TDP-43 protein (22%) can cause FTLD-TDP. Similarly, overexpression of wild-type TDP-43 causes motor neuron degeneration in yeast, mice and rats (15–17). Moreover, it has recently been reported that the C-terminal portion of TDP-43 shows sequence similarity to prion protein (18) and that truncated CTFs of TDP-43 readily form intracellular aggregates in cultured cells (19–21), suggesting that not only FL-TDP but also its CTFs contribute to the pathogenesis of TDP-43 proteinopathy. However, the molecular mechanisms through which FL-TDP or aggregated CTFs cause neuronal dysfunctions leading to cell death remain unknown. To identify the mechanisms involved, we examined whether overexpression of FL-TDP or its CTF induced cell death in human neuroblastoma line SH-SY5Y cells. We report here that two different pathways lead to cellular dysfunctions induced by FL-TDP and by its CTF. We observed striking apoptotic cell death and cell cycle arrest at the G2/M phase in cells overexpressing FL-TDP. In cells overexpressing TDP-43 CTF, RNA polymerase II (RNA pol II) and several transcription factors such as specificity protein 1 (Sp1) and cAMP-response-element-binding protein (CREB) were co-localized with cytoplasmic aggregates of TDP-43 CTF and their transcriptional activities were decreased, although apoptotic cell death was not significant. These results suggest that recruitment of these factors may cause transcriptional dysregulation, thereby leading to cellular dysfunctions. Dysregulation of FL-TDP expression and/or cytoplasmic accumulation of TDP-43 CTF may contribute to the pathogenesis of ALS and FTLD-TDP.

RESULTS

Overexpression of FL-TDP or its CTF causes cellular dysfunctions in SH-SY5Y cells

Gitcho *et al.* (14) reported a 2-fold increase in total expression of *TARDBP* in a 3′-untranslated region variant carrier of FTLD-TDP, and mice or yeasts overexpressing human TDP-43 develop various abnormalities, including cytotoxicity, neuronal loss and motor deficits (15,17). These findings suggest that overexpression of TDP-43 can cause neuronal degeneration *in vivo*. However, no striking cell death was detected in cultured SH-SY5Y cells transiently expressing a plasmid encoding FL-TDP (20,22). Therefore, to examine whether more substantial and stable expression of TDP-43 is required for induction of its cytotoxic effect in cultured cells, we used a lentiviral system for expression of TDP-43. We prepared SH-SY5Y cells stably expressing FL-TDP (FL-TDP), a deletion mutant of the nuclear localization signal [ΔNLS-TDP; 78–84 residues (23)] and CTF of TDP-43 [C-TDP; 162–414 residues (20)], by using a lentiviral expression system (Fig. 1A). At 3 days after infection, transfected cells were subjected to immunohistochemical and biochemical analyses. Confocal microscopic analyses (Fig. 1B) showed that FL-TDP was expressed mainly in nuclei, but was not phosphorylated or aggregated. ΔNLS-TDP was expressed mainly in cytoplasm, without phosphorylation or aggregation. In cells expressing C-TDP, phosphorylated and aggregated C-TDP was detected with phospho-TDP-43 specific antibody, anti-pS409/410 (Fig. 1B). Immunoblot analyses of transfected cell lysates also revealed that FL-TDP and ΔNLS-TDP were recovered mainly in Triton X-100 (TX)-sup fraction without phosphorylation, while C-TDP was phosphorylated and aggregated in TX-ppt fraction, as shown in Figure 1C.

We examined the cytotoxic effects of these TDP-43 constructs in SH-SY5Y cells. At 4 days after lentiviral infection, the viability of cells transfected with FL-TDP was strongly suppressed and striking cell death was observed (Fig. 2A–C), suggesting that expression of FL-TDP is highly cytotoxic for SH-SY5Y cells. As shown in Figure 2D, poly(ADP-ribose) polymerase (PARP), a well-known substrate for activated caspase-3, was cleaved in cells expressing FL-TDP. This result clearly indicates that expression of FL-TDP induces apoptotic cell death. On the other hand, expression of ΔNLS-TDP or C-TDP did not influence the viability at 4–7 days (Fig. 2B). We also found that expression of the N-terminal fragment of TDP-43 (N-TDP: 1–161 residues) has no cytotoxic effect (Supplementary Material, Fig. S1). In cells expressing C-TDP, however, the number of living cells was significantly less than that in mock cells or cells expressing ΔNLS-TDP at 7 days (Fig. 2C). These results suggest that expression of not only FL-TDP but also CTF of TDP-43 causes cellular dysfunctions in SH-SY5Y cells, and may indicate that expression of FL-TDP without aggregate formation and expression of CTF with inclusions induce cellular damage through distinct mechanisms.

Different modes by which FL-TDP and its CTF induce cellular dysfunctions

To examine this possibility, we performed BrdU incorporation assay in cells expressing green fluorescent protein (GFP)-tagged FL-TDP (GFP-FL-TDP) or CTF (GFP-C-TDP) (Fig. 3A). Since stable expression of TDP-43 using the lentiviral system often resulted in severe damage to SH-SY5Y cells, we mainly used cells transiently expressing TDP-43 plasmids in subsequent work. SH-SY5Y cells were transfected with a plasmid encoding GFP-FL-TDP or GFP-C-TDP for 3 days, then treated with BrdU. After 10 h incubation, the cells were fixed and stained with anti-BrdU antibody and observed with a confocal microscope. As shown in Figure 3B and C, incorporation of BrdU in cells expressing GFP-FL-TDP was significantly decreased when compared with that in cells expressing the empty vector (pEGFP), indicating that DNA synthesis and cell growth were suppressed by overexpression of GFP-FL-TDP. We also found that cells with diffuse expression of GFP-C-TDP were stained with anti-BrdU antibody, while cells including GFP-C-TDP aggregates were not (Fig. 3B, right). Quantitative analysis showed that BrdU incorporation was almost wholly suppressed in cells with GFP-C-TDP inclusions (Fig. 3C). These results indicate that suppression of cell growth owing to formation of GFP-C-TDP inclusions is more marked than that owing to expression of GFP-FL-TDP or GFP-C-TDP without inclusions.

Next, we analyzed the cell cycle by measuring the DNA content of propidium iodide (PI)-stained cells. SH-SY5Y cells were transfected with GFP-FL-TDP or GFP-C-TDP. Three days after transfection, cells were stained with PI and analyzed using a flow cytometer. As shown in Figure 4A and B, cells transfected with GFP-FL-TDP were extensively accumulated in the G2/M and subG1 phases when compared with those transfected with empty vector (pEGFP) or GFP-C-TDP. It is well known that apoptotic cells are accumulated in the subG1 phase, and an increase of cells in the G2/M phase reflects growth arrest, leading to apoptosis. This result shows that overexpression of GFP-FL-TDP induces apoptosis, which is consistent with the

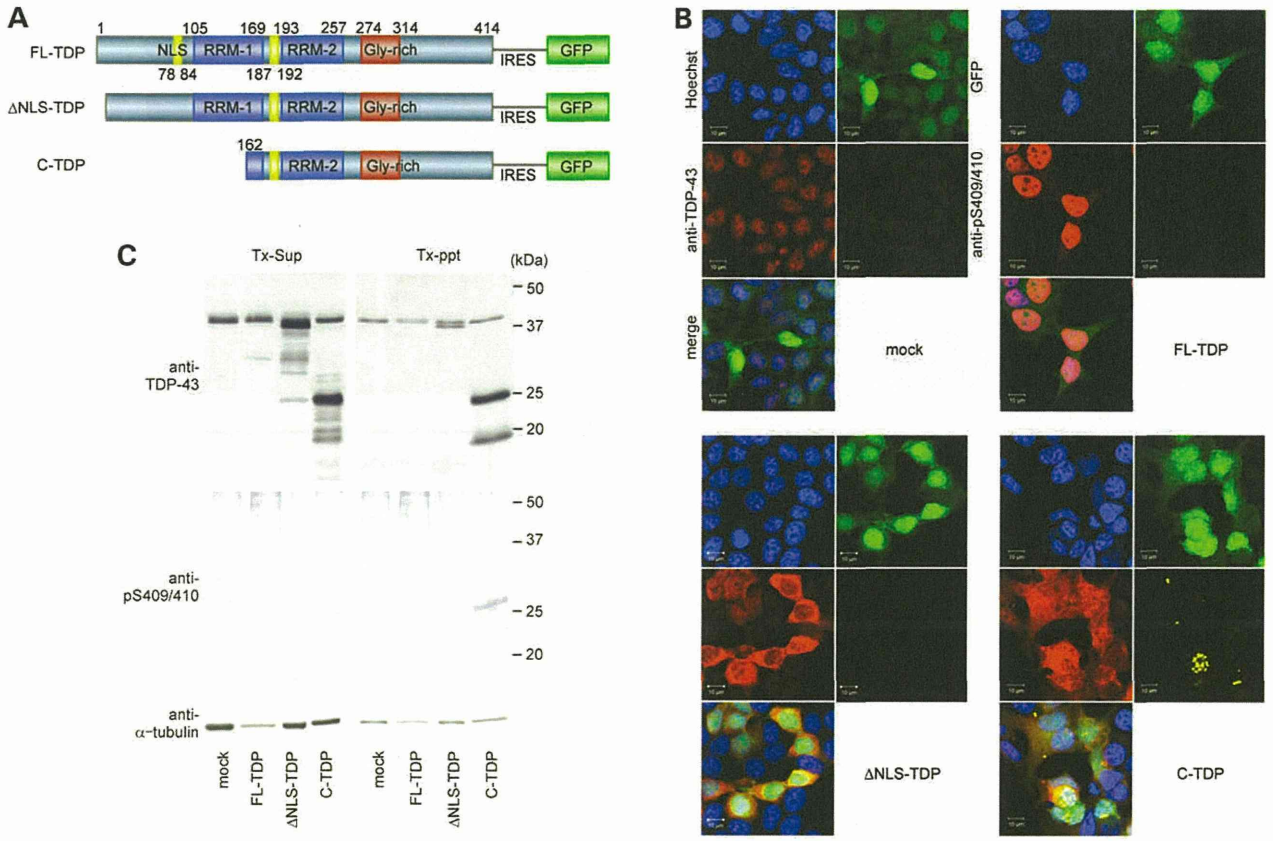

Figure 1. Overexpression of TDP-43 constructs in SH-SY5Y cells. (**A**) Schematic representation of FL-TDP, TDP-43 with deletion of the nuclear localization signal [78–84 residues (23)] (ΔNLS-TDP) and CTF (C-TDP) constructs in lentiviral expression system. RNA recognition motifs (RRM-1 and RRM-2: blue) and glycine-rich domain (Gly-rich: red) are shown. (**B**) SH-SY5Y cells were infected with each TDP-43 virus at 2×10^7 copies/well. At 4 days after infection, the cells were fixed and stained with anti-TDP-43 and anti-phospho-TDP-43 (anti-pS409/410) antibodies. Scale bars, 10 μm. (**C**) Transfected cells were also prepared for western blotting (20, 22). Cell lysates were extracted with 1% Triton X-100 (Tx), and the supernatants (Tx-sup) and insoluble pellets (Tx-ppt) after centrifugation at 100 000*g* for 20 min were analyzed by immunoblotting. The blots were probed with anti-TDP-43, anti-pS409/410 or anti-tubulin antibody.

result obtained in the case of lentiviral expression of FL-TDP (Fig. 2D). On the other hand, the cell cycle distribution of cells expressing GFP-C-TDP was not changed when compared with that of cells transfected with pEGFP empty vector, indicating that apoptosis is not induced in cells with formation of GFP-C-TDP aggregates. Taking these results together, it appears that overexpression of GFP-FL-TDP caused mild suppression of DNA synthesis and striking induction of apoptosis, whereas significant suppression of DNA synthesis and no abnormalities in the cell cycle were seen in cells including GFP-C-TDP aggregates. These results clearly indicate that cell death is induced via distinct molecular pathways upon overexpression of different TDP-43 species.

