α-Synuclein Aggregation and Transmission Are Enhanced by Leucine-Rich Repeat Kinase 2 in Human Neuroblastoma SH-SY5Y Cells

Kazunari Kondo,* Saemi Obitsu, and Reiko Teshima

National Institute of Health Sciences, Division of Novel Foods and Immunochemistry; 1–18–1 Kamiyoga, Setagaya-ku, Tokyo 158–8501, Japan. Received February 16, 2011; accepted March 30, 2011; published online April 14, 2011

Formation of α-synuclein aggregates is a key step in Parkinson's disease pathogenesis although the etiology remains elusive. α-Synuclein is accumulated in degenerating neurons, leading to the production of filamentous inclusions such as Lewy bodies. However, the *in vitro* overexpression of α-synuclein alone failed to induce inclusion bodies consisting of phosphorylated α-synuclein. The seeded aggregates-initiated polymerization of α-synuclein and tau has been reported elsewhere. What molecule is an initiator of filamentous inclusions remains to be defined. Here, we report that leucine-rich repeat kinase 2 (LRRK2)-cotransfection together with α-synuclein enhance the aggregate formation, phosphorylation, release to extracellular media of α-synuclein, and the cell-to-cell transmission into neighboring cells in human neuroblastoma SH-SY5Y cells. In cells transfected with α-synuclein alone, the proteins were distributed in the cytosol and did not form inclusions. On the other hand, the inclusions and phosphorylation of α-synuclein were formed in cells cotransfected with α-synuclein and LRRK2 G2019S mutant together. LRRK2 G2019S-cotransfected PC12 cells also induced the aggregates. Furthermore, the cell-to-cell transmission of α-synuclein and the cell toxicity were also enhanced by either LRRK2 wild type or G2019S mutant, whereas the cell viability was not decreased in cells transfected with α-synuclein alone. These results suggest that overexpression of LRRK2, especially G2019S mutant, whose functions remain unclear, initiate the aggregate formation, release and transmission of α-synuclein, resulting in the propagation of α-synuclein to neighboring cells and reduction of cell viability.

Key word α-synuclein; leucine-rich repeat kinase-2; aggregation; cell-to-cell transmission

Parkinson disease (PD) is the second most common neurodegenerative disease. The muscle rigidity, tremor, and bradykinesia that are characteristic of PD patients, are caused by dopaminergic neuron death in the substantia nigra. One of the well-known pathological hallmarks inside the cells is the presence of inclusion bodies called Lewy bodies (LB) that include aggregates of α-synuclein. Since α-synuclein and leucine-rich repeat kinase 2 (LRRK2) cause familial forms of PD that resembles sporadic PD pathologically, these genetic mutations provide important molecular tools to investigate PD pathogenesis.[1] Although the etiology of PD remains unclear, the production of aggregated α-synuclein is a key step in PD pathogenesis.[2]

α-Synuclein plays an important role in PD pathology and neuronal cell death.[3—5] Point mutations (A30P, E46K, and A53T) and multiplication of the gene α-synuclein are linked to the early-onset of PD pathology. The increased severity of PD and earlier age of onset have been reported to correlate with increased α-synuclein dosage.[6] The physiological function of α-synuclein found in pre-synapus remains undefined. Several studies have indicated that α-synuclein regulates intracellular transport of synaptic vesicles underlying neurotransmitter release.[7—14] α-Synuclein may also be involved in mitochondrial complex I function,[15,16] and has been reported to impair macroautophagy.[17]

Until recently, α-synuclein was considered to exert pathogenic effects inside the cells. However, α-synuclein can be detected in human cerebrospinal fluid (CSF) and plasma.[18] The accumulation of aggregated α-synuclein spreads from lower brainstem into the limbic system and neocortex, suggesting a mechanism underlying pathological propagation of PD such as Prion diseases.[19] Some groups have reported that aggregated α-synuclein can be released into extracellular media by exocytosis through exosomes and propagated by direct neuron-to-neuron transmission.[20—22]

LRRK2 is a large 2527 amino acid protein consisting of several functional domains including a Ras-like small GTPase domain (ROC), a carboxy-terminal of Roc (COR) domain, and a kinase domain. The various mutations in LRRK2 are involved in PD, such as R1441C, R1441G in the Roc domain, Y1699C in the COR domain, and G2019S, I2020T in the kinase domain. Among them, G2019S mutation clearly increases kinase activity, which is required for PD pathology.[23,24] G2019S mutation has been shown to increase kinase activity by 2 to 3 fold. However, normal function and kinase substrates of LRRK2 remain unclear. Although mutant LRRK2 was toxic when overexpressed in cultured cells[25,26] and *Drosophila*,[27,28] loss of neurons was not observed in transgenic mice overexpressing R1441G and R1441C mutants.[29,30] Loss of LRRK2 did not cause neurodegeneration and neuropathological changes. LRRK2 mutations cause clinically typical PD features, ranging from nigral degeneration without LB to nigral degeneration with widespread LB or neurofibrillary tangles.[31]

The neuronal cell death through neuron-to-neuron transmission of α-synuclein has been reported using mouse cortical neuron stem cells.[22] Coexpression of LRRK2 with A53T mutant of α-synuclein causes synergistic toxicity to neurons that accelerate the progression of α-synuclein-mediated pathology.[29] However, the underlying mechanism has not been yet defined. Thus, we have investigated the aggregate formation and cell-to-cell transmission of α-synuclein and the toxicity in the presence of LRRK2 wild type (WT) and G2019S mutant.

