We next examined the expression and distribution of mPGES-1 in brains of old-aged (16 months old) Tg2576 mice. mPGES-1-immunoreactive cells were observed in neuronal cells in limited areas of brains of Tg2576 and age-matched control mice. The expression level of mPGES-1 was higher in Tg-2576 mice than control mice (Fig. 1D). Immunostaining with anti-human Aβ antibody (82E1) revealed Aβ plaques in the hippocampus and cortex of the old-aged Tg2576 mice, although Aβ immunostaining was not observed in the age-matched control mice. The mPGES-1 expression was found around the Aβ plaques in Tg2576 mice, and staining was particularly evident in areas of dense Aβ plaques (Fig. 1E). Furthermore, double-immunostaining using antibodies specific to each cell marker showed that the mPGES-1-stained cells were found to be glial fibrillary acidic protein (GFAP)-positive reactive astrocytes (Fig. 1F). In our study, GFAP-immunoreactive cells were observed in both Tg2576 and controls, and astrocytes that were intensely immunoreactive for GFAP were found surrounding the Aβ plaques in Tg2576 mice. On the other hand, the mPGES-2- or cPGES- immunoreactive cells were observed in whole brain of Tg2576 and control mice (data not shown). Neither mPGES-2 nor cPGES was found to be associated with Aβ plaques.

These results indicated that among the three PGES isozumes, only mPGES-1 expression is induced and associated with the pathogenesis in both human AD patients and Tg2576 mice.

Induction of mPGES-1 Expression in Aβ–treated Neuronal Cells *in Vitro*. As Satoh et al. reported that Aβ induced mPGES-1 expression in rat astrocytes *in vitro* (19), we next used Aβ fragment 31-35 ($A\beta_{31\text{-}35}$) as Aβ peptide and examined whether mPGES-1 is induced in mouse

primary neuronal cells by Aβ. It has been reported that $A\beta_{31-35}$ was induce cytotoxic and pro-apoptotic effects in several neuronal cells (23, 24), although it does not exhibit aggregation phenomena (25). Primary cerebral neuronal cells were prepared from the cerebri of 16.5-day-old mouse embryos and then treated by Aβ. As shown in Fig. 2A, RT-PCR analysis revealed that mPGES-1 mRNA was barely detectable in the untreated cells but markedly increased by Aβ without alteration of COX-2 expression. Production of PGE_2 was also increased with increase in the mPGES-1 expression 48 h after Aβ treatment (Fig. 2B). On the other hand, neither induction of mPGES-1 nor increment in PGE_2 production was observed in Aβ-treated neuronal cells derived from mPGES-1 deficient mice. These results indicated that mPGES-1 contributes to the formation of PGE_2 in Aβ-treated neuronal cells.

Furthermore, to determine the cell type expressing mPGES-1, double-immunostaining of mPGES-1 and GFAP or microtubule-associated protein-2 (MAP-2) was carried out. The immunoreactivity of mPGES-1 was slightly but significantly detected near the nuclear membrane in untreated cells and its positive signals were enhanced after Aβ treatment (Fig. 2C). The mPGES-1-immunoreactive signals were observed in GFAP-positive astrocytes as well as a portion of MAP-2-positive neurons.

mPGES-1 Deficiency Attenuates Aβ-induced Neuronal Cell Death *in Vitro*. The effect of mPGES-1 deficiency on Aβ-induced neuronal cell death *in vitro*. As shown in Fig. 3A, the cell number of primary neuronal cells derived from wild-type mice was increased during the study period. After 48 h of Aβ treatment, the increase in cell number was significantly repressed. Double-immunostaining of GFAP and MAP-2 revealed that both of GFAP-positive astrocytes and

MAP-2-positive neurons proliferated during the period and both growth was suppressed by Aβ (Fig. 3B). On the other hand, proliferation of neuronal cells derived from mPGES-1 deficient mice was not suppressed by Aβ treatment. Conversely, Aβ modestly increased the growth of the mPGES-1 deficient cells (Fig. 3).

