**Down-regulation of Mortalin Exacerbates Aβ-mediated Mitochondrial Fragmentation and Dysfunction**

Received for publication, June 13, 2013, and in revised form, December 3, 2013 Published, JBC Papers in Press, December 9, 2013, DOI 10.1074/jbc.M113.492587

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**Background:** Mitochondrial dysfunction is associated with neuronal disorders, and mitochondrial dynamics are altered in neurodegenerative diseases.

**Results:** Inhibition of mortalin potentiates amyloid-β-mediated mitochondrial dysfunction and cytotoxicity.

**Conclusion:** Inhibition of mortalin could lead to mitochondrial dysfunction through mitochondrial fragmentation.

**Significance:** Activation of mortalin may antagonize the progression of Aβ-mediated neuronal injury in which mitochondrial dysfunction has a key role.

Mitochondrial dynamics greatly influence the biogenesis and morphology of mitochondria. Mitochondria are particularly important in neurons, which have a high demand for energy. Therefore, mitochondrial dysfunction is strongly associated with neurodegenerative diseases. Until now various post-translational modifications for mitochondrial dynamic proteins and several regulatory proteins have explained complex mitochondrial dynamics. However, the precise mechanism that coordinates these complex processes remains unclear. To further understand the regulatory machinery of mitochondrial dynamics, we screened a mitochondrial siRNA library and identified mortalin as a potential regulatory protein. Both genetic and chemical inhibition of mortalin strongly induced mitochondrial fragmentation and synergistically increased Aβ-mediated cytotoxicity as well as mitochondrial dysfunction. Importantly we determined that the expression of mortalin in Alzheimer disease (AD) patients and in the triple transgenic-AD mouse model was considerably decreased. In contrast, overexpression of mortalin significantly suppressed Aβ-mediated mitochondrial fragmentation and cell death. Taken together, our results suggest that down-regulation of mortalin may potentiate Aβ-mediated mitochondrial fragmentation and dysfunction in AD.

Mitochondria, essential organelles for both life and death, are highly dynamic. They continuously undergo balanced fusion and fission processes, which are termed mitochondrial dynamics. Mitochondrial dynamics greatly affect the mitochondrial functions such as biogenesis as well as their morphology (1, 2). Imbalanced mitochondrial dynamics are directly linked to many human diseases including cancer, diabetes, and neurodegenerative diseases (3–5). Neurons are particularly dependent on mitochondrial function because of their higher metabolic activity and complex morphology (6). Mitochondria are pivotal for synaptic plasticity and the primary producers of reactive oxygen species (ROS), which contribute to mitochondrial dysfunction. Thus, disruptions of mitochondrial function and mitochondrial dynamics are prominent early events in neurodegenerative diseases such as Alzheimer disease (AD), Parkinson disease (PD), Huntington disease, and amyotrophic lateral sclerosis (1, 6). Both the treatment of amyloid-β (Aβ) and the overexpression of either APP (Aβ precursor protein) or APPsw mutant efficiently induce mitochondrial fragmentation and synaptic injury in neuronal cells (7–9).

Mitochondrial fission and fusion processes are regulated by evolutionarily conserved molecular machinery. The large GTPase proteins, MFN1/2 (mitofusin-1/-2) and Opa1 (optic atrophy type 1) assists in the mitochondrial fusion process. Another GTPase protein, Drp1 (dynamin-related protein 1) promotes mitochondrial fission by interacting with mitochondrial outer membrane proteins such as Fis1 and mitochondrial fission factor. Loss-of-function mutations of MFN2 and Opa1 are directly linked to neurodegenerative diseases such as Charcot-Marie-Tooth subtype 2A and autosomal dominant optic atrophy (10, 11). Additionally, a mutation of Drp1 identified in an infant with lethal abnormal brain development also emphasizes the importance of mitochondrial dynamics in neurons (12). As a regulatory mechanism, various post-translational modifications such as phosphorylation, nitrosylation, sumoylation, ubiquitination, or GlcNAcylation of Drp1, proteolytic cleavage of Opa1, and ubiquitination or phosphorylation of

*This work was supported by Korean Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea Grant A092042 and the Korea-UK Collaborative Alzheimer’s Disease Research Project, Ministry of Health & Welfare, Republic of Korea Grant A120196.

