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Brain expression level and activity of HDAC6 protein in neurodegenerative dementia

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ABSTRACT

Histone deacetylase 6 (HDAC6) is a multifunctional cytoplasmic protein that plays an especially critical role in the formation of aggresomes, where aggregates of excess protein are deposited. Previous immunohistochemical studies have shown that HDAC6 accumulates in Lewy bodies in Parkinson's disease and dementia with Lewy bodies (DLB) as well as in glial cytoplasmic inclusions in multiple system atrophy (MSA). However, it is uncertain whether the level and activity of HDAC6 are altered in the brains of patients with neurodegenerative dementia. In the present study, we demonstrated that the level of HDAC6 was not altered in the temporal cortex of patients with Alzheimer's disease and DLB in comparison with controls. In contrast, the level of HDAC6 was significantly increased in the temporal cortex of patients with frontotemporal lobar degeneration with TDP-43 inclusions (FTLD-TDP) and in the cerebellar white matter of patients with MSA. However, the level of acetylated α-tubulin, one of the substrates of HDAC6, was not altered in FTLD-TDP and MSA relative to controls. These findings suggest that the induced level of HDAC6 in the brain is insufficient for manifestation of its activity in FTLD-TDP and MSA.

1. Introduction

Histone acetylation is a reversible post-translational modification that plays an important role in gene expression [1]. Recent large-scale analyses have revealed that numerous acetylated substrates exist in cells [2–4] and are involved in diverse biological functions such as metabolic regulation, the cell cycle, and the immune response [5–8]. Histone deacetylase 6 (HDAC6), a member of the HDAC family, has 18 isoforms in mammals and is present mainly in the cytoplasm, where it deacetylates α-tubulin and colocalizes with microtubules and dynein motor complexes [9,10]. HDAC6 also binds to polyubiquitinated proteins and recruits them to aggresomes through dynein motor-based transportation [10,11]. Furthermore, HDAC6 controls the fusion of autophagosomes and lysosomes through actin remodeling machinery [12]. Thus, one of the major functions of HDAC6 is probably the elimination or sequestration of abnormal molecules and aggregates such as huntingtin in Huntington's disease and α-synuclein in Parkinson's disease (PD) [13,14]. Accordingly, HDAC6 immunoreactivity is observed in Lewy bodies (LBs) in PD and dementia with LBs (DLB) as well as in glial cytoplasmic inclusions in multiple system atrophy (MSA) [10,15,16]. However, it is unclear whether the level and activity of HDAC6 in the brain are altered in neurodegenerative dementia. In this study, we assessed the level of HDAC6 expression and found that it was increased in the temporal cortex of patients with frontotemporal lobar degeneration with TDP-43 inclusions (FTLD-TDP), but not in patients with Alzheimer's disease (AD) or DLB, relative to controls. The level of HDAC6 was also increased in the cerebellar white matter of patients with MSA. Immunohistochemical analysis showed that HDAC6 immunoreactivity in the neuronal cytoplasm was increased in the temporal cortex of patients with FTLD-TDP and that glial cytoplasmic inclusions in the cerebellar white matter of patients with MSA were exclusively immunopositive for HDAC6. However, further analysis showed that the level of acetylated α-tubulin, one of the substrates of HDAC6, was not altered in the brains of patients with FTLD-TDP and MSA. These results suggest that the induced level of HDAC6 in the brain is insufficient for manifestation of its activity in FTLD-TDP and MSA.

2. Materials and methods

2.1. Antibodies

Rabbit polyclonal antibodies against HDAC6 (Santa Cruz Biotechnology, Santa Cruz, CA), β-actin (Sigma, Saint Louis, MO) and acetylated histone (Millipore, Bedford, MA), and mouse

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monoclonal antibodies against α-tubulin (Active motif, Carlsbad, CA; Gene Tex Inc., Irvine, CA) and acetylated α-tubulin (Sigma) were used in this study.