As it was shown that Aβ induces apoptosis in cultured neuronal cells (23-25), we next examined the effect of mPGES-1 deficiency on Aβ-induced apoptosis by TUNEL staining. As shown in Fig. 4A, the number of TUNEL-positive apoptotic cells were increased when wild-type neuronal cells were treated with Aβ for 72 h. On the other hand, apoptosis of mPGES-1 deficient neuronal cells was not induced by Aβ treatment. PGE_2 also could not induce apoptosis in mPGES-1 deficient cells as well as Aβ, but combination of Aβ and PGE_2 could induce apoptosis (Fig. 4B). These results indicated that Aβ-induced mPGES-1 dependent PGE_2 production is necessary but not sufficient for Aβ-induce neuronal cell apoptosis.

mPGES-1 Deficiency Mitigates the AD-like Pathology in Tg2576 Mice. To determine whether mPGES-1 contributes to Aβ-dependent behavioral deficits *in vivo*, we bred Tg2576 ($APPsw^{+/-}$) mice, which develop Aβ plaques starting at 9 to 10 months of age and impair spatial memory without neuron loss (20), with mPGES-1 deficient mice to generate $APPsw^{+/-}$/mPGES-$1^{+/-}$ (as Tg2576 mice), $APPsw^{+/-}$/mPGES-$1^{-/-}$ (as mPGES-1 deficient Tg2576 mice), $APPsw^{-/-}$/mPGES-$1^{+/-}$ (as control mice), and $APPsw^{-/-}$/mPGES-$1^{-/-}$ mice (as mPGES-1 deficient mice). Morris water-maze tests were performed using these mice at the age of 10 to 11 months. During the 5-day period of training trials, the latency in finding a target platform of all groups of mice was decreased (data not shown). Over the entire period, the Tg2576 mice did not

differ from the control mice in latency, distance of swimming and swimming speed. To measure spatial learning and memory retention, we next performed the probe trials on the day after the 5-day training was finished. As shown in Fig. 5A, in the probe trial, only APPsw$^{+/-}$/mPGES-1$^{+/-}$ mice significantly failed to favor the target platform location compared to control APPsw$^{-/-}$/mPGES-1$^{+/-}$ mice. On the other hand, APPsw$^{+/-}$/mPGES-1$^{-/-}$ mice tended to swim around the target location as well as control mice, indicating that mPGES-1 deficiency improved target crossing in Tg2576 mice.

Furthermore, we found that histopathological features of Tg2576 mice were also affected by mPGES-1 deficiency. As shown in Fig. 5B, around senile plaques of brain derived from APPsw$^{+/-}$/mPGES-1$^{+/-}$ mice at the age of 10 to 11 months, mPGES-1 protein was expressed and reactive atrocytes and swollen microglia were accumulated. On the other hand, in APPsw$^{+/-}$/mPGES-1$^{-/-}$ mice, only a small numbers of reactive astrocytes and no swollen microglia were observed around senile plaques, although there was no significant difference in Aβ plaque deposition between APPsw$^{+/-}$/mPGES-1$^{+/-}$ and APPsw$^{+/-}$/mPGES-1$^{-/-}$ mice. These results suggested that mPGES-1 deficiency might suppress inflammatory reactions occurred in brains of Tg2576 mice.

Discussion

In the present study, we found that mPGES-1 is induced in cerebral cortex from AD patients and Tg2576 mice *in vivo* (Fig. 1) and Aβ-treated mouse cerebri nerve cells *in vitro* (Fig. 2), and that mPGES-1 deficiency attenuates Aβ-induced apoptosis of neuronal cells *in vitro* (Figs. 3 and 4)

and mitigates AD-like pathology in Tg2576 mice (Fig. 5). It has been shown that mPGES-1 is up-regulated in many tissues by proinflammatory cytokines or growth factors (18). The increase in mPGES-1 expression is strongly correlated with the induction of COX-2 in many conditions, and mPGES-1 is shown to be preferentially coupled with COX-2 activity to increase the pathologic production of PGE_2 (16-18). However, these are not always the case. Sandee et al. reported that mPGES-1 was overexpressed and involved in PGE_2 production in both COX-1 and COX-2 deficient cells (27). We here also found that mPGES-1 mRNA was markedly increased by Aβ without alteration of COX-2 expression in mouse neuronal cells (Fig. 2A). Several studies have shown that COX-2 expression is increased in cerebral cortex of AD brain (9-13), but interestingly, decreased expression of COX-2 in end-stage AD has also been reported (28). In addition to this study, Chaudhry et al. recently reported that mPGES-1 levels were elevated in western blots of middle frontal gyrus tissue extracts from AD patients relative to age matched controls (29). mPGES-1 may play more critical roles in pathogenic PGE_2 production in AD brains than COX-2.