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MFN1/2 have explained the intricate mechanisms of mitochondrial dynamics in different signals (9, 13–27). Moreover, several other regulatory proteins have been identified. Knockdown of mitochondrial fission factor, GDA1 (ganglioside-induced differentiation-associated protein-1), MiD49/51 (mitochondrial dynamics protein 49/51), endophilin B1, or MTP18 (mitochondrial protein 18 kDa) resulted in elongated mitochondria, suggesting that these proteins are involved in the mitochondrial fragmentation processes (28–31). On the other hand, inhibition of prohibitin-2, SLP2 (stomatin-like protein 2), and mitofusin-binding protein promotes mitochondrial fragmentation, indicating that these proteins regulate the mitochondrial fusion process (32–34). Nonetheless, the precise mechanism that coordinates these complex processes of mitochondrial dynamics still remains unclear.

To further understand the regulatory machinery of mitochondrial dynamics, we established a cell-based functional screening system that identified mortalin as a potential regulatory molecule from siRNA library screening. The suppression of mortalin highly induced mitochondrial fragmentation and the suppression synergistically increased Aβ-mediated mitochondrial dysfunctions and cell death. However, up-regulation of mortalin remarkably reduced Aβ-mediated mitochondrial fragmentation and cytotoxicity. Importantly, we found that the expression level of mortalin was reduced in AD patients and in the AD-model mice. Taken together, our results suggest that down-regulation of mortalin exacerbates Aβ-mediated mitochondrial fragmentation and dysfunction in AD.

EXPERIMENTAL PROCEDURES

Cell Culture and Measurement of Mitochondrial Length—SK-N-MC and SH-SY5Y neuroblastoma cells were obtained from the American Type Culture Collection (ATCC). Drp1-deficient mouse embryo fibroblast (MEF) cells were generously provided by Dr. Katsuyoshi Mihara (Kyushu University, Japan) (35). All cells were cultured at 37 °C in a 5% CO2 incubator and provided by Dr. Renu Wadhwa (National Institute of Advanced Industrial Science and Technology, Japan) (37, 38). Amyloid-β (Aβ) was purchased from American Peptide Co. (Sunnyvale, CA). A MitoTracker® probe and Hoechst 33342 dye were purchased from Invitrogen. The validated siRNA targeting for mortalin (number 1, 5’-GCUUACUUCGUUUAUUGCU-3’ and number 2, 5’-AAACCGAAGUGGA-AUUUA-3’) and negative scrambled siRNA (5’-CCUACGC-CACCAUUUCGU-3’) were purchased from Dharmacon (Thermo Scientific) and previously validated Drp1 siRNA (5’-GAGGUUAUGAAGCAUCUA-3’) and Opal siRNA (5’-CUG-GAAAGACAGUGUGU-3’) were synthesized from Bioneer (Daejeon, Korea) (39).

Cell-based Functional Screening with siRNA Library—For the siRNA screening, we listed mitochondrial proteins based on a mitochondrial protein database (MitoProteom database). Among ~850 genes, we collected around 500 target genes by exclusion of unknown genes. Using the collected genes, we synthesized a custom siRNA library/mitochondrial siRNA library using the Dharmacon siGENOME SMART pool system (Dharmacon, Thermo Scientific). SK/mito-YFP (SK-N-MC stably expressing mito-YFP) cells were seeded in 96-well plates at ~1500 cells/well. After 24 h, each siRNA was transiently transfected into the cells with a final concentration ~50 pmol. After 3 and 7 days, mitochondrial morphology was observed under a fluorescence microscope to screen mitochondria dynamics regulator. siRNAs for OPAL1 and Drp1 were used as positive controls for each experiment. The screening experiment was repeated two times with consistent results.