2.2. Subjects and immunoblot analysis

For immunoblot analysis, tissue samples from 41 autopsy cases were obtained from the Department of Pathology, Brain Research Institute, University of Niigata, Niigata, Japan. Written informed consent for autopsy, collection of samples and subsequent analysis was obtained from the patients and the next of kin of the deceased involved. This study was approved by the Institutional Ethics Committee of Hirosaki University Graduate School of Medicine, Hirosaki, Japan. Brain tissues were dissected out at autopsy and rapidly frozen at −70 °C. The middle temporal cortex of patients with AD ($n = 7$), neocortical type DLB [17] ($n = 7$), FTLD-TDP type B [18] ($n = 4$) and normal controls ($n = 7$), and the cerebellar cortex and white matter of patients with MSA-C [19] (cortex, $n = 7$; white matter, $n = 5$) and controls (cortex, $n = 7$; white matter, $n = 5$) were used in this study. All the diagnoses had been confirmed by neuropathological examinations using immunohistochemistry for tau, β-amyloid, α-synuclein and TDP-43. Each tissue was weighed and homogenized with a 20-fold volume of loading buffer [75 mM Tris–HCl, pH 6.8, 4% sodium dodecyl sulfate (SDS), 25% glycerol, 5% β-mercaptoethanol].

Samples were subjected to SDS–polyacrylamide gel electrophoresis (PAGE), and Western blot analysis was performed as described previously [20]. Detection was performed according to