Our results indicated that mPGES-1-derived PGE_2 might be involved in Aβ-induced apoptosis of neuronal cells *in vitro* (Figs. 3 and 4). PGE_2 exerts its biological actions through its binding to four specific receptor subtypes known as EP1, EP2, EP3 and EP4 (30). Studies using EP2 deficient mice had shown that among these four EPs, EP2 that is present on microglia, plays an important role in Aβ-activated neurotoxicity (31, 32). Shie et al. used primary cultures of wild-type neurons and microglia from either wild-type or EP2 deficient mice, and found that Aβ-treated wild-type microglia enhanced Aβ-induced apoptosis of wild-type neurons but

Aβ-treated EP2 deficient microglia could not (31). Aβ-induced expression of inducible nitric oxide synthase (iNOS) that can cause neurotoxicity was also reduced in EP2 deficient microglia. Furthermore, Liang et al. showed that deletion of EP2 in APPswe-PS1ΔE9 mice, one of AD model mice, resulted in marked reduction in age-dependent lipid peroxidation in brain tissues (32). These two reports indicated that activation of EP2 by PGE_2 leads to an increase in the production of microglial superoxides and neurotoxins and then increased production of reactive oxygen species promotes lipid peroxidation and injury in neurons. We also found that deletion of mPGES-1 reduced the Aβ-induced generation of 8-isoprostane, a lipid peroxide, in a mixed culture of neurons, astrocytes and microglia (data not shown). mPGES-1-derived PGE_2 produced by astrocytes or neurons might act on microglial EP2 and promote oxidative damage in AD brain.

It has also been shown that PGE_2-EP2 signaling shows bidirectional effects enhancing Aβ-induced neuronal cell death and protecting neuronal cells against Aβ-induced cell death (33). Although PGE_2 and EP2 agonists have neurotoxic effects (34), lower concentrations of PGE_2 and EP2 or EP4 agonists were neuroprotective against Aβ toxicity (33). It was noteworthy that mPGES-1 deficient neuronal cells did not grow without Aβ during the study period (Fig. 3). These results suggested that small amount of mPGES-1-derived PGE_2 might be involved in neuronal cell maintenance and growth. We further found that Aβ did not suppress the growth of mPGES-1 deficient cells but conversely increased it. It was reported that Aβ induced arachidonic acid release by activation of phospholipase A_2 in primary neuronal cells (35). In mPGES-1 deficient cells, Aβ might enhance some arachidonate metabolites other than PGE_2, which stimulate neuronal cell growth. Further studies were needed to reveal the mechanism how Aβ

enhanced the growth of mPGES-1 deficient neuronal cells.

Although unlike AD patients, severe neuronal cell death is not observed, Tg2576 mice develop Aβ senile plaques starting at 9 to 10 months age and impair spatial memory (20). As shown in Fig. 5, we examined the effect of mPGES-1 deficiency on AD-like pathology in Tg2576 mice and found that deletion of mPGES-1 reduced accumulation of microglia and reactive astrocytes around senile plaques without affecting Aβ plaque deposition. Learning impairments in water maze probe trial were also attenuated in mPGES-1 deficient mice. It has been suggested that microglia might play different roles at different time points in the progression of AD pathogenesis (36). Early microglia accumulation, which begins before formation of visible Aβ deposits, might be beneficial. Microglia recruited at early stages of AD phagocytose and clear Aβ and, hence, protect the brain from the toxic effects of Aβ. As the disease progresses, however, and persistent production of proinflammatory cytokines, microglia lose their protective phenotype and their ability to keep up with Aβ deposition and become dysfunctional and unable to clear all of the Aβ (37). In addition to down regulating Aβ clearance, the products of activated microglia are neurotoxic and, hence, promote neuronal degeneration. Our results indicated that mPGES-1-derived PGE_2 positively regulates activation and migration of microglia after Aβ deposition in AD brain and then exacerbates AD pathology.

It was noteworthy that mPGES-1 deficiency did not affect Aβ plaque deposition. Studies using EP2 or EP4 deficient mice showed that both PGE_2 receptors are involved in cleavage of APP and Aβ deposition by (32, 38). mPGES-2- or cPGES-derived PGE_2 might regulate Aβ production from APP and promote AD progression in collaboration with

mPGES-1-derived PGE_2.

In conclusion, our results indicated that mPGES-1 is induced in AD brains and then plays a critical role in AD pathology. Although epidemiological and clinical studies have suggested that long-term use of NSAIDs moderate the onset or progression of AD, their intake is frequently associated with gastrointestinal side effects. Because COX expressed in gastrointestinal mucosa is mainly COX-1, in recent years efforts focused on the development of highly selective COX-2 inhibitors with an improved gastric tolerability profile. However, severe cardiovascular adverse reactions challenged the initial enthusiasm in this new class of anti-inflammatory drugs. Blockage of mPGES-1 could form the basis of a novel therapeutic strategy for AD, which may represent a safer approach than therapies involving NSAIDs.

