem. 275, 215–222
14. Murayama, T., Tanaka, S., Palacino, J., Murayama, O., Honda, T., Sun, X., Yasutake, K., Nishonmatsu, N., Wolszyn, B., and Takashima, A. (1998) FEBS Lett. 433, 73–77
15. Kang, D. E., Soriano, S., Proesch, M. P., Collins, T., Naruse, S., Sisodia, S. S., Leibowitz, G., Levine, F., and Koo, E. H. (1999) J. Neurosci. 19, 4229–4237
16. Dale, T. C. (1998) Biochim. J. 329, 209–223
17. Stahl, B., Diehmnn, A., and Sudhoff, T. C. (1999) J. Biol. Chem. 274, 9141–9148
18. Zhou, J., Liyanage, U., Medina, M., Ho, C., Simmons, A. D., Lovett, M., and Kosik, K. S. (1997) Neuron 8, 2085–2090
19. Nishimura, M., Yu, G., Levesque, G., Zhang, D. M., Ruel, L., Chen, F., Milam, P., Holmes, E., Liang, Y., Kawarai, T., J., E., Supala, A., Rogeva, E., Xu, D. M., Janus, C., Levesque, L., Bi, Q., Duthie, M., Rozmahel, R., Mattila, K., Lannfelt, L., Westaway, D., Grant, S. T., and Le, J. (2000) Nature 405, 750–754
20. Zhang, Z., Hartmann, H., Do, V. M., Abramowski, D., Sturchler-Pieratt, C., Staufenbhl, M., Sommer, B., van de Wetering, M., Clevers, H., Saftig, P., De Strooper, B., He, X., and Yankner, B. A. (1998) Nature 395, 698–702
21. Roose, J., and Clevers, H. (1999) Biochim. Biophys. Acta 1242, M32–M37
22. Laemmli, U. K. (1970) Nature 227, 680–685
23. Baeuerle, P. A., and Baltimore, D. (1988) Cell 53, 211–217
24. Korinek, V., Barker, N., Morin, P. J., van Wichen, D., de Weger, R., Kinzler, K. W., Vogelstein, B., and Clevers, H. (1997) Science 275, 1784–1787
25. Morin, P. J., Sparks, A. B., Korinek, V., Barker, N., Clevers, H., Vogelstein, B., and Kinzler, K. W. (1997) Science 275, 1787–1790
26. van Gassen, G., Annaert, W., and Van Broeckhoven, C. (2000) Neurobiol. Dis. 7, 135–151
27. van de Wetering, M., Cavallo, R., Douijes, D., van Beest, M., van Es, J., Loureiro, J., Yama, A., Hush, D., Jones, T., Bejsovec, A., Peifer, M., Martin, M., and Clevers, H. (1997) Cell 88, 789–799
28. Honda, T., Nishonmatsu, N., Yasutake, K., Ohtake, A., Sato, K., Tanaka, S., Murayama, O., Murayama, M., and Takashima, A. (2000) Neurosci. Res. 37, 101–111
29. Levesque, G., Yu, G., Nishimura, M., Zhang, D. M., Levesque, L., Yu, H., Xu, D., Liang, Y., Rogeva, E., Ikeda, M., Duthie, M., Furago, E., Wang, L., VanderVere, P., Bayne, M. E., Stadler, C. D., Rommens, J. M., Fraser, P. E., and St. George-Hyslop, P. (1999) J. Neurochem. 75, 999–1008
30. Takashima, A., Murayama, M., Yoh, K., Honda, T., Yasutake, K., Nishonmatsu, N., Mernik, M., Yamaguchi, H., Sugihara, S., and Wolszyn, B. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 9057–9061
31. Gantier, R., Gilbert, D., Dumanich, C., Campion, D., Davoust, D., Hama, T., and Frebourg, T. (2000) Neurosci. Lett. 283, 217–220
32. Kirschchenbaum, F., Hsa, G. C., Cordell, B., and McCarthy, J. V. (2001) J. Biol. Chem. 276, 7366–7375
33. Naruse, S., Thinakaran, G., Luo, J. J., Kusiak, W. J., Tomita, I., Iwatsubo, T., Qian, X., Ginty, D. D., Price, D. L., Borcherd, D. R., Wong, P. C., and Sisodia, S. S. (1998) Neuron 21, 1213–1221