MATERIAL AND METHODS

Materials The antibody (Ab) for α-synuclein (mouse

* To whom correspondence should be addressed. e-mail: kondo@nihs.go.jp
© 2011 Pharmaceutical Society of Japan

immunoglobulin G_1 (IgG_1)) was purchased from BD Biosciences. Anti-phospho α-synuclein (clone #64) was obtained from Wako Pure Chemical (Japan). Anti-β-actin was purchased from Santa Cruz Biotechnology. Extracellular signal-regulated kinase 1/2 (ERK1/2) was purchased from Cell Signaling. Anti-LRRK2 (C-terminal region) was obtained from Sigma-Aldrich. MitoTracker Red CMX-H_2Ros to stain mitochondria in live cells and Lipofectamin 2000 were purchased from Invitrogen. cDNA constructs for wild type α-synuclein, α-synuclein A53T mutant, and enhanced green fluorescent protein (EGFP)-α-synuclein were kindly gifted by Dr. Sang Myun Park (Ajou University School of Medicine, Korea). c-Myc-tagged (2×myc) LRRK2 WT, G2019S, and kinase dead (KD) were kindly gifted by Dr. Mark Cookson (NIH, Bethesda). All sequences of the plasmids were confirmed. Transfection was performed by the method of lipofection (Lipofectamin 2000, Invitrogen) or electroporation (Amaxa Nucleofactor II, Lonza).

Cell Culture Human neuroblastoma SH-SY5Y cells (American Type Culture Collection [ATCC], CRL-2266) were grown in Dulbecco's modified Eagle's medium (DMEM)/F-12 supplemented with 10% fetal calf serum (FCS) penicillin, and streptomycin (Invitrogen). The SH-SY5Y was induced to differentiate by the treatment with 10 μM all-*trans* retinoic acid (RA, Sigma-Aldrich) and maintained at 37 °C and 5% CO_2. PC12 (JCRB0266) cells were grown in DMEM supplemented with 10% horse serum and 5% FCS, penicillin, and streptomycin (Invitrogen). The cell viability was measured by a WST-8 assay (Nacalai Tesque, Japan). Briefly, SH-SY5Y cells were transfected and differentiated by RA for 24 h to 8 d for cell viability assay. At the indicated times, the cell count reagent (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2*H*-tetrazolium) for the WST-8 assay was added to the cells, and the cells were incubated for 2—4 h. Cell viability was measured by 450 nm and 650 nm (as a reference) absorbance. Cell viability data were obtained from three independent experiments performed in triplicate.

For α-synuclein transmission experiments, cells were transfected with EGFP-α-synuclein with or without LRRK2 by electroporation (1×10^6 cells/electroporation), and cultured for 24 or 48 h in 24-well culture plates. At 24 h, the conditioned media were collected, centrifuged at 3000 ***g*** for 10 min to remove dead cells and cell debris. The supernatants were transferred to separately prepared SH-SY5Y cells in the differentiation condition. The cells were cultured for another 24 h to 8 d, and the cell viabilities were measured by WST-8 assay.

Western Blot Analysis SH-SY5Y cells were cultured at 9×10^5 cells/dish in 10-cm dishes in the differentiation condition following the transfection of α-synuclein with or without LRRK2. At 24 or 48 h-posttransfection, the cells were rinsed with Tris-buffered saline (TBS), and cell lysates were prepared using Triton-based lysis buffer containing protease and phosphatase inhibitors. The cell lysates were resolved by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (SDS/PAGE) on 5—20% gradient gels, and transferred onto polyvinylidene fluoride (PVDF) membranes (ATTO, Japan). The membranes were incubated with primary Abs at 4 °C overnight, and then with alkaline phosphatase (AP)-labeled secondary Abs for 1 h at room temperature. The blots were detected by an AP-conjugated substrate kit (BioRad), and the images were scanned and analyzed using Photoshop CS5. For the detection of α-synuclein in the conditioned media, chemiluminescent ECL system was used to detect the blots (ECL plus, GE Healthcare). The blots were analyzed using ImageQuant LAS-4000 (Fujifilm, Japan). Western blot analyses were performed on data from three independent experiments.