Materials and Methods

Animal Experiments. Mouse studies were approved by the Institutional Animal Care and Use Committee of Fukuoka University and Showa University. Tg2576 mice ($APPsw^{+/-}$, C57BL/SJL), which contained the APP gene with the human Swedish mutations (K670N/M671L), were obtained from Taconic. For immunohitochemical analysis, brains were obtained from four Tg2576 mice and four age-matched control mice (C57BL/SJL) of 16 months of age. mPGES-$1^{-/-}$ mice were described previously (21). For *in vitro* analysis, we crossed mPGES-$1^{-/-}$ mice (21) with Balb/c mice. For *in vivo* analysis, we crossed Tg2576 ($APPsw^{+/-}$) mice with mPGES-$1^{-/-}$ mice (21) to generate $APPsw^{+/-}$/mPGES-$1^{+/-}$ and $APPsw^{-/-}$/mPGES-$1^{+/-}$ mice. Then, these mice were again

crossed with mPGES-1$^{-/-}$ mice to generate APPsw$^{+/-}$/mPGES-1$^{+/-}$ (as Tg2576 mice), APPsw$^{+/-}$/mPGES-1$^{-/-}$ (as mPGES-1 deficient Tg2576 mice), APPsw$^{-/-}$/mPGES-1$^{+/-}$ (as control mice), and APPsw$^{-/-}$/mPGES-1$^{-/-}$ mice (as mPGES-1 deficient mice).

Human Tissues. Brains from 9 AD cases (average age 85.8±9.5, range 68-98), 5 non-AD cases including individuals with Parkinson's disease and cerebrovascular disorders (average age 78.6±8.2, range 68-90) and 4 cases without neurological disorders (average age 89.3±4.6, range 83-94) defined as normal control were selected from the Choju Medical Institute Brain Bank of Fukushimura Hospital. We obtained informed consent from all subjects. The diagnosis of AD was confirmed in all cases by neuropathological evidence of loss of temporo-parietal neurons and the presence of SPs and NFTs. The control cases were without neurological signs or symptoms and no pathological changes were detected in their brains on post mortem examination.

Immunohistochemistry. For preparation of human brain tissues, about 7 mm thick transverse tissue blocks of the frontal, parietal, and hippocampal regions were fixed in 10% formalin solution and embedded in paraffin until processed for histological examination. For preparation of murine brain tissues, mice were anesthetized with ether, and then using peristaltic pump, perfused with saline followed by 4% paraformaldehyde in PBS. Their brains were removed from their skulls and immersed in 4% paraformaldehyde in PBS at 4°C overnight. The brains were then dehydrated, degreased with ethanol, and embedded in paraffin. Using a microtome, tissues were cut into 5 μm thick sections that were mounted onto glass slides and stored.

Brain tissue sections were deparaffinized in xylene, hydrated in an ascending ethanol and then autoclaved in 10 mM citrate buffer (pH 6.0) for 20 min at 120°C for antigen retrieval. After

cooling at room temperature, the sections were treated for 30 min with 0.3% H_2O_2 to eliminate endogenous peroxidase activity, and then treated with 5% skim milk in PBS for blocking for 30 min at room temperature. After washing PBS-Tween, the sections were incubated with each specific primary antibody (cPGES (diluted 1:500, Cayman, Ann Arbor, MI), mPGES-1 (1:500, Cayman) or mPGES-2 (1:500, Cayman)) overnight at 4℃. Following incubation with the primary antibodies, the sections were treated with the appropriate biotinylated secondary antibody (1:1000, Jackson Immuno Research, West Grove, PA) for 2 hr at room temperature, followed by incubation in avidin-biotinylated horseradish peroxidase complex (1:1000, Vector, Burlingame, CA) for 1 hr at room temperature. The sections were then washed three times with Tris-HCl (pH 7.6) for 5 min each time, and peroxidase labeling was visualized by incubation with a solution containing 0.01% 3,3'-diaminobenzidine (Dojindo, Kumamoto, Japan), 0.05 M imidazole (Wako, Osaka, Japan), 0.00015% H_2O_2 and 0.6% nickel ammonium sulfate (Sigma, St. Louis, MO) in 0.05 M Tris-HCl pH 7.6. At the same time, positive control sections were examined under a microscope to monitor the efficiency of the immunostaining. After dehydration, the sections were cover-slipped using HSR liquid (Sysmex, Kobe, Japan). The results of immunostaining were recorded as digital images, and analyzed by image analysis software (WinRoof, ver. 5.6.1, Mitani Co. Ltd., Osaka, Japan). Illuminated pixels were statistically analyzed by Dunnett's two-tailed test. A P value less than 0.05 was considered statistically significant.