RNA pol II and some transcription factors are co-localized with TDP-43 inclusions and their activities are suppressed

It has been reported that transcriptional dysregulation is one of the central pathogenic mechanisms in Alzheimer's disease, Parkinson's disease and Huntington's disease (24). A number of transcriptional regulators, such as Sp1 and CREB, interact with protein aggregates, resulting in functional disruption of these transcriptional factors in brain, followed by neurodegeneration (25–27). Therefore, to check whether these transcriptional factors are also associated with aggregates of TDP-43 CTF, we examined the localization of these factors as well as RNA pol II, which is a major enzyme responsible for transcription of protein-encoding genes. SH-SY5Y cells were transfected with GFP-C-TDP and incubated for 3 days, followed by immunohistochemical analyses. As shown in Figure 5A, inclusions composed of GFP-C-TDP were found to be positive for anti-RNA pol II antibody in cells expressing GFP-C-TDP. Furthermore, endogenous Sp1 and CREB were also co-localized with these inclusions. This result clearly shows that not only endogenous RNA pol II but also Sp1 and CREB are recruited to aggregated GFP-C-TDP. Next, we tried to confirm that endogenous RNA pol II interacts with TDP-43 biochemically. Cells expressing GFP, GFP-FL-TDP, GFP-N-TDP, GFP-C-TDP or GFP-ΔNLS-TDP were lyzed in RIPA buffer and the lysates were subjected to immunoprecipitation with anti-GFP antibody-tagged Dynabeads, followed by immunoblotting using several antibodies. We found that endogenous RNA pol II bound with phosphorylated form of GFP-C-TDP to a greater extent than did GFP-FL-TDP, GFP-N-TDP and

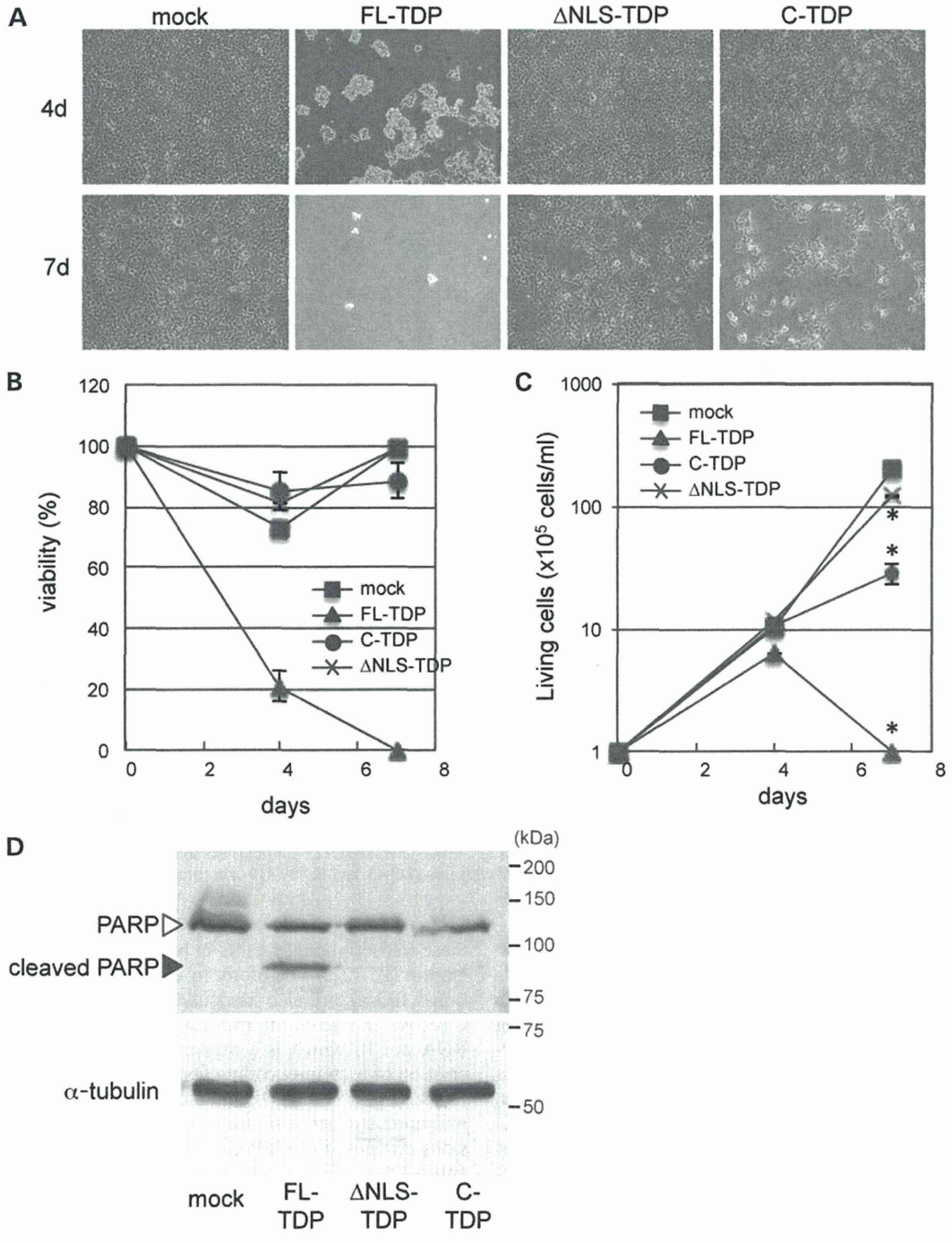

Figure 2. Cytotoxic effects of overexpressed TDP-43 in SH-SY5Y cells. SH-SY5Y cells were infected with each TDP-43 virus at 2×10^7 copies/well. At 4–7 days after infection, transfected cells were subjected to microscopic analyses, followed by cell death assay. They were assessed with light microscopy (×10 objective) at 4 and 7 days after transfection (**A**), and viability was examined by the trypan blue dye exclusion method (**B**) and living cells were counted using an automated cell counter TC10 (Bio-Rad) (**C**). The experiments were repeated three times; the illustrated results are typical. Data are means ± SEM. $^*P < 0.01$ versus 'mock' by Student's *t*-test. (**D**) Transfected cells were also subjected to immunoblot analyses using anti-PARP and anti-tubulin antibodies. Note that PARP was cleaved in cells expressing FL-TDP, indicating that apoptosis is induced in these cells.

GFP-ΔNLS-TDP (Fig. 5B). We also examined the transcriptional activities of Sp1 and CREB in cells containing inclusions of GFP-C-TDP by means of luciferase assay. SH-SY5Y cells were transfected with mCherry (mC)-tagged full-length (mC-FL), ΔNLS (mC-ΔNLS) or CTF of TDP-43 (162–414 residues, mC-C), followed by co-transfection of pFR-Luc together