Immunofluorescent Staining SH-SY5Y cells on glass chamber slides were washed twice with DPBS before the cells were fixed with 4% paraformaldehyde (Wako, Japan) for 30 min and permeabilized with cold 0.2% Triton X-100 for 10 min. After blocking with 2% bovine serum albumin (BSA) for 1 h, the cells were incubated with primary Abs against α-synuclein, phosphor-α-synuclein, LRRK2, ERK, or β-actin at 4 °C overnight. MitotrackerRed CMX-H_2Ros was used to stain mitochondria of the live cells. The cells were washed and incubated with Alexa 488- or Alexa 555-labeled secondary Abs (Invitrogen) and Hoechst 33342 (Invitrogen) at room temperature for 1 h. After washing, the cells were mounted with Prolong gold mounting media (Invitrogen). Fluorescent microscopy was performed using an IX71 microscope (Olympus, Japan) and CarlZeiss LSM-Pascal (Germany). Immunofluorescent staining data were obtained from more than three independent experiments.

Transfection of α-Synuclein and LRRK2 SH-SY5Y cells (1×10^6 cells/cuvette) were transfected with α-synuclein, α-synuclein A53T, or EGFP-α-synuclein (1.5 μg cDNA each), and LRRK2 (1.5 μg cDNA) using Amaxa (program #A023, Lonza, Switzerland). The transfected cells were seeded to 10-cm culture dishes for Western blotting or 24-well culture plates for immunofluorescence and cell viability assay, and cultured for 24 to 8 d in the differentiation condition. PC12 cells were seeded at 0.25×10^5 cells/ml into a polylysine-coated 24 well plate (BD Biosciences). The cells were transfected using cDNA (0.5 μg each) and lipofectamine 2000 (Invitrogen) in OPTI-MEM (Invitrogen), and cultured for 24 h. Then, the cells were differentiated with nerve growth factor (NGF) (50 ng/ml), and cultured for another 4 d.

Statistical Analysis All data are expressed as the mean±standard deviation (S.D.). The statistical significance was evaluated by one-way analysis of variance (ANOVA) followed by the Dunnett's test to compare the data from multiple groups against a common control group. Student's *t*-test was used to compare the data from two groups (SigmaPlot). Statistical significance was determined at $p<0.05$ (indicated with an asterisk in the figures).

RESULTS

Formation of α-Synuclein Aggregates and Cell-to-Cell Transmission SH-SY5Y cells were transfected with wild type (WT) α-synuclein fused with EGFP by lipofection or electroporation in the proliferating or differentiation conditions, respectively. In proliferating cells, EGFP-α-synuclein was distributed in the cytoplasm 24 h after lipofection. By 48 h, the α-synuclein-containing vesicles were increased around the cells. A larger amount of vesicles was observed on the surface and outside of the cells 72 h after transfection (Fig. 1A). In the differentiation condition, α-synuclein-containing vesicles were not produced in the cells transfected