Western Blotting—For Western blotting, all lysates were prepared with protein sample buffer (62.5 mM Tris-HCl, pH 6.8, 25% glycerol, 2% SDS, 5% β-mercaptoethanol, 0.01% bromphenol blue) (Bio-Rad). Then the samples separated by SDS-PAGE were transferred to PVDF membrane (Bio-Rad). After blocking with 4% skim milk in TBST (25 mM Tris, 3 mM 140 mM NaCl, 0.05% Tween 20), the membranes were incubated overnight with specific primary antibodies at 4 °C. Anti-Drp1 antibody was from BD Biosciences (San Jose, CA); anti-mortalin antibody was from BD Biosciences; anti-Actin antibody was from Millipore (Temecula, CA). For protein detection, the membranes were incubated with HRP-conjugated secondary antibodies (Pierce).

ROS Measurement—Intracellular ROS levels were assayed using a fluorescent dye, dichloro-dihydro-fluorescein diacetate (DCFH-DA) (Invitrogen), which is converted to highly fluorescent 2’,7’-dichlorofluorescein (DCF) in the presence of oxidant. Briefly, cells plated in 96-well plate were transfected with siRNA. After 3 or 7 days from transfection, the cells were further treated with Aβ for 24 h. Then, the cells were incubated with DCFH-DA (20 μM) in serum-free medium for 30 min and measured the fluorescence (excitation/emission wavelength...
Measurement of Mitochondria Membrane Potential and ATP Level—Mitochondrial membrane potential was examined with a unique fluorescent cationic dye, JC-1 (5,5′,6,6′-tetramethylrhodamine carbocyanine iodide, BD Biosciences) that detects loss of signal of mitochondrial membrane potential. The fluorescence intensity was measured using plate reader (PerkinElmer Life Sciences) at excitation and emission wavelengths of 485 and 535 nm, respectively, for the monomeric form as well as 535 and 590 nm for J-aggregate forms, respectively. Images were obtained using IX71 (Olympus, Tokyo, Japan) fluorescence microscopes and the cellular total ATP level was detected with an ATP bioluminescence detection kit (Promega, Madison, WI) according to the manufacturer’s protocol.

Cell Proliferation Analysis—For the cell proliferation assay, cells seeded in 96-well plates were transfected with mortalin siRNA. After transfection, the cell proliferation rate was measured daily using a Cell Counting Kit-8 (CCK8) solution reagent (10 μl) (Dojindo Laboratories, Kumamoto, Japan) for 2 h. The absorbance was measured with spectrophotometer (Victor-X3, PerkinElmer Life Sciences).

Apoptotic Cell Death Analysis—Apoptotic cell death was determined using an Annexin V-FITC/PI Apoptosis Detection Kit (BD Pharmingen) according to the manufacturer’s protocol. Briefly, cells transfected with mortalin siRNA or treated with MKT077 were exposed to Aβ for 24 h. Then, the cells were stained with Annexin V-FITC and propidium iodide (BD Pharmingen). After staining, the cell death ratio was analyzed using a flow cytometer (BD Pharmingen).

Mouse and Human AD Samples—Triple transgenic AD mice (3xTgAD mice; swAPP, PS1-M146V, Tau-P301L) (40) that had been backcrossed to C57BL/6 mice for 8 generations were used for this study. All patients with AD met both clinical diagnostic criteria and neuropathological diagnostic criteria of AD (41, 42). The control subjects had no history or neuropathological signs of a brain disorder. At autopsy, tissue specimens were rapidly removed and frozen, and were stored at −80 °C.

Statistical Analysis—Data were obtained from least three independent experiments, and presented as mean ± S.E. Statistical evaluation of the results was performed with one-way analysis of variance. Data were considered significant at a value of p < 0.05.

RESULTS

Down-regulation of Mortalin Induces Drp1-dependent Mitochondrial Fragmentation and Dysfunction—It has been previously reported that there are ~850 proteins in mitochondria (43). For cell-based functional screening, we generated a mitochondrial siRNA library as described under “Experimental Procedures.” In addition, we also established a cell-based functional screening system using SK-N-MC cells that stably expressed the YFP-fused mitochondria tracker, mito-YFP (SK/mito-YFP). Initially to identify novel genetic modulators of mitochondrial dynamics, we screened the siRNA library. Both Drp1 siRNA and Opa1 siRNA were used as positive controls in each experiment. Based on the screening results, we identified mortalin as a potent regulator of mitochondrial dynamics (Table 1).