For double immunostaining, sections were treated at the end of the first incubation cycle with 0.5% H_2O_2 for 30 min. The second cycle was carried out similarly to the first, expect that nickel ammonium sulfate was omitted from the DAB solution. A monoclonal antibody to

GFAP (1:1000, Chemicon, Billerica, MA) was used as a specific marker for astrocytes, and monoclonal antibodies to HLA-DR, DP, DQ (1:100, Dako, Glostrup, Denmark) were used as markers for microglia. Monoclonal antibodies 82E1 (diluted 1:500, IBL, Gunma, Japan) and AT8 (1:5000, Innogenetics, Gent, Belgium) were used to detect Aβ in SPs and phosphorylated tau in NFTs, respectively. For immunostaining of Aβ, the sections were pretreated with 100% formic acid (Wako, Osaka, Japan) for 30 sec before the blocking procedure.

For immunofluorescence staining, sections were blocked with skim milk. After they were incubated with mixed primary antibodies for 48 hr at 4°C, the following secondary antibodies were used: first, fluorescein isothiocyanate-conjugated donkey anti-rabbit IgG antibody (1: 1000, Jackson Immuno Research, West Grove, PA) for 2 hr at room temperature, and then biotinylated donkey anti-mouse IgG antibody (1: 1000, Jackson Immuno Research) for 2 hr at room temperature. The biotinylated secondary antibody treatment was followed by incubation with Texas-Red dye-conjugated streptavidin (1:1000, Jackson Immuno Research) for 1 hr at room temperature.

Primary culture of cerebral neuronal cells. Primary cerebral neuronal cells were prepared from the cerebri of 16.5-day-old embryos of wild-type and mPGES-$1^{-/-}$ mice using Nerve-Cell Culture System (Sumitomo Bakelite, Tokyo, Japan). In brief, cerebral tissues were cleaned of meninges, minced, and treated with protease mixure. After mechanical dissociation by pipetting and treatment with dissociation solution, we resuspended cells in nerve cell culture medium, and then plated onto poly-L-lysine-coated plates or glass dishes (Φ 35 mm) at a density of 2.4 x 10^6 cells/well. After 7 days of the culture, we used neuronal cells to examine the effects of $A\beta_{31\text{-}35}$

(Peptide Institute, Inc., Osaka, Japan) on the expression of mPGES-1 and the cell death. The viable cells were peeled off by pipetting and counted using the trypan blue exclusion method. Neuronal cell identity was confirmed by immunostaining with the neuron marker anti-MAP-2 (Sigma) and astrocyte marker anti-GFAP antibodies (Chemicon).

RT-PCR analysis. Total RNA was extracted from mouse primary cerebral neuronal cells using Trizol Reagent (Invitrogen). Synthesis of cDNA was performed with 2 μg of the total RNA isolated from mouse primary cerebral neuronal cells, oligo dT primers, and SuperScript III Reverse Transcriptase (Invitrogen). Subsequent amplifications of the partial cDNA fragments were performed by Ex-Taq Polymerase (TaKaRa) using the reverse-transcribed products as a template with a set of specific oligonucleotide primers as follows: mPGES-1 sense 5'-AATGGGAGACGGAGCCATGA-3' and antisense 5'-ACCAGTCACGTGTCTCTCCT-3'; COX-2 sense 5'-GGTCTGGTGCCTGGTCTGATGATG-3' and antisense 5'-GTCCTTCAAGGAGAATGGTGC-3'; GAPDH sense 5'-TCGTGGATCTGACGTGCCGCCTG-3' and antisense 5'-CACCACCCTGTTGCTGTAGCCGTAT. The PCR mixtures were subjected to 35 (for mPGES-1 and COX-2) or 25 (for GAPDH) cycles of amplification by denaturation (45 s at 94°C), annealing (60 s at 60°C) and elongation (60 s at 72°C). The PCR products were analyzed by 1% agarose gel electrophoresis with ethidium bromide.

TUNEL staining experiments. The TUNEL staining experiments were performed as previously described (39). Briefly, after the treatment of $A\beta_{31-35}$ and/or PGE_2, the cells were fixed for 25 min in 4% paraformaldehyde in PBS, permeabilized for 5 min in 0.2% Triton X-100, and

then stained by using the DeadEnd Fluorometric TUNEL system (Promega) according to the manufacturer's instructions. For the positive control experiments, the cells were treated with DNase I (TaKaRa, Japan) for 10 min before the staining. Three randomly chosen microscopic fields were captured, and the numbers of TUNEL-positive neuronal nuclei were calculated.