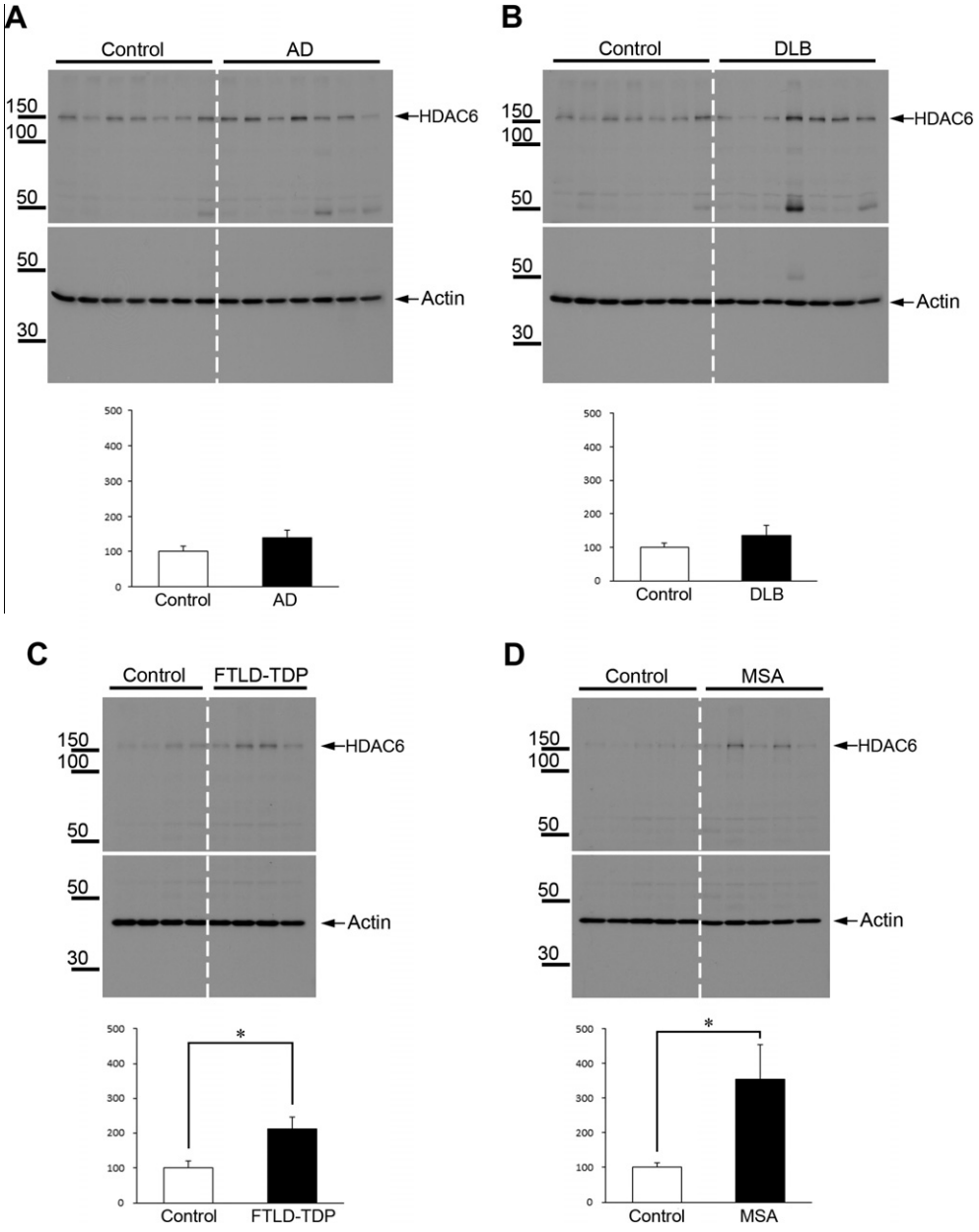

Fig. 1. Comparison of the expression level of HDAC6 in the brains of patients with various neurodegenerative diseases and normal controls. (A, B) Rabbit anti-HDAC6 antibody detects HDAC6 protein with a molecular mass of approximately 160 kDa. Immunoblotting shows that the level of HDAC6 is not altered in the brains of patients with AD or DLB relative to controls. (C) Immunoblotting shows that the level of HDAC6 is significantly increased 2.1-fold in the brains of patients with FTLD-TDP relative to controls ($p = 0.033$). (D) The level of HDAC6 is significantly increased 3.6-fold in the cerebellar white matter of patients with MSA relative to controls ($p = 0.0342$). Data are normalized by the level of actin protein in each sample and indicated as mean + SEM. Actin is used as a loading control. The control value is defined as 100%. $^{*}p < 0.05$.

the protocol provided with the ECL or ECL plus detection system (Amersham Pharmacia Biotech, Piscataway, NJ). Horseradish peroxidase-conjugated anti-mouse or anti-rabbit IgG (Santa Cruz Biotechnology) was used as a secondary antibody. Semi-quantitative analysis of protein was done by image analysis with the Image J software package from NIH.

2.3. Statistical analysis

All values are presented as mean + standard error of the mean (SEM). Statistical significance was evaluated using Student's *t* test when comparing two conditions. Probability values of $p < 0.05$ were considered to be significant.