To confirm the screening results, SK/mito-YFP cells were transiently transfected with a scrambled or specific siRNA against mortalin. The results showed that down-regulation of mortalin induced pronounced fragmentation of mitochondria (Fig. 1, A–D). Because Drp1 is a key regulator in mitochondrial fission, we next examined the effects of Drp1 on mortalin-mediated mitochondrial fragmentation. Mortalin siRNA was co-transfected with either scrambled or Drp1 siRNA in SK/mito-YFP cells. The knockdown of Drp1 was confirmed by Western blot analysis. The results showed that reduced Drp1 expression completely suppressed mitochondrial fragmentation induced by mortalin knockdown (Fig. 1E). Mitochondrial dynamics regulate mitochondrial functions as well as their morphology. Excessive mitochondrial fragmentation induces mitochondrial dysfunctions. Therefore, we addressed the effect of mortalin knockdown on mitochondrial function. Because the electrochemical gradient of mitochondrial membrane is essential for ATP synthesis, we detected both mitochondrial membrane potential and total cellular ATP levels following mortalin knockdown. The suppression of mortalin expression markedly induced mitochondrial membrane depolarization but reduced the cellular ATP levels, suggesting that knockdown of mortalin disrupts the mitochondrial membrane potential in neuroblastoma cells (Fig. 2, A and B). In addition, the loss of mitochondrial membrane potential was associated with excessive ROS generation. Thus, we further elucidated mitochondrial dysfunction in mortalin down-regulated cells. The suppression of mortalin expression by their siRNAs resulted in enhanced ROS production in neuroblastoma cells, whereas ROS inhibitors significantly suppressed ROS production, which was induced by down-regulation of mortalin (Fig. 2, C and D). Moreover, we investigated the effect of mortalin knockdown on proliferation. The down-regulation of mortalin suppressed cell proliferation in neuroblastoma cells, respectively (Fig. 2E). Taken together, our data suggest that down-regulation of reduced mortalin
induces mitochondrial fragmentation and mitochondrial dysfunction.

**MKT077, a Mortalin Chemical Inhibitor Induces Both Mitochondrial Fragmentation and Mitochondrial Dysfunction**—With the use of a chemical inhibitor, we investigated the effect of mortalin inhibition on mitochondrial fragmentation and dysfunction. MKT077 is a rhodacyanine dye analog that directly inhibits the function of mortalin (44). To investigate the effect of MKT077 on mitochondrial dynamics, SK/mito-YFP cells were transfected with either control scrambled siRNA (Sc) or a specific siRNA against mortalin (siMOT), and then mitochondrial morphology was observed under a fluorescence microscope. Both Drp1 siRNA (siDrp1) and Opa1 siRNA (siOpa1) were used as controls. Representative fluorescence pictures of mitochondrial morphology by siRNAs are shown (bar size, 20 μm) (Fig. 3A). SK/mito-YFP cells were transfected with either control scrambled siRNA (Sc) or a specific siRNA against mortalin (siMOT), and then the mitochondrial length was measured (Fig. 3B). Mortalin knockdown also significantly increased ROS generation compared with that of control cells following Aβ treatment (Fig. 5B). These data suggest that MKT077 promotes mitochondrial dysfunction via Drp1 activity.

**Down-regulation of Mortalin Potentiates Aβ-mediated Mitochondria Dysfunction and Cytotoxicity**—Aβ promotes mitochondrial dysfunction, which contributes to Alzheimer disease pathology. Moreover, it was recently reported that mitochondrial import of mortalin is influenced by Aβ (45). Thus, we examined the role of mortalin knockdown on Aβ-mediated mitochondria dysfunction. SK-N-MC cells transfected with either scrambled siRNA or mortalin siRNA were incubated with Aβ. Then, both the total cellular ATP level and ROS production were measured. Our results showed that the cellular ATP level decreased appreciably by down-regulation of mortalin compared with Aβ-treated control cells (Fig. 5E). Consistently, mortalin knockdown also significantly increased ROS generation compared with that of control cells following Aβ treatment (Fig. 5F).