Water Maze. Morris water-maze tests were performed to evaluate learning ability at the age of 10 to 11 months. The apparatus used in the tests consisted of a blue plastic circular pool 100 cm diameter enclosed by a 25-cm high wall. The pool was filled with water kept at a temperature of 19°C. A transparent plastic platform (8 cm in diameter) was placed 0.5 cm below the water surface and 18 cm from the wall of the pool. Visual cues for navigation were provided by four posters on the room wall.

In training trials for the Morris water-maze test, a mouse was placed at an area opposite the platform set in the pool and allowed to swim. The behavior was recorded by video. The time required to reach the platform (the latency period) and the distance of swimming were measured by using the analysis software. Such training trials were repeated for 5 days (3 trials/day). The starting point was randomly selected from three points.

A probe trial of the Morris water-maze test was performed on the day after the 5-day training was finished. The probe test was performed without the platform. The mouse was placed in the pool and swam for 60 s looking for a platform. The proportion of time spent in the different quadrants was recorded.

ACKNOWLEDGMENTS. We are grateful to all patients and their bereaved family members

who kindly gave their informed consent for enrollment in this study. This work was supported in part by Grants-in-Aid for Comprehensive Research on Aging and Health, and Research on Dementia from the Ministry of Health, Labour and Welfare of Japan, Grants-in-Aid for Scientific Research and "High-Tech Research Center" Project for Private Universities (matching fund subsidy (2004-2007)) from the Ministry of Education, Science, Culture, Sports and Technology of Japan, and a Showa University Special Grant-in-Aid for Innovative Collaborative Research Projects. D. Kamei was supported by Research Fellowships of the Japan Society for the Promotion of Science for Young Scientists.

References

1. Selkoe DJ (2001) Alzheimer's disease: genes, proteins, and therapy. *Physiol Rev* 81:741-766.
2. McGeer PL, McGeer EG (2007) NSAIDs and Alzheimer disease: epidemiological, animal model and clinical studies. *Neurobiol Aging* 28:639-647.
3. Townsend KP, Praticò D (2005) Novel therapeutic opportunities for Alzheimer's disease: focus on nonsteroidal anti-inflammatory drugs. *FASEB J* 19:1592-1601.
4. Akiyama H, et al. (2000) Inflammation and Alzheimer's disease. *Neurobiol Aging* 21:383-421.
5. Praticò D, Trojanowski JQ (2000) Inflammatory hypotheses: novel mechanisms of Alzheimer's neurodegeneration and new therapeutic target? *Neurobiol Aging* 21:441-445.
6. Lim GP, et al. (2000) Ibuprofen suppresses plaque pathology and inflammation in a mouse model for Alzheimer's disease. *J Neurosci* 20:5709-5714.

7. Yan Q, et al. (2003) Anti-inflammatory drug therapy alters β-amyloid processing and deposition in an animal model of Alzheimer's disease. *J Neurosci* 23:7504-7509.

8. Smith WL, Meade EA, DeWitt DL (1994) Interactions of PGH synthase isozymes-1 and -2 with NSAIDs. *Ann NY Acad Sci* 744:50-57.

9. Pasinetti GM, Aisen PS (1998) Cyclooxygenase-2 expression is increased in frontal cortex of Alzheimer's disease. *Neuroscience* 87:319-324.

10. Ho L, et al. (1999) Regional distribution of cyclooxygenase-2 in the hippocampal formation in Alzheimer's disease. *J Neurosci Res* 57:295-303.

11. Oka A, Takashima S (1997) Induction of cyclo-oxygenase 2 in brains of patients with Down's syndrome and dementia of Alzheimer type: specific localization in affected neurons and axons. *Neuroreport* 8:1161-1164.

12. Yasojima K, Schwab C, McGeer EG, McGeer PL (1999) Distribution of cyclooxygenase-1 and cyclooxygenase-2 mRNAs and proteins in human brain and peripheral organs. *Brain Res* 830:226-236.

13. Fujimi K, et al. (2007) Altered expression of COX-2 in subdivisions of the hippocampus during aging and in Alzheimer's disease: the Hisayama study. *Dement Geriatr Cogn Disord* 23:423-431.

14. Montine TJ, et al. (1999) Elevated CSF prostaglandin E_2 levels in patients with probable AD. *Neurology* 53:1495-1498.

15. Murakami M, Kudo I (2004) Recent advances in molecular biology and physiology of the prostaglandin E_2-biosynthetic pathway. *Prog Lipid Res* 43:3-35.

16. Murakami M, et al. (2000) Regulation of prostaglandin E_2 biosynthesis by inducible membrane-associated prostaglandin E_2 synthase that acts in concert with cyclooxygenase-2. *J Biol Chem* 275:32783-32792.