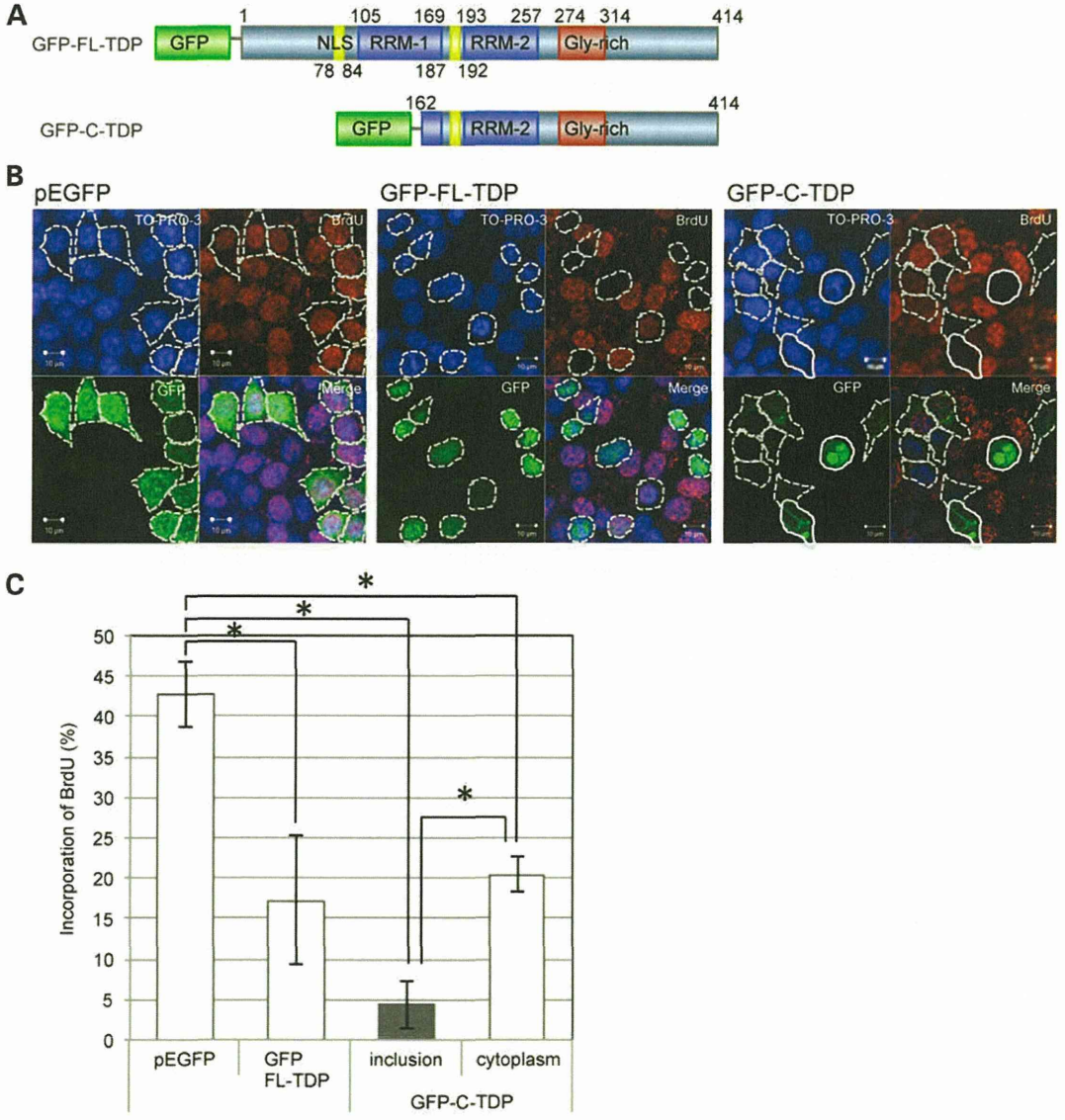

Figure 3. Comparison of cytotoxic effects of FL-TDP and its CTF. (**A**) Schematic representation of GFP-FL-TDP and GFP-C-TDP constructs in transient expression systems. (**B** and **C**) SH-SY5Y cells were transfected with the empty (pEGFP), GFP-FL-TDP or GFP-C-TDP vector for 3 days. After incubation, DNA synthesis was measured by BrdU uptake assay, using a confocal laser microscope (B). Scale bars, 10 μm. The positions of cells are indicated with broken white lines. Cells with GFP-C-TDP inclusions are indicated with white lines. The ratio (%) of the numbers of BrdU-positive cells to the numbers of GFP-positive cells was calculated as the incorporation ratio of BrdU (C). At least eight areas per sample were analyzed ($n = 8–16$), and the experiments were repeated three times; the illustrated results are typical. Data are means ± SEM. $^*P < 0.01$ by Student's *t*-test.

with pGAL4-Sp1 or pGAL4-CREB. At 48 h after transfection, cells were harvested and the luciferase activity was measured. Figure 5C shows that the transcriptional activities of Sp1 and CREB were significantly suppressed not only in cells transfected with mC-C but also in cells expressing mC-FL. We also observed co-localization of GFP-FL-TDP with Sp1, as well as co-localization of GFP-FL-TDP with CREB (Supplementary Material, Fig. S2). However, transcriptional dysregulation caused by the expression of GFP-FL-TDP appears to be due to its high toxicity (Fig. 2B), rather than the co-localization. Alternatively, it is possible that cellular damage resulting from overexpression of GFP-FL-TDP may cause transcriptional suppression of Sp1 and CREB.

RNA pol II co-localizes with TDP-43 inclusions in FTLD-TDP brain

To study the association of RNA pol II and transcriptional factors with TDP-43 aggregates in diseased brains, we carried out immunostaining of sporadic FTLD-TDP brain with several antibodies. As shown in Figure 6A and B, dystrophic neurites (DNs) were immunopositive for both anti-RNA pol II and

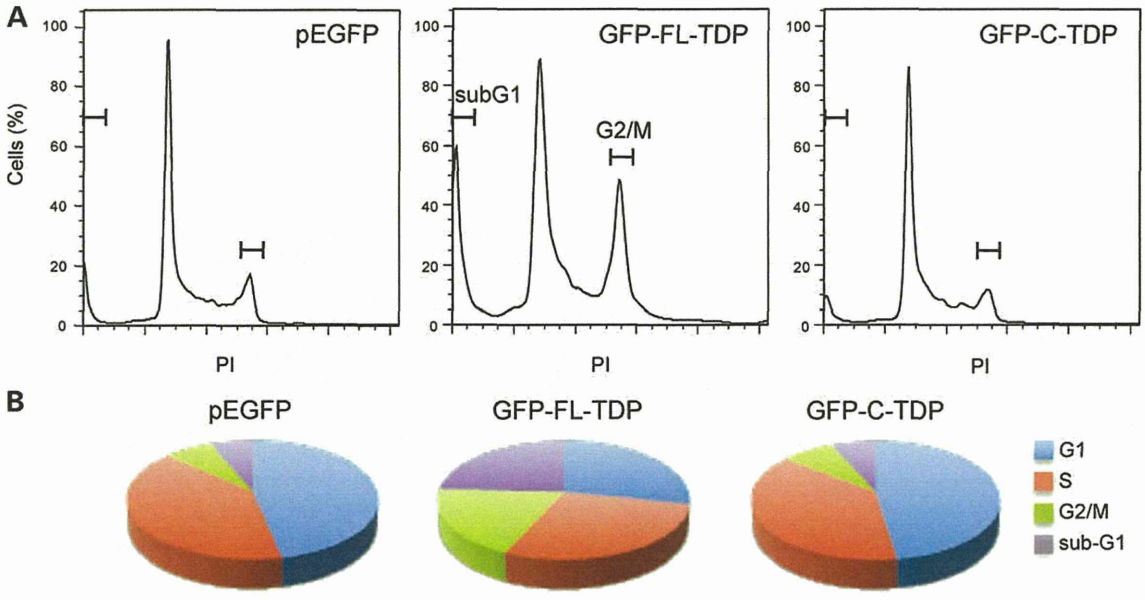

Figure 4. Analyses of the cell cycle distribution of cells transfected with GFP-tagged TDP-43. At 48 h after transfection of SH-SY5Y cells with GFP-tagged TDP-43 constructs or pEGFP empty vector, cells were stained with PI and analyzed with a flow cytometer. The results of flow-cytometric analyses are shown (**A**). The proportions of cells in G1, S, G2/M and subG1 phases were calculated using the Watson Pragmatic model (**B**). Note that growth arrest at G2/M phase was observed in GFP-FL-TDP-transfected cells, but not in GFP-C-TDP-transfected cells.

anti-pS409/410. In fluorescence-microscopic analyses, RNA pol II was co-localized with phosphorylated TDP-43 in DNs (Fig. 6D–F), clearly indicating that RNA pol II is indeed recruited to TDP-43 inclusions in FTLD-TDP brain as well as in cultured cells expressing GFP-C-TDP (Fig. 5A). On the other hand, unfortunately, antibodies to transcriptional factors Sp1, CREB and TAF II p130 failed to stain phosphorylated TDP-43 inclusions. These antibodies also failed to stain the nuclei of normal neurons in FTLD brains (data not shown). These results suggest the possibility that transcriptional activity is dysregulated *in vivo* as well as in cultured cells.

Apoptosis is not induced in FTLD-TDP brains

To test whether apoptosis is induced in FTLD-TDP brains, we performed immunoblot analyses of human brain lysates using anti-PARP and pS409/410 antibodies. As controls, we analyzed two control brains without any protein deposition and unaffected cerebellum of FTLD-TDP, in which no accumulation of TDP-43 and no atrophy has been reported. As shown in Figure 7, caspase-3-cleaved PARP was not detected in these control brains and the cerebellum of the FTLD-TDP case as well as in the temporal cortex of the FTLD-TDP case showing accumulation of TDP-43. This result indicates that apoptosis is not induced in affected neurons containing phosphorylated and aggregated TDP-43 *in vivo*.

DISCUSSION

Aberrant protein aggregates in affected neurons are well-known hallmarks of neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease, but the mechanisms by which these aggregates elicit neuronal degeneration remain unclear. In TDP-43 proteinopathy, inclusion bodies composed of phosphorylated, ubiquitinated and fragmented TDP-43 were found in neuronal cells of brains or spinal cords of patients. Recently, it was reported that prion-like propagation of aggregated TDP-43 is associated with the onset and progression of TDP-43 proteinopathy (22,28). So, in order to clarify the significance of TDP-43 inclusions in disease pathogenesis, we tried to establish cellular models with stable or transient TDP-43 expression in SH-SY5Y cells.

When we overexpressed FL-TDP in SH-SY5Y cells, significant cell death, suppression of cell growth, cleavage of PARP and increased cell populations at the G2/M and sub G1 phase were detected. However, intracellular inclusions of TDP-43 were not observed in these cells. These results suggest that an abnormally increased level of TDP-43, but not its aggregates, may be necessary for induction of cellular damage leading to apoptotic cell death. Indeed, several studies reporting that endogenous TDP-43 expression is tightly regulated and is critical for survival are consistent with this idea. For example, overexpression of wild-type TDP-43 caused motor neuron degeneration in yeast and rodents (15,17), knockout of TARDBP in mice led to embryonic lethality (29–32), heterozygous knockout mice develop motor impairments with age (30), and conditional knockout mice exhibit rapid postnatal lethality (29). TDP-43 is also regulated at the mRNA level through a negative feedback loop (3). These studies indicate that cellular TDP-43 levels are under tight control and perturbation of normal TDP-43 function is detrimental. Furthermore, it was reported that wild-type human TDP-43 expression causes mitochondrial aggregation in transgenic mice (33). This result suggests the possibility that apoptotic cell death found in cells expressing FL-TDP in this study may be caused by TDP-43-induced mitochondrial dysfunction.