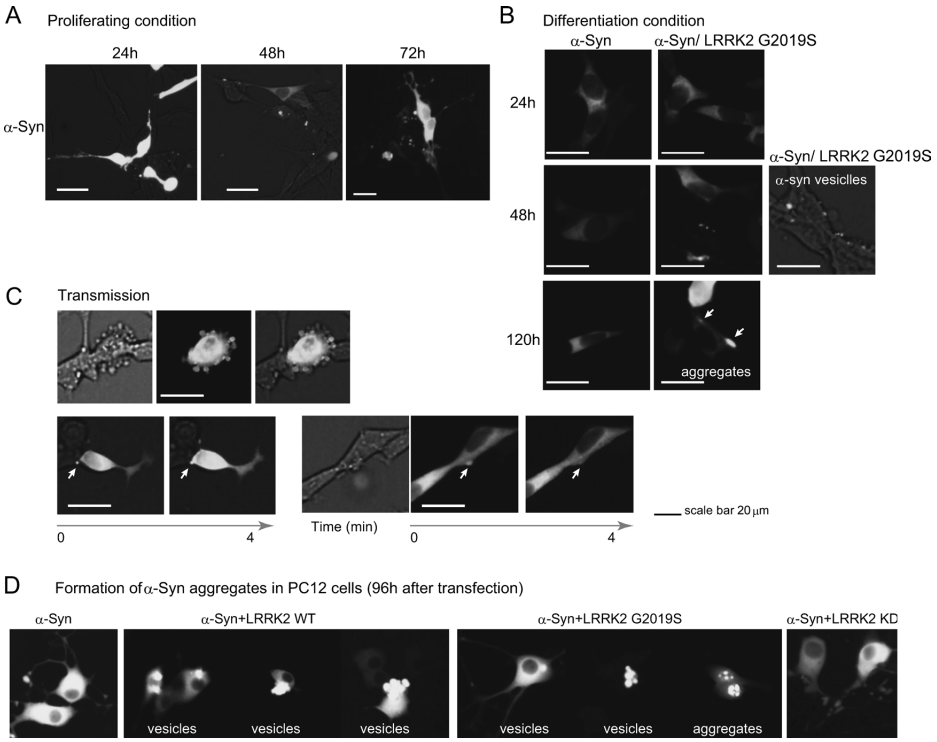

Fig. 1. Aggregate Formation and Vesicle Movement of α-Synuclein

SH-SY5Y cells were transfected with α-synuclein alone or with LRRK2 G2019S mutant in the proliferating and differentaion conditions. (A) α-Synuclein was localized in the cytoplasm in α-synuclein-transfected cells. (B) α-Synuclein was distributed in the cytoplasm, and many vesicles were formed and on the surface of neighboring cells within 48 h after transfection in RA-differentiated cells. In the presence of LRRK2 G2019S, α-synuclein aggregates were produced inside the cells after 5 d-culture (white arrow). (C) α-Synuclein-containing vesicles were moved into neighboring cells by cell contact (bottom left) or direct transmission through the conditioned media (bottom right). (D) Similar experiments were performed using the differentiated PC12 cells. LRRK2 WT-cotransfected cells have large vesicles containing α-synuclein, which appear to be effluxed from the cells. Cells cotransfected with LRRK2 G2019S did have the aggregates of α-synuclein.

with α-synuclein alone, whereas the vesicles and aggregates were formed in the cells cotransfected with α-synuclein and LRRK2 G2019S (Fig. 1B). The aggregated α-synuclein was also observed in RA-differentiated SH-SY5Y cells. The large aggregates were formed inside the cell 120 h after cotransfection of α-synuclein and LRRK2 G2019S mutant (Fig. 1B, white arrow). Since the aggregated proteins and vesicles of α-synuclein were found inside and on the surface of the cells, transmission of α-synuclein from one cell to neighboring cells was next investigated in the differentiated SH-SY5Y cells. A single EGFP-labeled α-synuclein was moving toward the neighboring cell or through the conditioned media, and appeared to be internalized on the cell surface (Fig. 1C). Many vesicles were exocytosed through plasma membrane (Fig. 1C, top). For further studies, we used rat adrenal pheochromocytoma PC12cells to evaluate the effect of LRRK2 on α-synuclein aggregation. In cells cotransfected with α-synuclein and LRRK2 WT, large vesicles releasing from the cells at the very moment were observed at 96 h posttransfection. Although similar vesicles containing α-synuclein were seen in PC12 cells cotransfected with LRRK2 G2019S mutant, distinct aggregates were also observed in the nucleus. In contrast, LRRK2 KD-transfected cells did not have any large vesicles and aggregates.

Taken together, α-synuclein-containing vesicle formation was enhanced by LRRK2 WT or G2019S mutant. α-synuclein aggregates were induced only by LRRK2 G2019S mutant.

α-Synuclein Transmission Is Enhanced in the Presence of LRRK2 G2019S Mutant Next, we investigated the effect of LRRK2 G2019S mutant on α-synuclein transmission. α-Synuclein alone or with LRRK2 WT or G2019S mutant was transfected into SH-SY5Y cells by electroporation, and cultured for 48 h in the differentiation condition. The supernatant of the conditioned media collected from the transfected cells was added to separately prepared and RA-differentiated SH-SY5Y cells without any concentration. Then, the cells were incubated for another 24—48 h to observe the expression of α-synuclein in the untransfected cells (Fig. 2A). The ratio of α-synuclein-expressed cells to total untransfected cells in the area that includes 300 cells was measured. Approximately 5% of cells were GFP-positive ($4.7\pm1.2\%$) when the conditioned media from α-synuclein-transfected cells was transferred into the separately prepared cells, whereas 11% of cells were GFP-positive ($10.7\pm2.1\%$) when the media from cells cotransfected with α-synuclein and LRRK2 G2019S was used as shown in Fig. 2A. The number of GFP-positive cells in the presence of LRRK2 G2019S mutant was greater than that in the absence of LRRK2 mutant.

To confirm that α-synuclein protein was internalized by the separately prepared cells, separately prepared and RA-differentiated SH-SY5Y cells were labeled with MitotrackerRed CMX-H_2Ros in advance, which is sensitive to mitochondrial membrane potential. α-Synuclein was indeed incorporated into the untransfected cells (Fig. 2B). The expression of α-synuclein was increased in a time-dependent manner, and there was no difference in α-synuclein expression between α-synuclein wild type and A53T mutant at 24 and 48 h (Fig. 2C). α-Synuclein expression was increased in