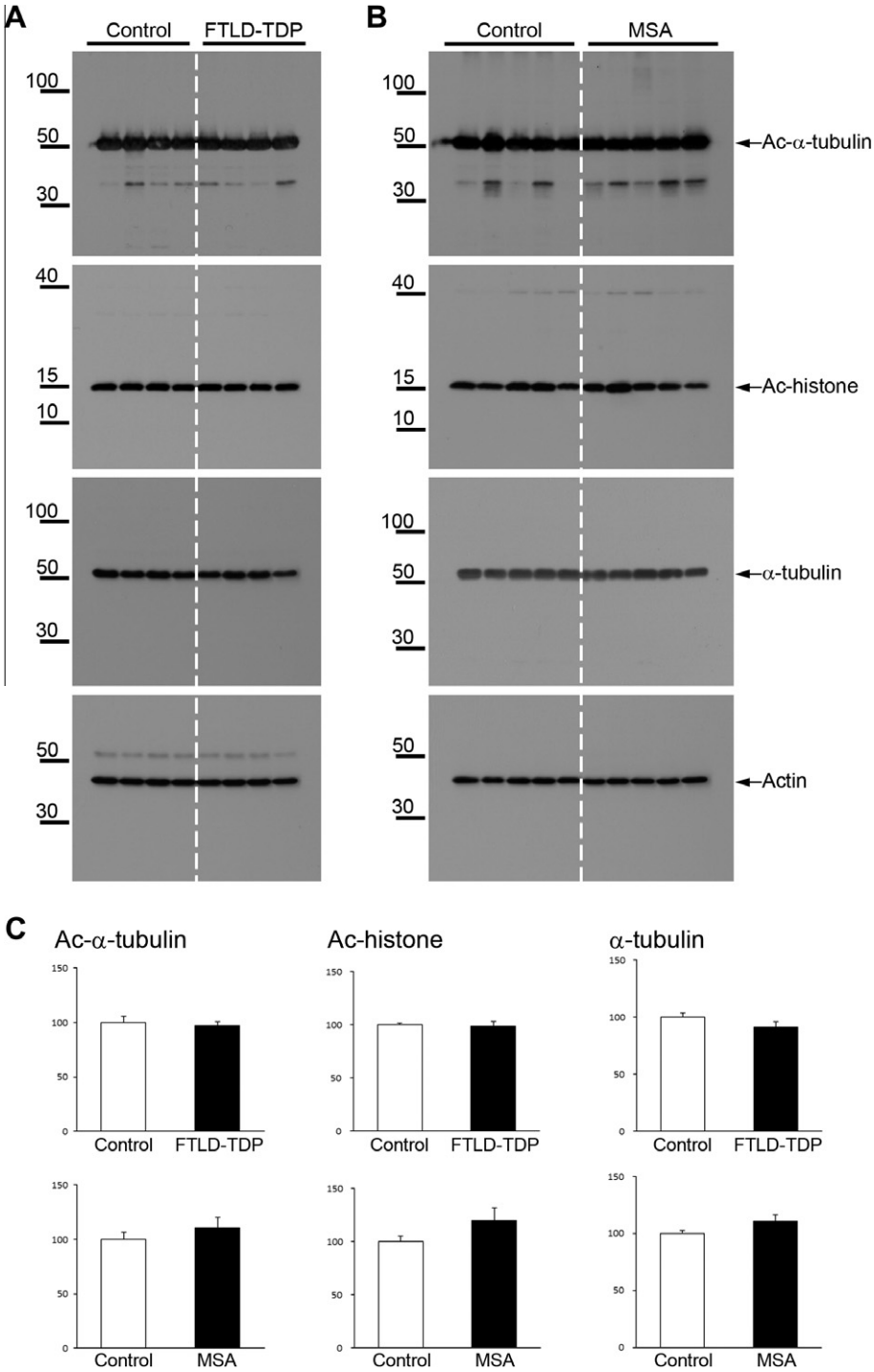

Fig. 2. Comparison of the expression level of HDAC6 substrate, acetylated α-tubulin (Ac-α-tubulin), acetylated histone (Ac-histone) and α-tubulin in the brains of patients with MSA and FTLD-TDP, and controls. (A) There is no significant difference in the amount of total and Ac-α-tubulin and Ac-histone in the middle temporal cortex between FTLD-TDP and controls. (B) The levels of Ac-α-tubulin and Ac-histone are not altered in MSA and controls. Actin is used as a loading control. (C) Quantification of relative levels of Ac-α-tubulin, Ac-histone and α-tubulin in the brains of patients with FTLD-TDP and MSA (*closed bar*) and controls (*open bar*). Data are normalized by the level of actin protein in each sample and indicated as mean + SEM. The control value is defined as 100%.