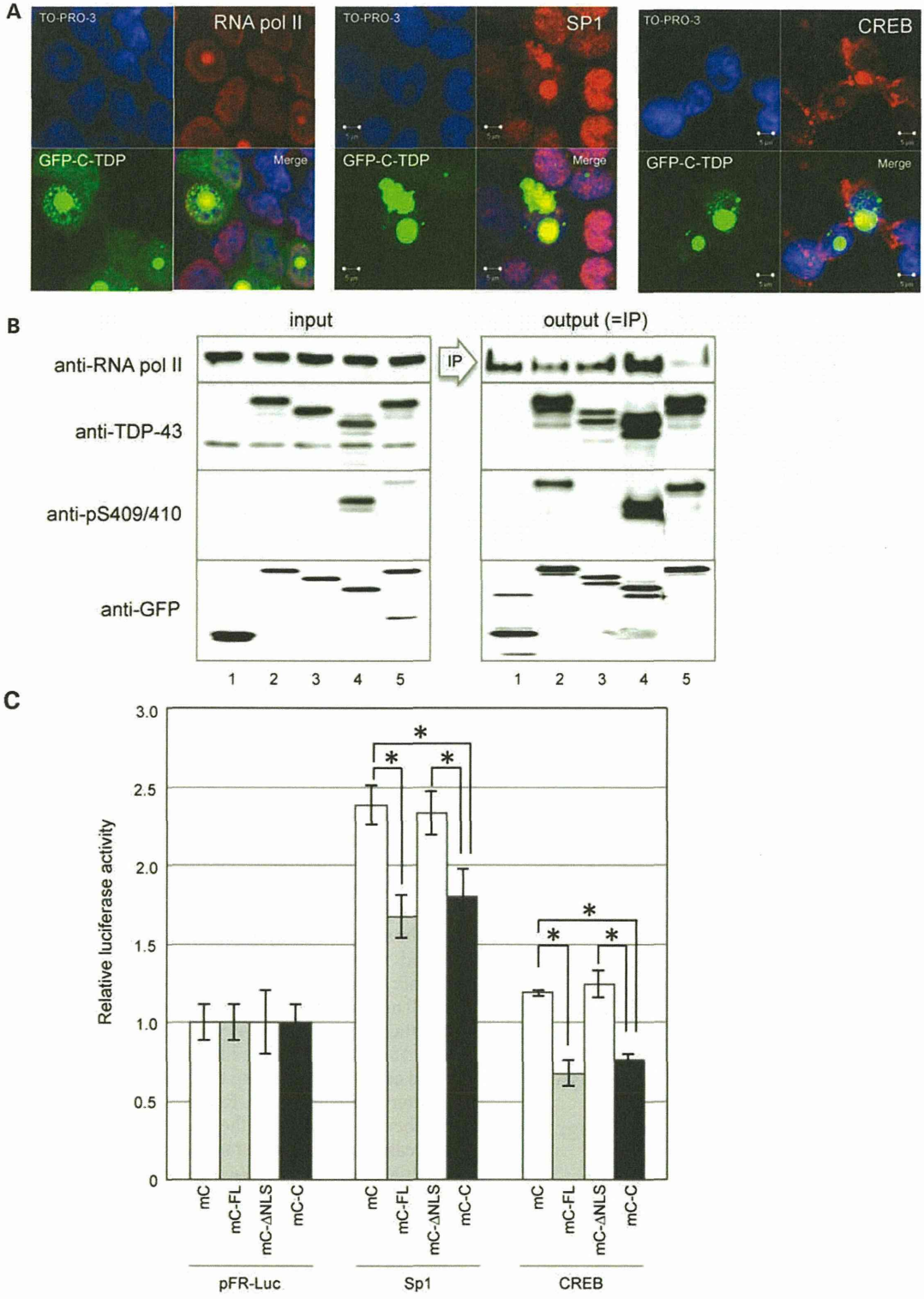

Figure 5. Co-localization of RNA pol II, Sp1 and CREB with intracellular inclusions of TDP-43 CTF. (**A**) At 72 h post-transfection with GFP-C-TDP, SH-SY5Y cells were stained with antibodies for RNA pol II, Sp1 and CREB, and observed with a confocal laser microscope. Scale bars, 5 μm. (**B**) Immunoprecipitation of cells transfected with GFP-tagged TDP-43 was performed with anti-GFP, and each sample was subjected to immunoblotting with anti-RNA pol II, TDP-43, pS409/410 and GFP antibodies. 1: pEGFP; 2: GFP-FL-TDP; 3: GFP-N-TDP (TDP-43 N-terminal fragment of 1–161 residues); 4: GFP-C-TDP and 5: GFP-ΔNLS-TDP. (**C**) SH-SY5Y cells were transfected with mCherry (mC)-tagged ΔNLS-TDP-43 (mC-ΔNLS) or CTF (mC-C). On the next day, cells were co-transfected with pFR-Luc together with pGAL4-Sp1 or pGAL4-CREB. At 48 h after the second transfection, cells were collected and luciferase activity was measured. At least three points were measured for each sample ($n = 3–6$), and the experiment was repeated three times; the illustrated results are typical. Data are means ± SEM. $^*P < 0.01$ by Student's *t*-test.

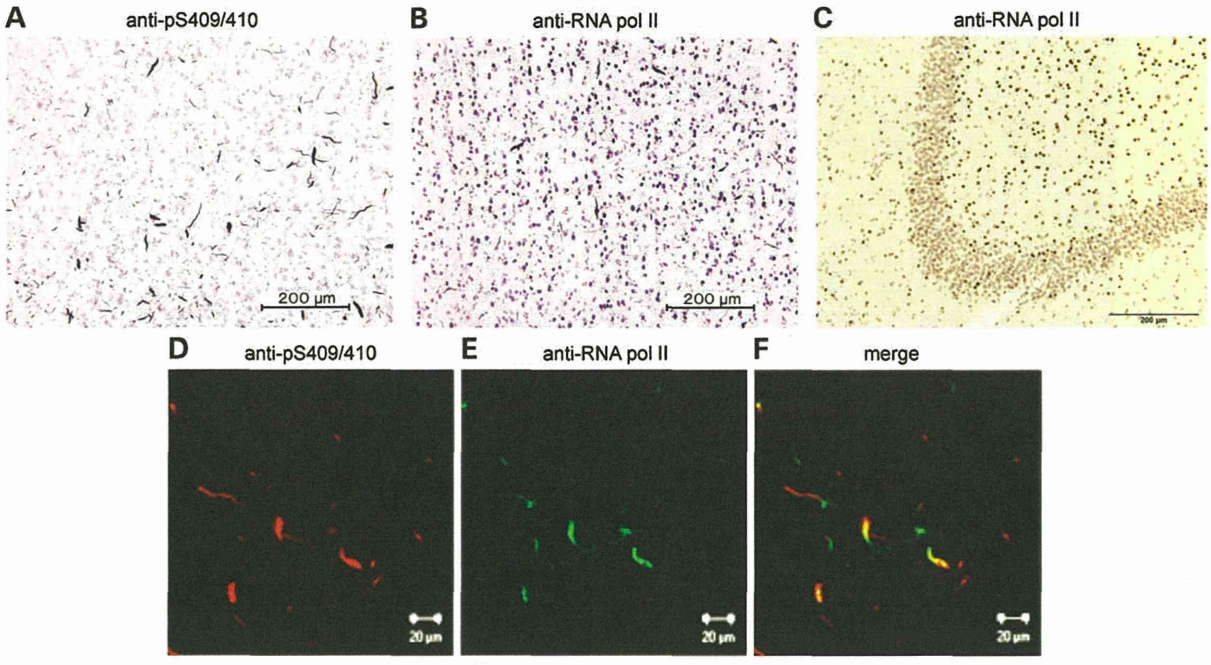

Figure 6. RNA pol II co-localizes with DNs in FTLD-TDP brain. Immunohistochemical stainings of sporadic FTLD-TDP (**A** and **B**) and control brain (**C**) were performed with anti-pS409/410 and anti-RNA pol II antibodies. (A–C) Bright-field images. Scale bars, 200 μm. (**D**–**F**) fluorescence images of sporadic FTLD-TDP. (A and D), anti-pS409/410; (B, C and E), anti-RNA pol II; (F), merge of (D) and (E). Scale bars, 20 μm.

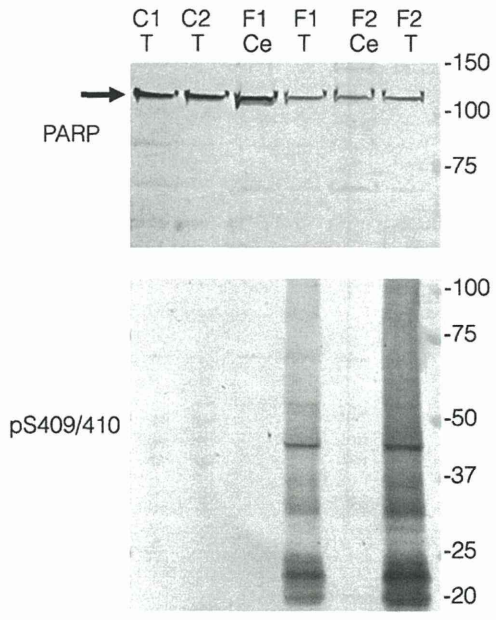

Figure 7. Apoptosis is not induced in affected neurons of FTLD-TDP brains. Immunoblot analyses of Tris-soluble (upper) and Sarkosyl-insoluble fraction (lower) prepared from human brain tissues (C1 and C2: control brains, F1 and F2: FTLD-TDP brains) were performed using anti-PARP (upper) and anti-pS409/410 (lower) antibodies. Ce, cerebellum; T, temporal cortex. Bands of endogenous whole PARP (~120 kDa: arrow) were detected in controls and patients, but no caspase-cleaved band (~95 kDa) was detected in affected or non-affected brains.

It remains unclear, however, how TDP-43 induces neuronal apoptotic cell death.

TDP-43 is a heterogeneous nuclear ribonucleoprotein and functions in RNA transcription and pre-mRNA splicing (34–38). In several genes, TDP-43 has been shown to bind directly to pre-mRNAs and regulate their splicing (36–42). In fact, widespread dysregulation of pre-mRNA splicing has been found in TDP-43-depleted cultured cells, TDP-43-depleted mouse brain and affected tissues from ALS patients (36–38, 41–44). These results suggest that dysregulation of pre-mRNA splicing is associated with ALS pathogenesis (38). Pre-mRNA splicing is mainly regulated by the spliceosome, which is a complex of small nuclear ribonucleoproteins (snRNPs) (38). The biogenesis of spliceosomes is regulated in Gemini of coiled bodies (GEMs) (38, 45–47). TDP-43 is likely to associate with GEMs in cultured cells (7), suggesting that TDP-43 contributes to GEM formation or function (38). Recently, it has been reported that the number of GEMs and the level of uridine-rich snRNA were decreased in spinal motor neurons of ALS patients (38, 48), suggesting that abnormal splicing caused by spliceosome disruption results in motor neuron death in ALS. Furthermore, several RNA processing genes have been shown to be mutated or genetically associated with ALS, including not only TDP-43, but also FUS/TLS, again suggesting that disordered RNA processing may be a key pathogenic mechanism in development of ALS (49). To our knowledge, the evidence presented here is the first to show that the formation of inclusions composed of TDP-43 CTF is cytotoxic: in cells with these inclusions, we observed a significant decrease of BrdU uptake, sequestration of RNA pol II, Sp1 and CREB into cytoplasmic aggregates of TDP-43 CTF, and

decreased transcriptional activities of Sp1 and CREB. Furthermore, RNA pol II was co-localized with these inclusions both in cultured cells and FTLD-TDP brain. These results also support the idea that transcriptional deregulation plays a critical role in the degenerative cascade in TDP-43 proteinopathy.