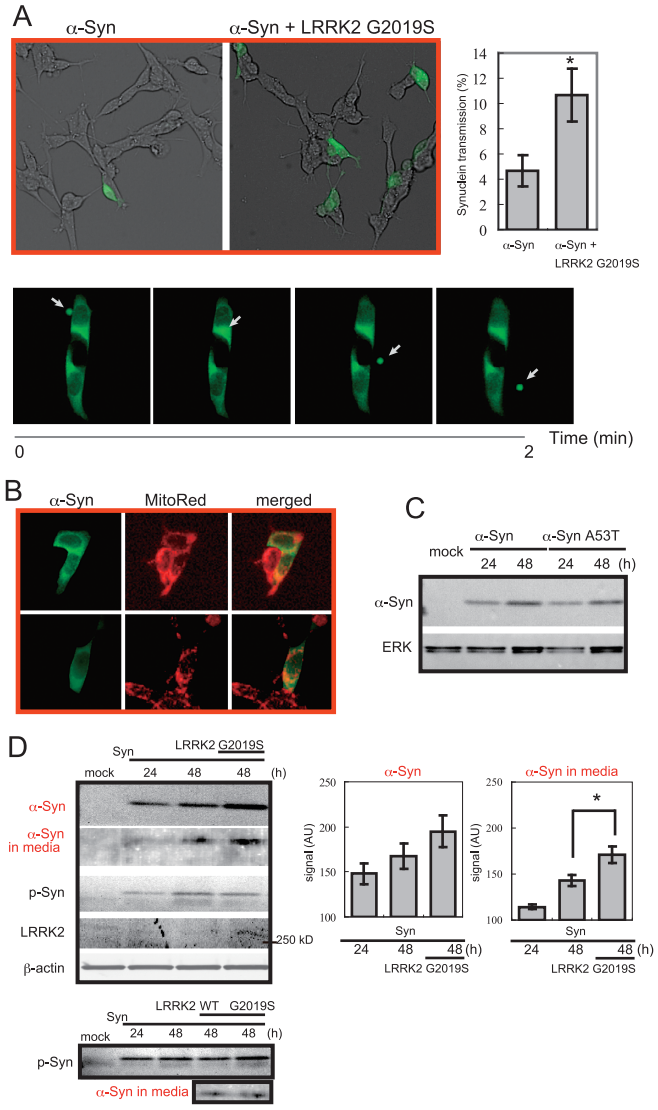

Fig. 2. α-Synuclein Transmission Is Enhanced by LRRK2

The conditioned media from α-synuclein-transfected cells in the presence or absence of LRRK2 were added to the separately prepared cells, and cultured for another 24 h to clarify whether α-synuclein proteins are incorporated into neighboring cells through the conditioned media. (A) The untransfected SHSY5Y cells were cultured in the conditioned media collected from α-synuclein-transfected cells. Transmission of α-synuclein from α-synuclein-transfected cells to separately prepared and untransfected cells was enhanced by LRRK2 G2019S mutant. The ratio of transmission in the presence of LRRK2 was greater than that in the absence of LRRK2. Three hundred cells from different wells of a 24-well culture plate were counted in each experiment. The data were obtained from three independent experiments ($n=3$, $*p<0.05$). One α-synuclein-containing vesicle is moving and crossing over the cells in the conditiond media. (B) α-Synuclein proteins were incorporated into the untransfected and MitotrackerRed-labeled cells, showing the transmission through the media. Two different fields were shown here. (C) α-Synuclein was expressed in the α-synuclein-transfected cells in a time-dependent manner. There was no difference between α-synuclein wild type and A53T mutant in the expression. (D) α-Synuclein expression inside the cells and in the conditioned media in the presence of LRRK2 WT and G2019S. A larger amount of α-synuclein was detected in the conditioned media from LRRK2 G2019S-expressing cells. There was no difference between LRRK2 WT and G2019S-expressing cells. The amounts of phosphorylated α-synuclein were not significant in transfected cells. The data were obtained from two to three independent experiments ($n=2$ or 3). Representatives were shown in Western blots.

cells cotransfected with LRRK2, but not significant (Fig. 2D). The expression of α-synuclein was also confirmed in the conditioned media from SH-SY5Y cells transfected with α-synuclein alone or with LRRK2 WT or G2019S. α-Synuclein was detected in the media within 24 h and increased with the time (Fig. 2D). LRRK2 G2019S mutant did significantly enhance the α-synuclein release into the conditioned media. However, the amount of α-synuclein in the conditioned media was not significantly different between LRRK2 WT and G2019S mutant. The phosphorylation state was not different between the two groups either (Fig. 2D, bottom).

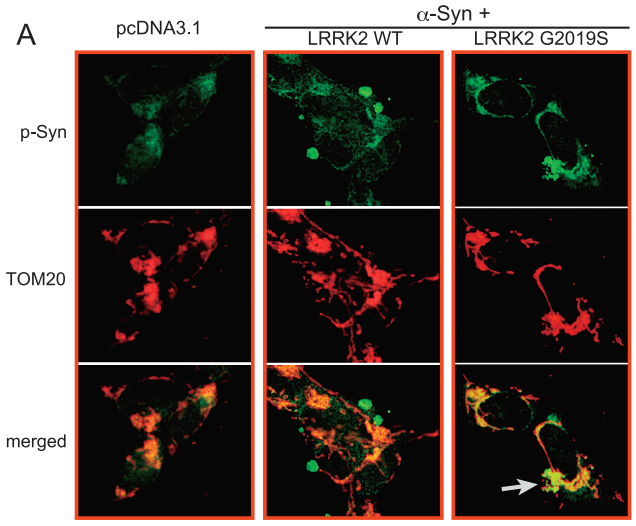

Fig. 3. α-Synuclein Localization and Its Phosphorylation

α-Synuclein (green) aggregates were phosphorylated and localized in mitochondria (red) in LRRK2 G2019S-trasnfected cells (white arrow). Large vesicles of α-synuclein were phosphorylated but not localized in LRRK2 WT-transfected cells. Cells transfected with pcDNA 3.1 did not have any aggregates and vesicles.