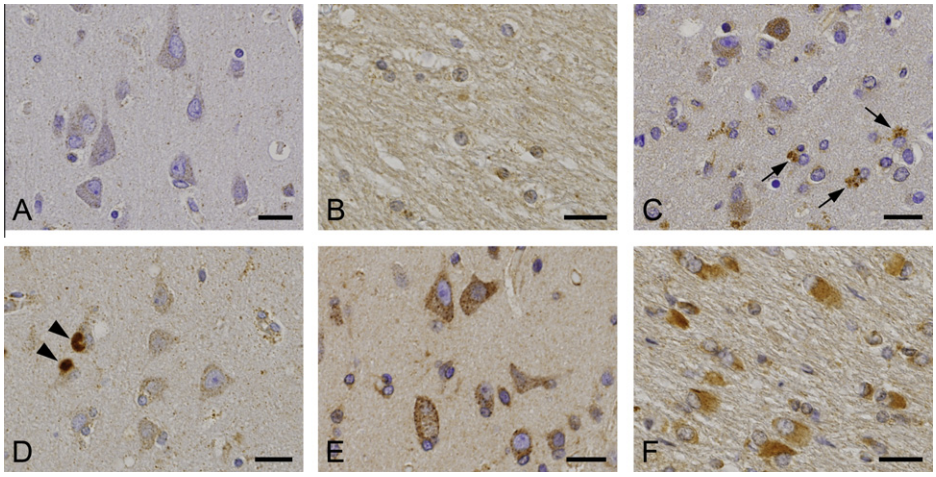

Fig. 3. HDAC6 immunoreactivity in the brains of control subjects (A, B) and patients with AD (C), DLB (D), FTLD-TDP (E) and MSA (F). (A, B) Weak HDAC6 immunoreactivity in the neuronal cytoplasm in the temporal cortex (A) and glial cells in the cerebellar white matter (B). (C) Weak immunoreactivity in the neuronal cytoplasm. Reactive astrocytes (arrows) are immunolabeled with anti-HDAC6. (D) Cortical LBs (arrowheads) are intensely immunolabeled with anti-HDAC6, whereas HDAC6 immunoreactivity in the neuronal cytoplasm is weak. (E) In FTLD-TDP, the neuronal cytoplasm is moderately immunolabeled with anti-HDAC6 in a diffuse granular pattern. (F) Glial cytoplasmic inclusions in the cerebellar white matter are exclusively immunopositive for HDAC6. *Bars* = 20 μm.

2.4. Immunohistochemistry

For immunohistochemical analysis, tissue samples from 32 autopsy cases were obtained from the Department of Neuropathology, Institute of Brain Science, Hirosaki University Graduate School of Medicine, Hirosaki, and the Department of Pathology, Brain Research Institute, University of Niigata, Niigata, Japan. Patients with AD ($n = 7$), neocortical type DLB ($n = 5$), FTLD-TDP type B ($n = 8$), MSA-C ($n = 5$) and normal controls ($n = 7$) were the subjects of this study. For routine histological investigations, the brain was fixed with 10% buffered formalin for 3–4 weeks. Blocks were cut from various cortical and subcortical regions, and embedded in paraffin. In all cases except for AD, the concomitant AD-related neurofibrillary changes were less than stage IV [21].

Four-micrometer-thick sections were cut from the middle temporal cortex and cerebellum of patients with AD, DLB, FTLD-TDP, MSA and controls, and immunostained using the avidin–biotin–peroxidase complex method with diaminobenzidine as the chromogen. Rabbit polyclonal anti-HDAC6 (c11420; Santa Cruz Biotechnology, 1:100) was used as a primary antibody. The sections were pretreated in an autoclave for 10 min in 10 mM citrate buffer (pH 6.0).

3. Results

3.1. Immunoblot analysis

We assessed the total level of HDAC6 in the temporal cortex of patients with AD, DLB and FTLD-TDP by immunoblotting. Immunoblot analysis showed that the level of HDAC6 was not altered in AD and DLB relative to controls (Fig. 1A and B). In contrast, the level of HDAC6 was significantly increased in FTLD-TDP relative to controls ($p = 0.033$) (Fig. 1C). Since previous immunohistochemical studies had demonstrated that HDAC6 was preferentially accumulated in neuronal and glial cytoplasmic inclusions in α-synucleinopathy (PD, DLB and MSA) [15], we next investigated whether the level of HDAC6 was altered in the cerebellum of patients with MSA. Immunoblot analysis showed that there was no significant difference in the level of HDAC6 in the cerebellar cortex between MSA and controls (data not shown). We further used cerebellar white matter samples to examine the level of HDAC6, because the occurrence of glial cytoplasmic inclusions in the cerebellar white matter is a pathological hallmark of MSA. Immunoblot analysis revealed that the level of HDAC6 was significantly increased 3.6-fold in the cerebellar white matter of MSA patients relative to controls ($p = 0.0342$) (Fig. 1D).

Acetylated α-tubulin is known to be one of the substrates of HDAC6. In order to determine whether the brain activity of HDAC6 is also increased in FTLD-TDP and MSA, we assessed the level of acetylated α-tubulin. Immunoblot analysis showed that the levels of acetylated α-tubulin were similar in the brains of control, FTLD-TDP and MSA patients (Fig. 2). Similarly, the levels of α-tubulin and acetylated histone were not altered in the brains of FTLD-TDP and MSA patients compared with controls. Based on these results, we calculated the activity of HDAC6 by normalization with the level of HDAC6 protein in each sample and concluded that the activity of HDAC6 was lower in the brains of patients with FTLD-TDP and MSA relative to controls.