Our results have shown that perturbation of the expression of FL-TDP elicits apoptotic cell death, and intracellular TDP-43 aggregation causes aberrations in RNA metabolism. The overproduction of TDP-43 might also lead to formation of intracellular TDP-43 aggregates, while decreased levels of TDP-43 protein could also influence TDP-43 expression, because TDP-43 itself auto-regulates its mRNA levels through a negative feedback loop (3). Intracellular TDP-43 aggregate formation may cause aberrant TDP-43 mRNA levels due to decreased levels of normal TDP-43 in nuclei, and this is known to be one of pathological characteristics found in brains of TDP-43 proteinopathy patients. On the other hand, lacking of apoptosis in FTLD-TDP brains containing phosphorylated and accumulated TDP-43 observed in this study suggests that non-apoptotic cytotoxicity induced by TDP-43 aggregates rather than soluble TDP-43 may be closely related to the neurodegenerative mechanisms of TDP-43 proteinopathy. Therefore, it is likely that the loss of function and the gain of toxic function of TDP-43 are mutually associated with the onset of TDP-43 proteinopathy.

We conclude that dysregulation of FL-TDP expression causes neuronal apoptosis, while formation of intracellular aggregates of TDP-43 CTF induces defects in RNA metabolism. Our results suggest that plural pathways lead to TDP-43-induced cellular dysfunction, contributing to the degeneration cascades associated with onset of TDP-43 proteinopathy.

MATERIALS AND METHODS

Antibodies

A monoclonal antibody specific for TDP-43 (anti-TDP-43) was purchased from ProteinTech. An antibody specific for phosphorylated TDP-43 at both Ser409 and Ser410 antibodies (anti-pS409/410) were prepared as described (12,50). Anti-PARP antibody (#9542) was purchased from Cell Signaling. A monoclonal anti-RNA poly II antibody, which recognizes both the phosphorylated and non-phosphorylated forms of the C-terminal heptapeptide repeat region of RNA pol II, was purchased from Active Motif. A polyclonal anti-Sp1 antibody was purchased from Bethyl Laboratories and a monoclonal anti-CREB antibody (M01) was purchased from Abnova. Anti-GFP antibody was obtained from MBL (Nagoya, Japan). Anti-tubulin α antibody was purchased from Sigma-Aldrich. Anti-mouse IgM conjugated with Alexa-568 (A-21043) and anti-rabbit IgG conjugated with Alexa-568 (A-11011) were obtained from Molecular Probes.

Viral transduction of TDP-43 constructs

FL-TDP, NTF (1–161 residues: N-TDP) and CTF [162–414 residues: C-TDP (20)] of TDP-43 were subcloned into the pCL36-C1L-CMp-IRES-GFP lentivirus expression vector (51). HEK 293T cells were transfected with vector containing the insert or the empty vector along with Packing Mix (pCAG-kGP4.1R, pCAG4-RTR2 and pCAGGS-VSV-G vectors) for 40 h (with medium replacement after 6 h). Virus particles were pelleted by ultra-centrifugation (5800*g*, Beckman SE28 rotor, 16 h, 4°C). Viruses were then suspended in Hanks Balanced Salt Solution and stored at −80°C until use. For transfection, virus (1×10^7 copies/ml) was added to 2×10^5 SH-SY5Y cells/ml.

Cell culture and transfection

SH-SY5Y cells were cultured in DMEM/F12 medium (Sigma) supplemented with 10% fetal calf serum, penicillin–streptomycin–glutamine (Invitrogen), and MEM non-essential amino acid solution (Invitrogen). The cells were maintained at 37°C under a humidified atmosphere of 5% (v/v) CO_2 in air. They were grown to 50% confluence in six-well culture dishes for transient expression, and transfected with expression plasmids using FuGENE6 (Roche) according to the manufacturer's instructions. TDP-43 expression plasmids for FL-TDP and C-TDP were constructed as previously described (20,23).

Cell proliferation assay

Cell proliferation was determined with a 5-Bromo-2′-deoxyuridine Labeling and Detection Kit II (Roche). Transfected SH-SY5Y cells were grown on coverslips for 3 days, and then incubated for 10 h at 37°C in culture medium containing 10 μM BrdU. After incubation, cells were washed briefly, fixed and processed for immunostaining according to the manufacturer's instructions.

Cell cycle analysis

Cells were harvested at 72 h after transfection, fixed in 70% ethanol, treated with RNase A (1 mg/ml) for 30 min, and then stained with PI (50 μg/ml). DNA content was analyzed using an EPICS XL flow cytometer (Beckman Coulter).

Immunohistochemical analysis

SH-SY5Y cells were grown on coverslips and transfected as described above. After incubation for the indicated times, cells were fixed with 4% paraformaldehyde and stained with primary antibody (anti-TDP-43, anti-pS409/410, anti-RNA pol II, anti-Sp1 and/or anti-CREB antibody) at 1:1000 dilution. The cells were washed and further incubated with anti-rabbit IgG-conjugated Alexa-568 (1:1000), and then with TO-PRO-3 (1:3000, Invitrogen) or Hoechst 33342 (1:2000, Lonza) to counterstain nuclear DNA. Finally, they were analyzed using a LSM5 Pascal confocal laser microscope (Carl Zeiss).

Human brain tissues were obtained from Tokyo Metropolitan Institute of Medical Science (Tokyo, Japan). This study was approved by the local research ethics committee of Tokyo Metropolitan Institute of Medical Science (approval no. 12-3). Small blocks of human brain were dissected at autopsy or from fresh-frozen brain samples and fixed in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for 2 days. Following cryoprotection with 15% sucrose in 0.01 M phosphate-buffered saline (pH 7.4), blocks were cut on a freezing microtome at 30 μm thickness. The free-floating sections were incubated with anti-pS409/410 or anti-RNA pol II antibody for 72 h.

Following treatment with the appropriate secondary antibody, labeling was detected using the avidin-biotinylated horseradish peroxidase (HRP) complex system coupled with diaminobenzidine (DAB) reaction to yield a brown precipitate. In some sections, the DAB reaction was intensified with nickel ammonium sulfate to yield a dark purple precipitate. Moreover, the sections were incubated with secondary antibodies, labeled with FITC for anti-pS409/410 or with Rhodamine for RNA pol II and then observed under a fluorescence microscopy.

Immunoprecipitation and western blotting

SH-SY5Y cells grown in a six-well plate were transfected with GFP-tagged TDP-43 expression vectors (20). After incubation for 3 days, cells were harvested and lyzed in TX buffer [50 mM Tris–HCl (pH 7.5), 150 mM NaCl, 5 mM ethylenediaminetetraacetic acid, 5 mM ethylene glycol tetraacetic acid (EGTA), 1% TX and protease inhibitor cocktail (Roche)] by brief sonication on ice. The lysates were incubated with anti-GFP antibody-linked Dynabeads (Invitrogen) for 4 h at 4°C. The immunoprecipitated Dynabeads complexes were washed five times with TX buffer. Proteins were eluted by boiling in SDS sample buffer and then processed for western blot analysis. Each sample was separated by 12% (v/v) SDS-PAGE using Tris–glycine buffer system, and proteins were transferred onto polyvinylidene difluoride membrane (Millipore). The blots were incubated overnight with each primary antibody at room temperature, followed by incubation with HRP-conjugated secondary antibody. Signals were detected using the ECL plus Western Blotting Detection System (GE Healthcare).

Luciferase assay

In the GAL4-Sp1 expression vectors, the 147 N-terminal codons of the yeast transcription factor GAL4 containing its DNA-binding domain were fused to fragments coding for the N-terminal regions of Sp1 and CREB. The expression vectors of pGAL4-Sp1 and pGAL4-CREB, and luciferase reporter plasmid pFR-Luc were prepared as described previously (52). SH-SY5Y cells were transfected with mCherry-tagged TDP-43 constructs. Next day, these cells were co-transfected with pFR-Luc together with pGAL4-Sp1 or pGAL4-CREB. At 48 h after the second transfection, cells were collected and luciferase activity was measured with a Luciferase Assay kit (Stratagene) according to the manufacturer's instructions. At least three points of each sample were measured ($n = 3–6$), and the experiment was repeated three times; the illustrated results are typical.

Preparation of human brain homogenates

Brain samples for immunoblot analyses were prepared as previously described (12,22). Briefly, frozen brain tissue from two controls or two patients with FTLD-TDP (type C) was homogenized in 10 volumes (w/v) of homogenization buffer (HB: 10 mM Tris–HCl, pH 7.4, 0.8 M NaCl, 1 mM EGTA and 10% sucrose). Aliquots of the homogenates were ultracentrifuged at 100 000g for 20 min at 4°C, and the supernatant was recovered as Tris-soluble fraction for immunoblotting analyses. Remaining lysate homogenates were incubated at 37°C for 30 min in HB buffer containing 2% Sarkosyl, and centrifuged at 20 000g for 10 min. The supernatants were ultracentrifuged at 100 000g for 20 min and the resulting pellets were used as Sarkosyl-insoluble fraction for immunoblotting analyses.