Because mitochondrial dysfunction is highly associated with cytotoxicity, we next investigated the effect of mortalin knockdown on Aβ-induced cytotoxicity. SH-SY5Y cells transfected with mortalin siRNA were exposed to Aβ, and the cell death rate was determined by flow cytometry. The results suggested that down-regulation of mortalin more enhanced the Aβ-induced cell death than that of control cells (Fig. 5C). We further investigated the effect of mortalin inhibition on Aβ-induced cell death and mitochondrial dysfunction using a chemical
inhibitor (MKT077). SK-N-MC cells were exposed to MKT077 in the presence or absence of A/H9252, and then the cellular ATP and ROS levels as well as cytotoxicity were examined. Similar to genetic inhibition, MKT077 treatment synergistically affected the A/H9252-mediated reduction of ATP levels, induction of ROS generation, and cell death (Fig. 5, D–F). Collectively, these data suggest that mortalin inhibition exacerbates A/H9252-induced mitochondrial dysfunction in neuroblastoma cells.

Over-expression of Mortalin Prevents Aβ-mediated Cytotoxicity in Neuroblastoma Cells—Our previous results imply that the inhibition of mortalin is involved in mitochondrial dysfunction, which is associated with cytotoxicity. To demonstrate the effect of mortalin over-expression on mitochondrial morphology and cell death, we established a cell line (SH-SY5Y/mortalin) stably expressing mortain (Fig. 6A). Then we examined the effect of mortalin on Aβ-mediated cell death as well as mitochondrial fragmentation. Consistent with mortalin knockdown experiments, both Aβ-mediated mitochondrial fragmentation and cell death were significantly suppressed in mortalin overexpressing cells compared with that of control cells (Fig. 6, B and C). These results further suggest that over-expression of mortalain prevents Aβ-induced cytotoxicity in neuroblastoma cells.

Mortalin Is Down-regulated in Brain Samples from 3xTg-AD Mice and Human Alzheimer Patients—In recent reports, mortalain has been implicated in neurodegenerative diseases, such as Parkinson disease (46, 47). However, the implication of mortalain expression with AD has not been elucidated. Therefore, we investigated the expresional regulation of mortalain by employing a triple transgenic mouse model of AD (3xTg-AD) harboring three mutant genes: Aβ precursor protein (APPSwe), presenilin-1 (PSTM146V), and tauP301L (40). The endogenous expression of mortalain gradually increased during normal brain development in mice. In contrast, mortalain expression was not increased in the brain of a 3xTg-AD mouse by 12 months (Fig. 7A). Importantly, the expression of mortalain in brain tissues from human AD patients was considerably decreased compared with that of age-matched normal controls (Fig. 7B). Taken together, these results suggest that mortalain is down-regulated in human AD patients as well as in the AD mice models.