17. Stichtenoth DO, et al. (2001) Microsomal prostaglandin E synthase is regulated by proinflammatory cytokines and glucocorticoids in primary rheumatoid synovial cells. *J Immunol* 167:469-474.

18. Samuelsson B, Morgenstern R, Jakobsson PJ (2007) Membrane prostaglandin E synthase-1: a novel therapeutic target. *Pharmacol Rev* 59:207-224.

19. Satoh K, et al. (2000) Expression of prostaglandin E synthase mRNA is induced in beta-amyloid treated astrocytes. *Neurosci Lett* 283:221-223.

20. Hsiao K, et al. (1996) Correlative memory deficits, Aβ elevation, and amyloid plaques in transgenic mice. *Science* 274:99-102.

21. Kamei D, et al. (2004) Reduced pain hypersensitivity and inflammation in mice lacking microsomal prostaglandin E synthase-1. *J Biol Chem* 279:33684-33695.

22. Kamei D, et al. Microsomal prostaglandin E synthase-1 in both cancer cells and hosts contributes to tumor growth, invasion and metastasis. *Biochem J* in press?.

23. Misiti F, et al. (2005) Aβ(31-35) peptide induce apoptosis in PC 12 cells: contrast with Aβ(25-35) peptide and examination of underlying mechanisms. *Neurochem Int* 46:575-583.

24. Misiti F, et al. A crucial role for the redox state of methionine-35 residue. *Neurochem Int* 49:525-532.

25. Yan XZ, Qiano JT, Dou Y, Qiao ZD (1999) β-amyloid peptide fragment 31-35 induces

apoptosis in cultured cortical neurons. *Neuroscience* 92:177-184.

26. Behl C, Davis JB, Lesley R, Schubert D (1994) Hydrogen peroxide mediates amyloid β protein toxicity. *Cell* 77:817-827.

27. Sandee D, Sivanuntakorn S, Vichai V, Kramyu J, Kirtikara K (2009) Up-regulation of microsomal prostaglandin E synthase-1 in COX-1 and COX-2 knock-out mouse fibroblast cell lines. *Prostaglandins Other Lipid Mediat* 88:111-116.

28. Yermakova AV, O'Banion MK (2001) Downregulation of neuronal cyclooxygenase-2 expression in end stage Alzheimer's disease. *Neurobiol Aging* 22:823-386.

29. Chaudhry UA, Zhuang H, Crain BJ, Doré S (2008) Elevated microsomal prostaglandin-E synthase-1 in Alzheimer's disease. *Alzheimers Dement* 4:6-13.

30. Sugimoto Y, Narumiya S (2007) Prostaglandin E receptors. *J Biol Chem* 282:11613-11617.

31. Shie FS, Breyer RM, Montine TJ (2005) Microglia lacking E prostanoid receptor subtype 2 have enhanced Aβ phagocytosis yet lack Aβ-activated neurotoxicity. *Am J Pathol* 166:1163-1172.

32. Liang X, et al. (2005) Deletion of the prostaglandin E_2 EP2 receptor reduces oxidative damage and amyloid burden in a model of Alzheimer's disease. *J Neurosci* 25:10180-10187.

33. Echeverria V, Clerman A, Doré S (2005) Stimulation of PGE_2 receptors EP2 and EP4 protects cultured neurons againsat oxidative stress and cell death following β-amyloid exposure. *Eur J Neurosci* 22:2199-2206.

34. Takadera T, Shiraishi Y, Ohyashiki T (2004) Prostaglandin E_2 induced caspase-dependent apoptosis possibly through activation of EP2 receptors in cultured hippocampal neurons.

Neurochem Lett 45:713-719.

35. Sanchez-Mejia RO, et al. (2008) Phospholipase A_2 reduction ameliorates cognitive deficits in a mouse model of Alzheimer's disease. *Nat Neurosci* 11:1311–1318.

36. El Khoury J, Luster AD (2008) Mechanisms of microglia accumulation in Alzheimer's disease: therapeutic implications. *Trends Phamacol Sci* 29:626-632.

37. Hickman SE, Allison EK, El Khoury J (2008) Microglial dysfunction and defective beta-amyloid clearance pathways in aging Alzheimer's disease mice. *J Neurosci* 28:8354-8360.

38. Hoshino T, et al. (2007) Involvement of prostaglandin E_2 in production of amyloid-β peptides both *in vitro* and *in vivo*. *J Biol Chem* 282:32676-32688.

39. Zhang H, et al. (2008) Full length mutant huntington is required for altered Ca^{2+} signaling and apoptosis of striatal neurons in the YAC mouse model of Huntington's disease. *Neurobiol Dis* 31:80-88.