Aggregated α-Synuclein Is Phosphorylated and Localized in Mitochondria We next examined the phosphorylation state and its localization of aggregated α-synuclein by staining with a mitochondria outer-membrane marker TOM20. As shown in Fig. 3, most of the aggregated α-synuclein were phosphorylated and colocalized with mitochondria in the differentiated SH-SY5Y cells coexpressing α-synuclein and LRRK2 G2019S mutant (white arrow). The phosphorylated α-synuclein-containing vesicles were seen in LRRK2 WT-transfected cells, but not colocalized with mitochondria. The pcDNA3.1-transfected cells did not have any vesicles and aggregates.

Cell Viabilities in α-Synuclein and LRRK2-Cotransfected Cells The cell viabilities of SH-SY5Y cells cotransfected with α-synuclein and LRRK2 wild type or LRRK2 G2019S mutant were investigated. When the cells were transfected with α-synuclein alone, the viability was not significantly different from that of the cells treated with pcDNA3.1 as a control. Like wild type, α-synuclein A53T mutant alone did not show the decrease in the cell viability during 8 d-culture. These results suggest that α-synuclein alone do not lead to the cell death for several days (Fig. 4). However, when the cells were cotransfected with α-synuclein and LRRK2 WT or G2019S mutant, the viabilities decreased by 12%, suggesting that LRRK2 enhance the cell toxicity by α-synuclein (Fig. 4). In addition, the cell toxicity was not observed in LRRK2 G2019S-mutant transfected SH-SY5Y. These results indicate that LRRK2 synergistically reduce the cell viability of α-synuclein-tranfected cells.

DISCUSSION

Until several years ago, α-synuclein was considered an

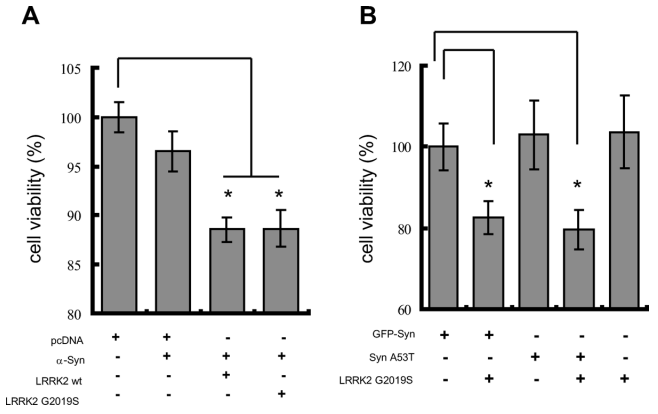

Fig. 4. Cell Viability of the Cells Transfected with α-Synuclein Alone or with LRRK2

(A) Cell viability was significantly decreased in the presence of LRRK2 WT or G2019S. There is no difference between WT and G2019S of LRRK2. (B) α-Synuclein WT and A53T mutant alone had no effect on the cell viability, whereas the viability was decreased in the presence of LRRK2 G2019S mutant. The data were three (A) and four (B) independent experiments in triplicates ($*p<0.05$).

intracellular protein and to function cell-autonomously. Recently, α-synuclein was reported to be present in human cerebrospinal fluid (CSF) and human plasma at a concentration of nanomolar in PD and normal subjects.[18,32] Two studies showed host-to-graft propagation of α-synuclein-positive Lewy-like pathology in long-term mesencephalic transplants in PD.[33,34] Most recently, cell-to-cell transmission of α-synuclein and the resulting cell death in neuronal cells has been demonstrated by Desplats *et al.*[22] In addition, Lin *et al.* has reported that LRRK2 enhances α-synuclein-mediated cytotoxicity.[29] Therefore, these results above now raise the question how LRRK2 give rise to the synergistic effect in cytotoxicity. Here, we have investigated the effect of α-synuclein aggregation and transmission in the presence of LRRK2 WT, G2019S, and KD.