Statistical analysis

All values in the figures are shown as mean ± SEM. Statistical analysis was performed using the unpaired, two-tailed Student's t-test. A P value of 0.01 or less was considered to be statistically significant.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

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Relative Ratio and Level of Amyloid-β 42 Surrogate in Cerebrospinal Fluid of Familial Alzheimer Disease Patients with Presenilin 1 Mutations

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Key Words
Alzheimer disease · Amyloid-β peptide · Amyloid-β-like peptide · Amyloid precursor-like protein-1-derived Aβ-like peptide · Presenilin · γ-Secretase · γ-Secretase inhibitor

Abstract
Background: Presenilin 1 (PS1) mutations associated with familial Alzheimer disease (FAD) generally increase the amyloid-β 42 (Aβ42) to Aβ40 ratio secreted in cultured cells. Some of these mutants reduce the secretion of Aβ40 rather than increase that of Aβ42. Since it has been difficult to estimate Aβ42 secretion in brains of PS1-FAD patients due to substantial Aβ42 accumulation, it remains unknown whether the enhanced Aβ42 to Aβ40 ratio in brains of FAD patients is caused by elevated Aβ42 secretion or by reduced secretion of Aβ40. ***Objective/Methods:*** Cerebrospinal fluids (CSF) of PS1-FAD patients and neurological control patients (controls) were collected. Levels of CSF amyloid precursor-like protein-1-derived Aβ-like peptide (APL1β), including APL1β28, an Aβ42 surrogate marker, were quantified by liquid chromatography tandem mass spectrometry, and Aβ42 secretion in the brain was estimated. ***Results:*** The relative ratio of CSF APL1β28 to total APL1β was higher in PS1-FAD patients than in controls. Importantly, CSF APL1β28 was not significantly higher. However, C-terminally shorter CSF APL1β25 and APL1β27 were significantly lower in PS1-FAD patients and, as expected, so were CSF Aβ40 and Aβ42. ***Conclusion:*** A higher relative ratio of the CSF Aβ42 surrogate in PS1-FAD patients is not due to its increase in CSF, suggesting that massive Aβ42 accumulation in the PS1-FAD brain occurs without an apparent increase in Aβ42 secretion.

Introduction

The relative ratio of amyloid-β 42 peptide (Aβ42) to total Aβ (the Aβ42 ratio) in secreted Aβ is elevated in cultured media of cells expressing presenilin 1 (PS1) familial Alzheimer disease (FAD) mutations [1]. An in vitro assay demonstrated that purified mutant PS1/γ-secretases also increase the Aβ42 ratio in de novo Aβ [2]. Since substantial amounts of Aβ42 accumulate in brains of FAD patients with PS1 mutations (PS1-FAD patients), it is easy to

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Table 1. Clinical information and levels of CSF APL1β and Aβ peptides of FAD patients and controls

Mutation	Diagnosis	Age at onset, years	APL1β25, pM	APL1β27, pM	APL1β28, pM	Total APL1β, pM	APL1β28 ratio	Aβ40, pM	Aβ42, pM	Aβ42 ratio
PS1 L85P	early-onset AD with spastic paraplegia (variant type AD)	26	806.5	1,009	821.8	2,638	0.3116	396	6.163	0.01531
PS1 G206V	early-onset AD with psychosis	37	886.4	727	868.2	2,481	0.3499	282	12.65	0.04299
PS1 L286V	early-onset AD	40	1,202	809	1,006	3,017	0.3335	253	11.53	0.04361
PS1 L381V	early-onset AD with spastic paraplegia (variant type AD)	29	935.2	1,057	814.9	2,807	0.2903	123	8.919	0.06759
None	neurological control patients	n.r.	1,953± 683.8	1,649± 683.8	948.4± 402.3	4,551± 1,721	0.2080± 0.033	1,009± 456.3	90.91± 41.93	0.0840± 0.016

The values of the controls represent the means ± SD. n.r. = Not recorded.

assume that the accumulation is caused by increased Aβ42 secretion in the brain. However, some PS1 mutations reportedly affect the activity of PS/γ-secretase to secrete Aβ [3–5]. This finding suggests that exogenous expressions of PS1 mutants in cultured cells can increase the Aβ42 ratio in secreted Aβ without elevating the level of Aβ42 secretion; that is, the mutant-expressing cells reduce secretion of major Aβ species such as Aβ40. If this is also the case in mutant-bearing brains, no increase in the level of Aβ42 secretion but an increase in the Aβ42 ratio plays an essential role in the promotion of brain Aβ42 accumulation.

Although this interpretation seems mysterious [3–5], the findings of some studies do not contradict this notion. First, a small increase in the Aβ42 ratio is more crucial for neurotoxicity than the absolute amounts of Aβ42 [6]. Second, amyloid pathology is exacerbated in the brain of knock-in mice with an artificial PS1 mutation which reduces the level of Aβ40 but not of Aβ42 in an in vitro γ-secretase assay [7]. Third, additional Aβ40 expression in the Tg2576 mouse brain was found to diminish Aβ42 accumulation [8].

The Aβ42 level in cerebrospinal fluid (CSF) may be the best indicator to determine whether Aβ42 secretion is elevated in FAD brains. However, it is quite common that both the ratio and the level of CSF Aβ42 are lowered in FAD patients [9]. Although it is still controversial [10], it may be the result of brain Aβ42 accumulation [11].

Previously, we reported that amyloid precursor-like protein-1-derived Aβ-like peptide 28 (APL1β28) in CSF is a non-amyloidogenic surrogate marker for brain Aβ42 production. The relative ratio of CSF APL1β28 to total APL1β (the APL1β28 ratio) is higher in PS1-FAD cases than in non-AD cases [12]. In this study, we analyzed CSF APL1β levels, including those of APL1β28, in PS1-FAD patients and in neurological control patients and tried to estimate whether the absolute values of newly generated Aβ42 levels are comparatively elevated in the brains of the PS1-FAD patients.

Results and Discussion

The CSF Aβ40 Level Is Lower in PS1-FAD Patients

CSF samples of 4 FAD patients with a PS1 mutation (PS1 L85P, L286V, L381V and G206V; PS1-FAD patients) and 22 neurological control patients (controls) were used (table 1) [12]. CSF samples of the 2 other FAD patients with a PS1 mutation (PS1 H163R and M233L) were excluded from this study, because these two mutations do not affect γ-cleavage of amyloid precursor-like protein 1 [12].

First, we quantified CSF Aβ40 and Aβ42 by means of ELISA. As expected, both Aβ40 and Aβ42 levels in the PS1-FAD patients were significantly lower than those in the controls ($p < 0.0005$ for Aβ40 and $p < 0.0005$ for Aβ42; fig. 1a, b; table 1) [13]. Aβ40 is the most predominant Aβ species and much less amyloidogenic than Aβ42. Thus, a lowered Aβ40 level in CSF of PS1-FAD patients may to some extent reflect a reduction in the total Aβ secretion level in the brain.

In accordance with previously published results, the ratios of CSF Aβ42 to total Aβ of PS1-FAD patients were significantly lower than those of the controls ($p < 0.005$; fig. 1c; table 1) [9].

The CSF APL1β28 Level, a Surrogate Marker of Brain Aβ42 Production, Is Not Elevated in PS1-FAD Patients

We next quantified levels of CSF APL1β25, APL1β27 and APL1β28 by means of liquid chromatography tandem mass spectrometry (LC/MS/MS) [12]. Because all three APL1β species are non-amyloidogenic, the level of each APL1β species in CSF is considered to reflect the secretion level of the corresponding species in the brain [12]. Moreover, the APL1β28 and Aβ42 ratios change in parallel [12].

The APL1β28 ratios to total APL1β of the PS1-FAD patients were significantly higher than those of the control ($p < 0.0005$; fig. 2a; table 1) [12]. Importantly, CSF APL1β28 levels of the PS1-FAD patients were not higher than those of the control ($p = 0.8291$; fig. 2b; table 1). Moreover, levels of CSF APL1β25 and APL1β27 were lower in the PS1-FAD patients than in the controls ($p < 0.05$ for APL1β25 and $p < 0.05$ for APL1β27; fig. 2c, d; table 1). The summed levels of three peptides, that is, the total level of APL1β, were also lower in the PS1-FAD patients ($p < 0.05$; fig. 2e; table 1). These results suggest that the higher APL1β28 ratio in CSF of the PS1-FAD patients is not due to an increase in the absolute level of APL1β28 generated but to a decrease in the absolute levels of C-teminally shorter APL1β species generated in brains.

These data for the Aβ42 surrogate marker lead us to speculate that in the brains of the PS1-FAD patients (1) the Aβ42 ratio is elevated, (2) the absolute values of newly generated Aβ42 levels are not elevated, and (3) the absolute values of newly generated C-terminally shorter Aβ species such as Aβ40 are lowered. Thus, Aβ42 accumulation in brains of the PS1-FAD patients may be induced not by an increase in Aβ42 generation but by a reduction in the secretion of C-terminally shorter Aβ species.

How does such a change, i.e. a decrease in C-terminally shorter APL1β species without an accompanying increase or decrease in the longer APL1β28 in CSF of the PS1-FAD patients, occur? Recently, we found that PS/γ-secretase cleaves elongated Aβ and Aβ-like peptides into shorter species, i.e. Aβ42 into Aβ38, Aβ43 into Aβ40/38, and APL1β28 into APL1β25 [14]. Because mutant PS1/γ-secretases diminish Aβ42 cleavage [14], the ratios of longer Aβ and Aβ-like peptides to the corresponding shorter species inevitably increase. Thus, a reduction in cleavages of APL1β28 by the mutant PS/γ-secretases may at least in part account for the increase in the CSF APL1β28 ratio to total APL1β in the PS1-FAD patients.

The very small number of CSF samples analyzed is a limitation to our study. However, it should be noted that patients with several different PS1 mutations all showed the similar phenotype.