FIGURE LEGENDS

Fig. 1. High expression of mPGES-1 in brains of human AD (A – C) and Tg2576 mice (D – F). (A) Representative images of anti-mPGES-1-stained sections in CA2-3 region of the hippocampus from NC (Left) and AD (Right). (B) Values along the vertical axis of quantitative analysis graphs represent total pixel counts. *, $P < 0.05$ by Dunnett's two-tailed test (vs. NC). (C) Double immunostaninig with anti-mPGES-1 and AT-8 antibodies. AT-8 positive, dark purple; mPGES-1-positive, brown. (D) Representative images of anti-mPGES-1-stained sections in the

brain from control wild-type (Left) and Tg2576 mice (Right). (E) Double-immunostaining of mPGES-1 expression (brown) associated with Aβ plaques (dark purple). (F) Double-immunofluorescent staining with anti-mPGES-1 and anti-GFAP antibodies. Immunofluorescent staining of cells for mPGES-1 expressions (green), anti-GFAP antibody staining of reactive astrocytes (red) and the merged image.

Fig. 2. Induction of mPGES-1 expression and PGE_2 production in Aβ-treated mouse cerebri neuronal cells. (A) RT-PCR analysis for mPGES-1 expression in neuronal cells. RNA was prepared from neuronal cells cultured with or without Aβ for the indicated times, and then RT-PCR analysis for mPGES-1, COX-2 and GAPDH (as a control) were performed. (B) Immunocytostaining analysis for mPGES-1 expression in neuronal cells. mPGES-1 and GFAP or MAP-2 were double immunostained in neuronal cells cultured with or without Aβ for 72 h. (C) PGE_2 production from neuronal cells. Neuronal cells were prepared from wild-type (Left) and mPGES-1 KO mice (Right), and then cultured with or without Aβ for the indicated times. Amounts of PGE_2 in culture medium from the cultured neuronal cells were assayed with enzyme immunoassay kit. All values are means ± SD for at least 4 independent experiments.

Fig. 3. Effects of mPGES-1 deficiency on changes in cell numbers of Aβ-treated mouse cerebri neuronal cells. (A) Neuronal cells prepared from wild-type (Left) and mPGES-1 KO mice (Right) were cultured with or without Aβ for the indicated times, and the cell numbers were counted. All values are means ± SD for at least 3 independent experiments. (B) GFAP and MAP-2 were double immunostained in wild-type mice- (Left) or mPGES-1 KO mice-derived (Right) neuronal cells cultured with or without Aβ for 72 h.

Fig. 4. Effects of mPGES-1 deficiency on Aβ-induced apoptosis in mouse cerebri neuronal cells. (A) Neuronal cells prepared from wild-type (Left) and mPGES-1 KO mice (Right) were cultured with or without Aβ for 72 h, and the apoptotic cells were TUNEL staining. All values are means ± SD for at least 5 independent experiments. (B) mPGES-1 KO mice-derived neuronal cells were cultured with or without Aβ and PGE_2 for 72 h, and the apoptotic cells were TUNEL staining. All values are means ± SD for at least 3 independent experiments.

Fig. 5. Effects of mPGES-1 deficiency on learning impairments and histopathologic changes in Tg2576 mice. (A) Learning impairments in mPGES-1 deficient Tg2576 mice. Morris water-maze tests were performed using $APPsw^{+/-}$/$mPGES\text{-}1^{+/-}$ (as Tg2576 mice), $APPsw^{+/-}$/$mPGES\text{-}1^{-/-}$ (as mPGES-1 deficient Tg2576 mice), $APPsw^{-/-}$/$mPGES\text{-}1^{+/-}$ (as control mice), and $APPsw^{-/-}$/$mPGES\text{-}1^{-/-}$ mice (as mPGES-1 deficient mice) at the age of 10 to 11 months. The probe test was performed without the platform on the day after the 5-day training was finished. While the mouse swam for 60 s looking for a platform, the proportion of time spent in the different quadrants was recorded. All values are means + SD for at least 3 independent experiments. (B) Histopathologic changes in mPGES-1 deficient Tg2576 mice. Brain tissues were prepared from $APPsw^{+/-}$/$mPGES\text{-}1^{+/-}$ (as Tg2576 mice) and $APPsw^{+/-}$/$mPGES\text{-}1^{-/-}$ (as mPGES-1 deficient Tg2576 mice) at the age of 10 to 11 months. Representative images of anti-mPGES-1, anti-Aβ, anti-GFAP or anti-Iba1-stained sections were shown.