α-Synuclein was distributed in the cytosol and many secreted vesicles including α-synuclein were observed outside and on the surface of SH-SY5Y cells by 48 h after transfection. The large aggregates were produced by 5 d in the cells cotransfected with α-synuclein and LRRK2 G2019 mutant, whereas such a large aggregate was not produced in the cells transfected with α-synuclein alone or with LRRK2 WT. The accumulation and propagation of amyloid β proteins are thought to occur through nucleation-dependent polymerization. Recently, the seed-dependent aggregate formation of α-synuclein has been reported using lipofection of seeded aggregation.[35] This may be an excellent model. However, the question where the first aggregates come from remains to be answered. We present here that LRRK2 G2019S initiates and enhances the formation of α-synuclein aggregates. Furthermore, vesicles including α-synuclein were transmitted to neighboring cells through the conditioned media especially in the presence of LRRK2. Intercellular transmission of exogenous protein aggregates has been well documented in Prion disorder. Similar mechanisms of pathological propagation have been suggested in other neurodegenerative diseases such as Alzheimer's and Polyglutamine diseases.[36,37] Extracellular tau aggregates also has been shown to induce the aggregation of intracellular protein.[38] Therefore, cell-to-cell transmission of aggregated proteins may be the common feature underlying progressive neurodegenerative diseases. Lin *et al.* has recently reported an important functional interplay between α-synuclein and LRRK2 in the development of neurodegeneration. LRRK2 accelerates the progression of neuropathological abnormality.[29] Our results were well consistent with this report. In the present study, larger amounts of α-synuclein were present in the conditioned media upon cotransfection of α-synuclein and LRRK2, compared to the amounts upon transfection of α-synuclein alone. These results suggest that LRRK2 enhance the α-synuclein release to extracellular media and the aggregate formation of α-synuclein. Tong *et al.* have recently reported that loss of LRRK2 causes the accumulation of α-synuclein only in kidney, not in neuronal cells.[30] The effect of LRRK2 might be dependent on cell types. We need to investigate how only LRRK2 G2019S initiates the aggregates for further study.

Here we show an interesting finding that LRRK2 G2019S enhances aggregate formation and transmission of α-synuclein and the cell toxicity.

Acknowledgements This work was supported by Grant from the Japan Health Sciences Foundation (KHB1201 to R. T.) and Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (20590132 to K. K.).

REFERENCES

1) Hardy J., *Mov. Disord.*, **2006**, 1790—1791 (2006).
2) Trojanowski J. Q., Goedert M., Iwatsubo T., Lee V. M.-Y., *Cell Death Differ.*, **5**, 832—837 (1998).
3) Dawson T. M., Dawson V. L., *Science*, **302**, 819—822 (2003).
4) Shults C. W., *Proc. Natl. Acad. Sci. U.S.A.*, **103**, 1661—1668 (2006).
5) Lees A. J., Hardy J., Revesz T., *Lancet*, **373**, 2055—2066 (2009).
6) Ross O. A., Braithwaite A. T., Skipper L. M., Kachergus J., Hulihan M. M., Middleton F. A., Nishioka K., Fuchs J., Gasser T., Maraganore D. M., Adler C. H., Larvor L., Chartier-Harlin M. C., Nilsson C., Langston J. W., Gwinn K., Hattori N., Farrer M. J., *Ann. Neurol.*, **63**, 743—750 (2008).
7) Abeliovich A., Schmitz Y., Farinas I., Choi-Lundberg D., Ho W. H., Castillo P. E., Shinsky N., Verdugo J. M., Armanini M., Ryan A., Hynes M., Phillips H., Sulzer D., Rosenthal A., *Neuron*, **25**, 239—252 (2000).
8) Ben Gedalya T., Loeb V., Israeli E., Altschuler Y., Selkoe D. J., Sharon R., *Traffic*, **10**, 218—234 (2009).
9) Cabin D. E., Shimazu K., Murphy D., Cole N. B., Gottschalk W., Mc Ilwain K. L., Orrison B., Chen A., Ellis C. E., Paylor R., Lu B., Nussbaum R. L., *J. Neurosci.*, **22**, 8797—8807 (2002).
10) Cooper A. A., Gitler A. D., Cashikar A., Haynes C. M., Hill K. J., Bhullar B., Liu K., Xu K., Strathearn K. E., Liu F., Cao S., Caldwell K. A., Caldwell G. A., Marsischky G., Kolodner R. D., Labaer J., Rochet J. C., Bonini N. M., Lindquist S., *Science*, **313**, 324—328 (2006).
11) Murphy D. D., Rueter S. M., Trojanowski J. Q., Lee V. M., *J. Neurosci.*, **20**, 3214—3220 (2000).
12) Nemani V. M., Lu W., Berge V., Nakamura K., Onoa B., Lee M. K., Chaudhry F. A., Nicoll R. A., Edwards R. H., *Neuron*, **65**, 66—79 (2010).
13) Sousa V. L., Bellani S., Giannandrea M., Yousuf M., Valtorta F., Meldolesi J., Chieregatti E., *Mol. Biol. Cell*, **20**, 3725—3739 (2009).
14) Yavich L., Tanila H., Vepsäläinen S., Jäkälä P., *J. Neurosci.*, **24**, 11165—11170 (2004).
15) Devi L., Raghavendran V., Prabhu B. M., Avadhani N. G., Anandatheerthavarada H. K., *J. Biol. Chem.*, **283**, 9089—9100 (2008).
16) Loeb V., Yakunin E., Saada A., Sharon R., *J. Biol. Chem.*, **285**, 7334—7343 (2010).
17) Winslow A. R., Chen C. W., Corrochano S., Acevedo-Arozena A., Gordon D. E., Peden A. A., Lichtenberg M., Menzies F. M., Ravikumar B., Imarisio S., Brown S., O'Kane C. J., Rubinsztein D. C., *J. Cell*