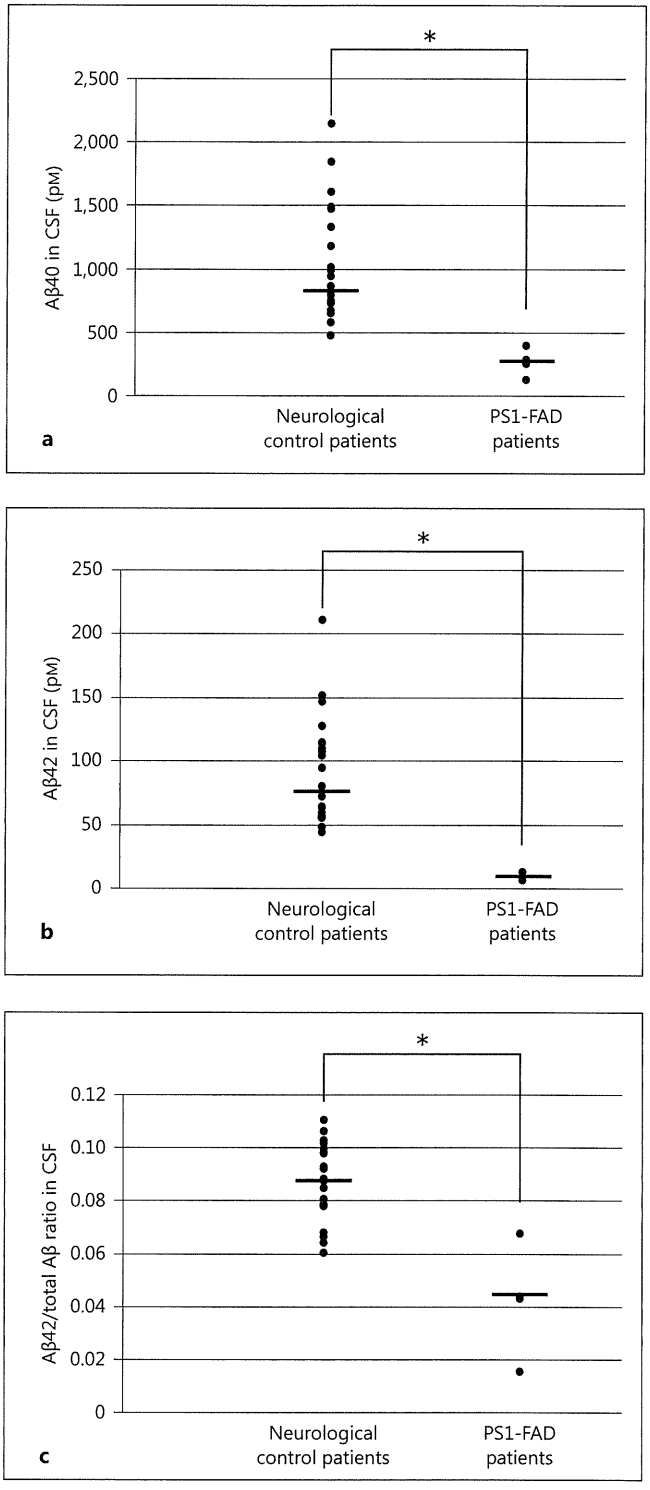

Fig. 1. Measurement of Aβ species levels in CSF. CSF Aβ40 levels (**a**), Aβ42 levels (**b**) and Aβ42 to total Aβ ratios (**c**) of the PS1-FAD patients and the controls. The bars represent median values. The asterisks indicate statistical significance (nonparametric statistical analysis).

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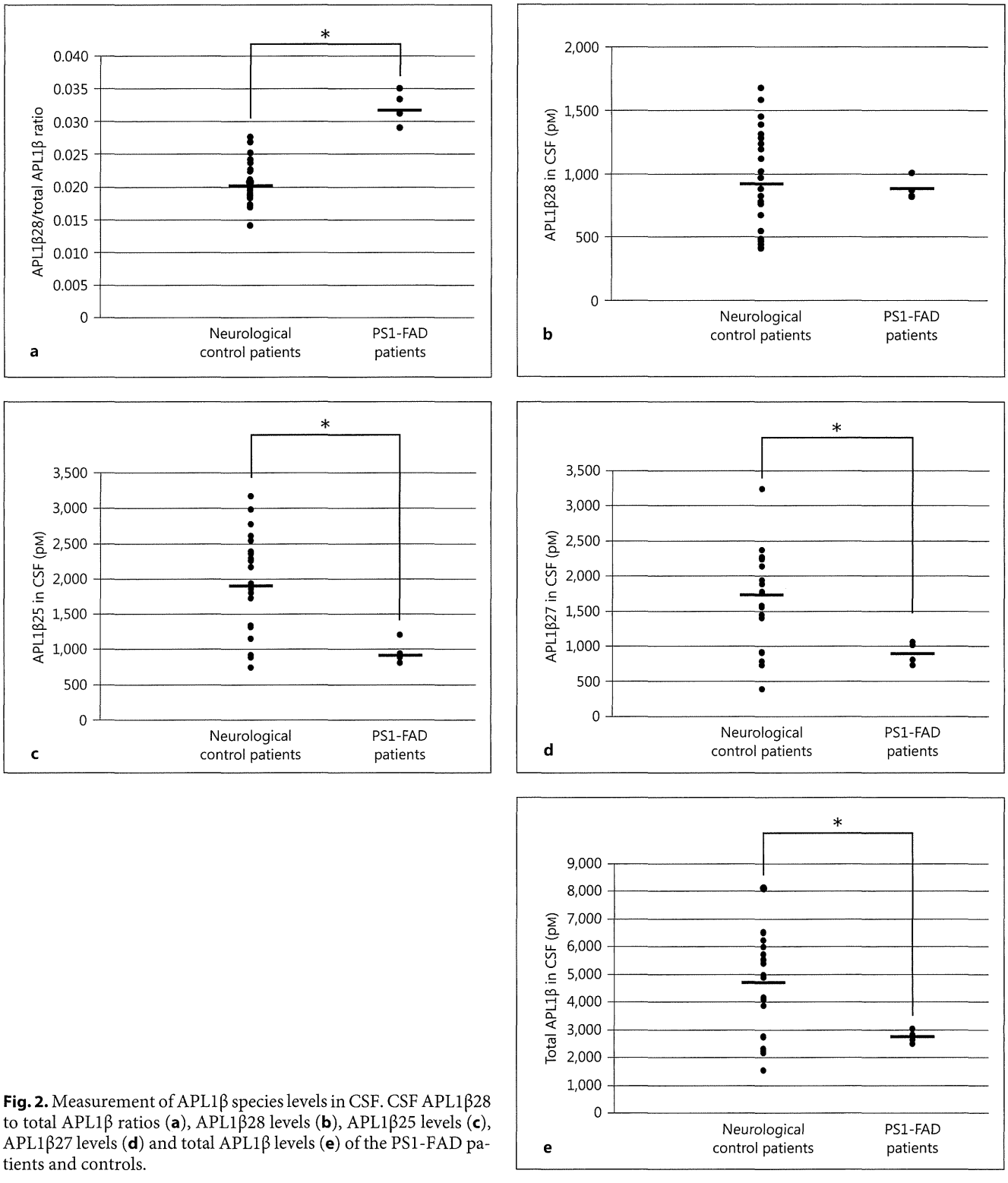

Fig. 2. Measurement of APL1β species levels in CSF. CSF APL1β28 to total APL1β ratios (**a**), APL1β28 levels (**b**), APL1β25 levels (**c**), APL1β27 levels (**d**) and total APL1β levels (**e**) of the PS1-FAD patients and controls.

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γ-Secretase inhibitors (GSIs) are one of the main disease-modifying drugs being developed. Our findings point to the need for caution regarding GSI development because GSIs inhibit Aβ42 cleavage as well as total γ-secretase activity. Thus, GSI administration will inevitably increase the Aβ42 ratio unless it completely blocks Aβ production [15]. The Aβ42 ratio will thus remain higher with GSI administration, which leads to a risk of enhancing disease progression even if total Aβ secretion is reduced.

Experimental Procedures

Aβ ELISA

Aβ40 or Aβ42 levels in human CSF were quantified with commercial sandwich ELISA kits (Wako Pure Chemical Industries, Ltd.). Human CSF was appropriately diluted with the standard diluents provided in the kit.

LC/MS/MS Analysis

The levels of APL1β25, APL1β27 and APL1β28 were quantified by means of LC/MS/MS analysis as described previously [12].

Acknowledgements

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7 BPSD 対応の現状と展望

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認知症疾患医療センター，精神科

Abstract

BPSD は，アルツハイマー病患者の地域での生活を阻む最大の要因の一つである。BPSD への対応の第一選択は非薬物療法および環境調整であり，それでも効果が不十分な場合に薬物療法を行うのが原則である。今後，認知症初期集中支援チームと身近型認知症疾患医療センターの整備により，BPSD への早期・事前的な対応が可能となり，地域での生活が維持されることが期待される。同時に，病態機序に基づいた BPSD の評価法および治療プログラムの開発が不可欠である。

はじめに

BPSD には，幻覚，妄想，興奮，叫声，不穏，焦燥，徘徊，社会文化的に不適切な行動，性的脱抑制，収集癖，暴言，つきまとい，不安，抑うつなどが含まれる。治療法を考える上で有用であることから，BPSD を，興奮，易刺激性，焦燥，幻覚，妄想などの陽性症状と，無気力，無関心，抑うつなどの陰性症状に分ける考え方がある。認知症患者の約 60-90% が，少なくとも 1 つ以上の BPSD 症状を呈するとされ，その対応に苦慮することも多い。2012 年の厚生労働省による新たな認知症施策により，患者の地域での生活を強化する方針が示されたが，BPSD はこの施策を阻害する最大の要因の一つである。したがって，BPSD の病態機序の解明とそれに基づく対応法の確立が急務であるが，これらについては未だ十分とは言えない。本稿では，アルツハイマー病（AD）に出現する BPSD への対応の現状と今後の展望について述べる。

1．BPSD 対応の現状

BPSD の出現により自宅あるいは施設での対応が困難となった場合，精神科に受診あるいは入院となることが多い。一旦入院となると，退院先が見つからない，合併した身体疾患の増悪，BPSD が改善しないなどの理由で入院が長期化する例があり，いかに早期に退院させるか，さらには入院させずに対処できるかが課題となっている。

筆者らは，厚生労働省の認知症対策総合研究事業の一環として，茨城県の地域型認知症疾患医療センターに指定されている精神科病院に平成 24 年度に入院した 107 名の認知症患者の実態調査を行った。診断の内訳は，AD が最も多く（44%），次いで混合型認知症（13%），レビー小体型認知症（12%），血管性認知症（5%），前

Current issues and future prospects of BPSD：Tetsuaki Arai [1,2]
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頭側頭葉変性症（5%），軽度認知機能障害（4%）であった。94%の症例にBPSDが認められ，その内訳は，暴言・暴力（68%），妄想（47%），徘徊（41%），睡眠障害（38%），幻覚（24%），介護への抵抗（21%），不安・焦燥（11%），せん妄（11%）などであった（図1）。平均在院日数は112日であり，自宅退院群と長期入院（6ヶ月以上）継続群を比較すると，不安・焦燥の頻度が後者で有意に高かった。Kitamuraらは，BPSDのために入院となった認知症患者の入院期間に影響を与える因子として，男性，独居，介護者が子供，MMSEの点数などを同定している[1]。Taniguchiらは，精神科の認知症治療病棟における入院治療のBPSDへの有効性を検討し，妄想と睡眠異常の改善度が高いことを報告した[2]。