DISCUSSION

Mitochondrial dysfunction is associated with neuropathies, and mitochondrial dynamics are altered in neurodegenerative diseases (1, 48). In this study, we synthesized a siRNA library that consisted of mitochondrial proteins and screened the library to identify novel regulators of mitochondrial dynamics. From the screening, we found several already known mitochondrial dynamics modulators such as prohibitin-2, LETM-1 (leucine zipper-EF hand containing transmembrane protein-1), VCP (valosin-containing protein), VDAC, and some mitochondrial ATPase subunits as well as mortalain (Table 1) (33, 49–51). In this article, we demonstrated that the inhibition of mortalain expression with AD has not been elucidated. Therefore, we investigated the expresional regulation of mortalain by employing a triple transgenic mouse model of AD (3xTg-AD) harboring three mutant genes: Aβ precursor protein (APPSwe), presenilin-1 (PSTM146V), and tauP301L (40). The endogenous expression of mortalain gradually increased during normal brain development in mice. In contrast, mortalain expression was not increased in the brain of a 3xTg-AD mouse by 12 months (Fig. 7A). Importantly, the expression of mortalain in brain tissues from human AD patients was considerably decreased compared with that of age-matched normal controls (Fig. 7B). Taken together, these results suggest that mortalain is down-regulated in human AD patients as well as in the AD mice models.
FIGURE 3. MKT077, a mortalin chemical inhibitor induces Drp1-mediated mitochondrial fragmentation and mitochondrial dysfunction. A, SK/mito-YFP cells were treated with MKT077 (MKT, 10 μM) for 4 h and then imaged using a fluorescence microscope. B, SK/mito-YFP cells were incubated with increasing concentrations of MKT, and the fragmented mitochondria were observed after 4 h. C, SK/mito-YFP cells transfected with either scrambled siRNA (Sc) or siRNA against Drp1 (siDrp1) were exposed to MKT (10 μM) for 6 h. D, mitochondria in wild type MEF (WT) and Drp1-deficient MEF (Drp1−/−) treated with MKT077 (10 μM) were labeled with a fluorescence MitoTracker (100 nM). Then, cells with fragmented mitochondria were observed by fluorescence microscopy. E, depolarized mitochondrial membrane was analyzed by MitoProbe JC-1 dye in WT and Drp1−/− MEF cells after MKT077 (10 μM) treatment. F, WT and Drp1−/− MEF cells treated with MKT077 (10 μM) were stained with DCF-DA, a ROS labeling dye and the intracellular ROS level was assessed by flow cytometric analysis. Data are represented as the mean ± S.E. (n > 3; *, p < 0.01 or **, p < 0.05).

FIGURE 4. MKT077 also induces mitochondrial fragmentation and mitochondrial dysfunction in primary cultured neuronal cells. A and B, pure cortical neuronal cultures (DIV 7) were treated with MKT077 (10 μM) for 4 h and stained with a MitoTracker probe. Mitochondrial morphology by MKT077 (A) and mitochondrial length was measured (B) by fluorescence. C and D, pure cortical neuronal cultures (DIV 7) were treated with MKT077 (10 or 20 μM) for 4 h and stained with CM-H2DCFDA (2 μM) for 20 min. The cellular ROS level was observed (C), and measured with a fluorescence microplate reader (D). E and F, MKT077-treated cells were stained with JC-1 staining solution and mitochondria dysfunction was measured by a fluorescence microplate reader (F), and images were observed under fluorescence microscope (E). Data are represented as the mean ± S.E. (n > 3; *, p < 0.02; **, p < 0.05).
strongly induced mitochondrial fragmentation and dysfunction. In addition, the expression of mortalin was reduced in brain tissues from the AD mice and AD patients. Mortalin is a mitochondrial chaperone protein and a member of the heat shock protein 70 (HSP70) family (52, 53). However, unlike most other HSP70 members, mortalin is not inducible by heat shock but is sensitive to oxidative stress, glucose deprivation, low-level of radiation, and some cytotoxins (54), suggesting that mortalin is a multifunctional protein in various stress conditions. Moreover, mortalin is a key regulatory protein for the import of mitochondrial ATPase components (55). In fact, HSP70 chaperone activity is important to protein quality control and function in mitochondria (56). According to this notion, our results indicated that mortalin has a crucial role in mitochondrial function. The mitochondrial fragmentation and dysfunction caused by mortalin inhibition were dependent on Drp1, suggesting that mitochondrial chaperon activity modulates function of the mitochondrial dynamics (Figs. 1–3). The immune precipitation assay, to examine the direct interaction between Drp1 and mortalin, suggested that mortalin was not directly interacted with Drp1 (data not shown). However, increased ROS by mortalin inhibition may be a key mediator in Drp1 activation in mortalin inhibition-induced mitochondrial fragmentation (Figs. 2D, 3F, and 4C).