Biol., **190**, 1023—1037 (2010).
18) El-Agnaf O. M., Salem S. A., Paleologou K. E., Cooper L. J., Fullwood N. J., Gibson M. J., Curran M. D., Court J. A., Mann D. M., Ikeda S., Cookson M. R., Hardy J., Allsop D., *FASEB J.*, **17**, 1945—1947 (2003).
19) Frost B., Diamond M. I., *Nat. Rev. Neurosci.*, **11**, 155—159 (2010).
20) Emmanouilidou E., Melachroinou K., Roumeliotis T., Garbis S. D., Ntzouni M., Margaritis L. H., Stefanis L., Vekrellis K., *J. Neurosci.*, **30**, 6838—6851 (2010).
21) Lee H. J., Patel S., Lee S. J., *J. Neurosci.*, **25**, 6016—6024 (2005).
22) Desplats P., Lee H. J., Bae E. J., Patrick C., Rockenstein E., Crews L., Spencer B., Masliah E., Lee S. J., *Proc. Natl. Acad. Sci. U.S.A.*, **106**, 13010—13015 (2009).
23) Cookson M. R., Dauer W., Dawson T., Fon E. A., Guo M., Shen J., *J. Neurosci.*, **27**, 11865—11868 (2007).
24) Cookson M. R., *Nat. Rev. Neurosci.*, **11**, 791—797 (2010).
25) Greggio E., Jain S., Kingsbury A., Bandopadhyay R., Lewis P., Kaganovich A., van der Brug M. P., Beilina A., Blackinton J., Thomas K. J., Ahmad R., Miller D. W., Kesavapany S., Singleton A., Lees A., Harvey R. J., Harvey K., Cookson M. R., *Neurobiol. Dis.*, **23**, 329—341 (2006).
26) Smith W. W., Pei Z., Jiang H., Dawson V. L., Dawson T. M., Ross C. A., *Nat. Neurosci.*, **9**, 1231—1233 (2006).
27) Imai Y., Gehrke S., Wang H. Q., Takahashi R., Hasegawa K., Oota E., Lu B., *EMBO J.*, **27**, 2432—2443 (2008).
28) Liu Z., Wang X., Yu Y., Li X., Wang T., Jiang H., Ren Q., Jiao Y., Sawa A., Moran T., Ross C. A., Montell C., Smith W. W., *Proc. Natl. Acad. Sci. U.S.A.*, **105**, 2693—2698 (2008).
29) Lin X., Parisiadou L., Gu X. L., Wang L., Shim H., Sun L., Xie C., Long C. X., Yang W. J., Ding J., Chen Z. Z., Gallant P. E., Tao-Cheng J. H., Rudow G., Troncoso J. C., Liu Z., Li Z., Cai H., *Neuron*, **64**, 807—827 (2009).
30) Tong Y., Yamaguchi H., Giaime E., Boyle S., Kopan R., Kelleher R. J. 3rd, Shen J., *Proc. Natl. Acad. Sci. U.S.A.*, **107**, 9879—9884 (2010).
31) Tong Y., Shen J., *Neuron*, **64**, 771—773 (2009).
32) Borghi R., Marchese R., Negro A., Marinelli L., Forloni G., Zaccheo D., Abbruzzese G., Tabaton M., *Neurosci. Lett.*, **287**, 65—67 (2000).
33) Li J. Y., Englund E., Holton J. L., Soulet D., Hagell P., Lees A. J., Lashley T., Quinn N. P., Rehncrona S., Björklund A., Widner H., Revesz T., Lindvall O., Brundin P., *Nat. Med.*, **14**, 501—503 (2008).
34) Kordower J. H., Chu Y., Hauser R. A., Freeman T. B., Olanow C. W., *Nat. Med.*, **14**, 504—506 (2008).
35) Nonaka T., Watanabe S. T., Iwatsubo T., Hasegawa M., *J. Biol. Chem.*, **285**, 34885—34898 (2010).
36) Frost B., Diamond M. I., *Nat. Rev. Neurosci.*, **11**, 155—159 (2010).
37) Caughey B., *Nat. Med.*, **6**, 751—754 (2000).
38) Frost B., Jacks R. L., Diamond M. I., *J. Biol. Chem.*, **284**, 12845—12852 (2009).