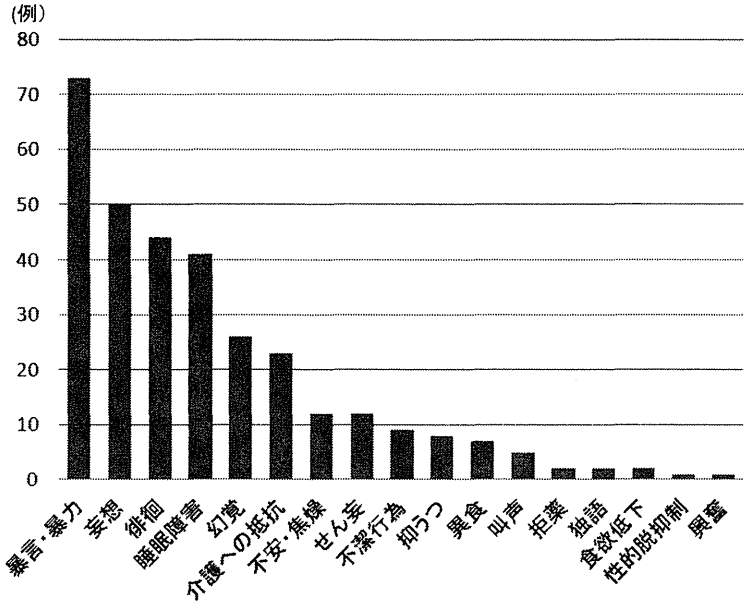

図1　平成24年度に茨城県の地域型認知症疾患医療センターに入院した107名の認知症患者に認められたBPSDの内訳

2．BPSD対応の原則

BPSDの病因は単純ではなく，身体的，心理的，環境的ないくつかの誘発因子あるいは増悪因子が組み合わさって生じると考えられる[3]（図2）。したがって，BPSDが出現した場合，まずこれらの因子の有無を評価しなければならない。身体的要因としては，便秘，脱水，感染，疼痛，薬剤などが，心理的・環境的要因としては，転居，入院，施設入所，身内や友人の病気や死，介護者の介護能力低下，家族との諍いや不仲などが挙げられる。これらを評価した上でBPSDへの対応の第一選択は非薬物療法および環境調整であり[4]，それでも効果が不十分な場合に薬物療法を行うのが原則である。患者に対応する際の基本は，訴えを傾聴し，受容的態度で接し，共感を示すことである。

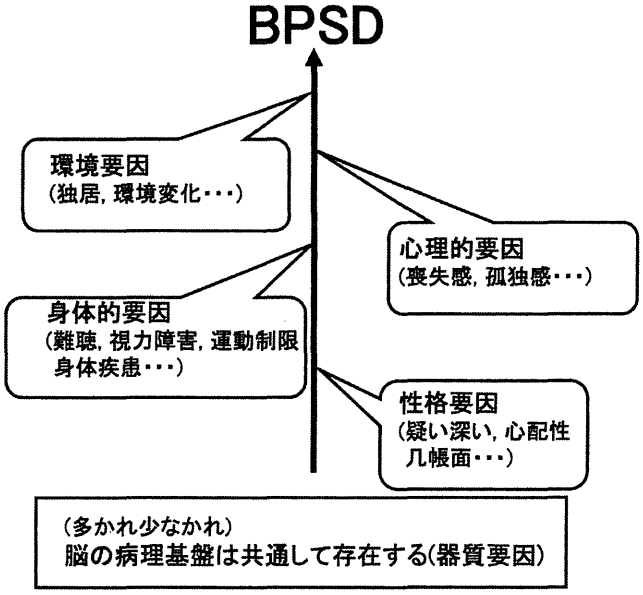

図2　BPSDの病態機序の模式図
（香川大学炎症病理・池田研二先生のご厚意による）

1．環境調整

入院や施設入所の環境では，静かな環境の確

保，写真や思い出の品などの個人の背景・文化の維持，照明の調節，音楽を流す，居室の変更などが有効である場合がある。

家族や施設スタッフに対する疾患教育は，BPSDの軽減に有効であることが一致して示されている[5]。デイサービスやショートステイの利用などのケアプランの調整が必要な場合もある。

2. 非薬物療法

ADに対する非薬物療法は，1）認知/感情に焦点を当てたもの（回想法，バリデーション療法，認知刺激療法，simulated presence therapyなど），2）感覚刺激に焦点を当てたもの（鍼，アロマセラピー，光療法，マッサージ，音楽療法，芸術療法，感覚刺激法など），3）行動に焦点を当てたもの（behavior management techniques），4）その他の心理社会的介入（ペット療法，運動など）などに分類される[3]。これらのBPSDへの効果については，厳密な二重盲検や無作為抽出の比較試験はなされていないが，Livingstonらによるシステマティックレビューでは，behavior management techniquesと認知刺激療法の有効性が高いことが指摘されている[5]。BPSDの症状別の効果では，音楽療法およびアロマセラピーの焦燥への有効性や運動療法の抑うつへの効果などが報告されている[3,5]。

3. 薬物療法

1）BPSDの陽性症状に対する薬物療法

A. 抗認知症薬

ドネペジル，ガランタミン，リバスチグミン，メマンチンは，基本的に認知症の中核症状の進行を抑制する薬剤であるが，最近BPSDに対する種々の効果が報告されている。特に陽性症状に対するメマンチンの効果については一致した見解が得られており，例えばGauthierらによれば投与12週間後にプラセボに比してメマンチン投与群では，妄想，幻覚，焦燥/攻撃性が，さらに24週間後では妄想，焦燥/攻撃性，易刺激性/不安定性などに有意な改善あるいは悪化の阻止が認められている。アセチルコリンエステラーゼ阻害剤（AChEI）も陽性症状を改善させる場合がある。例えばガランタミンの3つの臨床試験に関するメタ解析では，激越，不安，脱抑制，異常行動などへの有意な効果が示されている[6]。ただし，AChEIは易怒性や攻撃性などを増悪させる場合もあり，AChEIがどのような症例のどのようなBPSDに有効であるかについてはさらなる検討が必要である。いずれにしても，BPSDの陽性症状に対し，安易に他の薬剤を追加する前に，抗認知症薬の効果を見極めることが先決であると言える。

B. 漢方薬

抑肝散のBPSDに対する効果は，原が，易怒性，興奮，不眠，せん妄に有効であることを報告したことが最初である。その後Iwasakiらは，52例の軽度から高度認知症患者に対して無作為化対照試験を行い，抑肝散が幻覚，興奮，焦燥，異常行動などに有効であることを報告した。Mizukamiらも，AD，混合型認知症，レビー小体型認知症（Dementia with Lewy Bodies : DLB）を含む106例において，興奮や易刺激性への抑肝散の有効性を報告した[7]。抑肝散は副作用が少なく使いやすい薬だが，低カリウム血症を来す場合がある点に注意が必要である。

C. 気分安定薬

カルバマゼピンとバルプロ酸は，ADの焦燥や興奮の治療に有効であることが海外では多数報告されている。Mizukamiらは，AD，VD，DLBを含む外来患者110名を対象に調査し，バルプロ酸100～200mgの低容量でも，易刺激性，興奮，攻撃性，不眠などに有効であることを報告した[8]。バルプロ酸の注意点としては，眠気やふらつきが出ることがあることと，血清アン

モニアの上昇や血小板数の減少などがみられることである。

D. 抗不安薬

AD 剖検脳の検討から，セロトニン 1A 受容体の減少と易怒性が関連することが報告され，海外ではセロトニン 1A 受容体の部分作動薬であるブスピロンの効果が報告されている。

本邦では，同様の薬理作用を示す薬としてタンドスピロンがあり，ベンゾジアゼピン系抗不安薬と異なり，筋弛緩作用，過鎮静，常用量依存などの問題がないため高齢者に使いやすい。Sato らは，AD および VD を含む 13 例に対し，タンドスピロンを最大 30mg/ 日まで使用し，8 週間観察したところ，平均使用量 20mg/ 日で，焦燥，うつ，不安，易刺激性が改善し，特に焦燥や攻撃性に有効であることを報告した[9]。

E. 抗精神病薬

BPSD に対する非定型抗精神病薬の臨床治験はこれまで多く行われ，リスペリドン，オランザピン，クエチアピン，ペロスピロン，アリピプラゾールなどについて，概ね陽性症状に対する有意な効果が認められている。

非定型抗精神病薬の副作用としては，耐糖能異常，高脂血症，不整脈，脳血管系のリスク，過沈静，死亡率の増加などが挙げられる。特に問題となったのは，2005 年 4 月に FDA よりなされた警告であり，非定型抗精神病薬を投与された群はプラセボ投与群よりも死亡率が 1.6-1.7 倍高く，死因の多くは心臓障害（心不全，突然死等）か感染症（肺炎）であることが示されている。また，その後の検討で，定型抗精神病薬は非定型抗精神病薬よりも有意に死亡リスクが高く，その代替薬として使用はできないことも判明している。

なお，BPSD に対する抗精神病薬の使用は保険適応外であるが，厚生労働省保険局医療課長による平成 23 年 9 月 28 日付の通達により，「器質的疾患に伴うせん妄・精神運動興奮状態・易怒性」に対して，クエチアピン，ハロペリドール，ペロスピロン，リスペリドンの保険適応外使用が認められた。

2) BPSD の陰性症状に対する薬物療法

AChEI は，AD の抑うつ症状に対する効果が報告されているため[10]，まずはその効果を見定める。特に，悲哀感に乏しく，自発性や意欲が低下するアパシーに対しては抗うつ薬は無効なことが多く，AChEI の方が有効であり，またドパミン伝達系を賦活する塩酸アマンタジンやプラミペキソールが有効な場合がある。それらで効果がみられない場合，抗うつ薬を試みる。副作用が少ないという点から SSRI が推奨され，Lyketsos らはサートラリン，Mizukami らは，セロトニン・ノルアドレナリン再取込み阻害剤（Serotonin-Norepinephrine Reuptake Inhibitor : SNRI）であるミルナシプランの有効性を各々報告している。

3) 睡眠障害に対する薬物療法

短時間作用型で筋弛緩作用の少ない睡眠導入剤が第一選択であり，ブロチゾラム，ゾルピデム，ゾピクロンなどが使いやすい。メラトニン製剤であるラメルテオンは，ふらつきの副作用が少なく，高齢者に比較的安全に使用できる。睡眠薬を 2-3 種類併用しても効果がない患者に対する併用薬として，クエチアピンや抗うつ薬（トラゾドン，ミアンセリン，ミルタザピン）などの少量処方の有効性が指摘されている。

3．BPSD 対応の今後の展望

平成 24 年，厚生労働省は，「今後の認知症施策の方向性」として具体的な対応策を打ち出したが，その中で「これまでのケアは認知症の人が BPSD 等により危機が発生してからの事後的な対応が主眼となっていたが，今後は新たに早