Interestingly, recent proteomic analysis with post-mortem PD substantia nigra showed down-regulation of mortalin in PD brain, and mortalin is also deceased in the 6-hydroxydopamine-treated PD rat models (57, 58). Moreover, several PD-associated genetic variants of mortalin have been identified in PD patients (49). The PD-related mutants of mortalin increase
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FIGURE 7. Mortalin expression is down-regulated in Alzheimer disease patient and mice model. A, whole brain extracts from wild type mice (3, 6, and 12 months) and age-matched triple transgenic mice were assessed by Western blotting with mortalin and actin antibodies (upper panel). The relative expression value was calculated using densitometer analysis (lower panel, n = 5). B, the expression level of mortalin was examined in brain tissues from normal and age-matched Alzheimer disease patients (upper panel) and the relative expression value was analyzed using densitometry (lower panel).

both mitochondrial fragmentation and mitochondrial oxidative stress, which eventually impair mitochondrial homeostasis. The mitochondrial dysfunction mediated by down-regulation of mortalin is recovered by over-expression of Parkin, implying that the dysfunction of mortalin is highly associated with PD (59). Moreover, we found that mitochondrial translocation of Parkin was slightly enhanced by treatment of MKT077 (data not shown). However, the roles of mortalin in AD are not well understood. In this study, we examined the expressional regulation of mortalin in AD patients. Our expression analysis revealed that mortalin is down-regulated in brain tissues from 3xTg-AD mouse and AD patients (Fig. 7). Recently, it was shown that ectopic expression of mortalin attenuates Aβ-mediated oxidative stress and neurotoxicity, whereas the suppression of mortalin promotes mitochondrial dysfunction and neuronal injury (60, 61). According to previous results (60, 61), we also observed that the knockdown (by siRNA) and inhibition (by MKT077) of mortalin potentiated Aβ-induced mitochondrial dysfunction and cell death (Fig. 5). Although, the underlying mechanism for mortalin down-regulation in AD and the role of mortalin in Aβ-related neuronal injury are needed to further elucidation in neurodegenerative diseases, our results suggest that down-regulation of mortalin potentiates Aβ-mediated mitochondrial fragmentation and dysfunction in AD. Mortalin expression was differentially regulated in the hippocampus of human APOE4-targeted replacement mice, and the oxidation of mortalin was increased in APOE knock-out mice (62, 63). Oxidized mortalin induced mitochondrial aggregation and resulted in cell death in yeast (64). Therefore, future investigation of the transcriptional and translational regulation of mortalin in AD is needed.

Unlike PD or AD, mortalin is abundantly expressed in many human cancers (65). The up-regulation of mortalin in primary cells reduces cellular senescence and apoptosis but increases the life span of worms (66, 67). Indeed, we observed that over-expression of mortalin suppressed Aβ-induced cell death in neuroblastoma cells (Fig. 5C). As a chaperon, mortalin negatively regulates p53, a tumor suppressor protein (65, 68). MKT077 inhibits the chaperone function of mortalin and disrupts the binding of mortalin and p53, resulting in p53 accumulation (44, 69). The p53 protein is up-regulated in the superior temporal gyrus of AD patients and that increases Tau phosphorylation (70, 71). Although the relationship between mortalin and p53 in AD remains unclear, previous results suggest that up-regulation of mortalin reduces neuronal damage in neurodegenerative diseases. Indeed, Wang et al. (72) recently concluded that activation of HSP70 reduces neurotoxicity mediated by aggregation of the polyglutamine (poly-Q) protein, which is associated with Huntington disease.

In conclusion, we have suggested that mortalin is down-regulated in AD that could lead to mitochondrial dysfunction through mitochondrial fragmentation. Thus, up-regulation or activation of mortalin through drug targeting may antagonize the progression of AD in which mitochondrial dysfunction plays a key role.

Acknowledgments—We deeply thank Dr. Gyesoon Yoon (Ajou University, Korea) and Dr. Renu Wadhwa (NIAIST, Japan) for kindly providing mito-YFP plasmid and mortalin cDNA. We also appreciate to Dr. M. Katsuyoshi (Kyushu University, Japan) for providing Drp1-deficient MEF cells.

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