3.2. Immunohistochemical analysis

We examined immunohistochemically the brains of patients with AD, DLB, FTLD-TDP, MSA and normal controls using anti-HDAC6 antibody. In normal controls, the cytoplasm of neurons, astrocytes and oligodendrocytes was barely immunostained with anti-HDAC6 antibody (Fig. 3A and B), as reported previously [15,22]. No significant difference of HDAC6 immunoreactivity was noted in the neuronal cytoplasm between controls (Fig. 3A) and AD (Fig. 3C) or DLB (Fig. 3D). However, cortical LBs observed in DLB were intensely immunolabeled with anti-HDAC6 antibody (Fig. 3D), as reported previously [15]. In FTLD-TDP, the neuronal cytoplasm were moderately immunolabeled with anti-HDAC6 in a diffuse granular pattern (Fig. 3E). In MSA, almost all glial cytoplasmic inclusions were exclusively immunopositive for HDAC6 (Fig. 3F). No HDAC6 immunoreactivity was found in neurofibrillary tangles in AD or TDP-43-positive neuronal and glial inclusions in FTLD-TDP.

4. Discussion

HDAC6 acts as a novel component of the compensatory autophagic system when the ubiquitin–proteasome system is

disrupted [23]. HDAC6 controls the fusion of autophagosomes and lysosomes through actin remodeling machinery [12]. Indeed, HDAC6 is localized in LBs in PD and DLB and glial cytoplasmic inclusions in MSA [10,15,16]. Autophagosome-related proteins are also present in these inclusions [24–29]. These observations raise the question of whether the level and activity of HDAC6 are altered in the brains of patients with neurodegenerative dementia. In this study, we assessed the level of HDAC6 in neurodegenerative dementia and revealed that there were no differences in the level of HDAC6 between controls and AD or DLB patients. In contrast, Ding et al. [30] reported that the level of HDAC6 was increased in the hippocampus of AD patients relative to controls. Because we used the temporal neocortex as a sample, the discrepancy between the results of Ding et al., and ours may have been attributable to the choice of brain regions examined.

Notably, we observed a significant increase in the level of HDAC6 protein in the brains of patients with FTLD-TDP compared with the controls. This result was supported by the immunohistochemical finding that the neuronal cytoplasm in the temporal neocortex of FTLD-TDP patients was more intensely immunolabeled with anti-HDAC6 antibody relative to the controls. TDP-43 accumulates in ubiquitin-positive cytoplasmic inclusions and is post-translationally modified in the brains of patients with FTLD-TDP. Interestingly, TDP-43 regulates the transcription of HDAC6 mRNA [31,32]. In addition, microarray data have indicated that the level of HDAC6 mRNA is repressed 3.5-fold in embryonic stem cells derived from TDP-43-knockout mice relative to controls [33]. Based on these reports, we examined the level of TDP-43 in the brains of patients with FTLD-TDP and found that there was no difference between FTLD-TDP and controls (data not shown). However, this result is not surprising, because various experiments using TDP-43 gene-modified animal models have shown that the level of TDP-43 protein is kept strictly constant by an unknown mechanism [34,35]. Since TDP-43 is abnormally phosphorylated and truncated in the brains of patients with FTLD-TDP, it is possible that TDP-43 modification might affect the brain level of HDAC6 in such patients.

MSA is one of the α-synucleinopathies characterized by abnormal accumulation of α-synuclein. In order to assess the level of HDAC6, we first sampled the cerebellar cortex and found that there was no difference between MSA patients and controls. Since HDAC6 is exclusively accumulated in the oligodendroglial cytoplasm of the cerebellar white matter in MSA patients [15,16], we next sampled the cerebellar white matter. Immunoblotting demonstrated that the level of HDAC6 was increased 3.6-fold in the cerebellar white matter of patients with MSA relative to controls. This increase would be due to the accumulation of HDAC6 protein in MSA oligodendrocytes. Using the same samples, we further examined the expression level of acetylated α-tubulin to assess the activity of HDAC6 and found that there was no statistically significant difference between MSA patients and controls. Similarly, there was no alteration in the level of α-tubulin in the brains of patients with FTLD-TDP relative to controls. These findings suggest that the increased level of HDAC6 is not enough to exert its activity in the brains of patients with FTLD-TDP and MSA. However, we cannot rule out the possibility that the level of acetylated α-tubulin is kept strictly constant, as is that of TDP-43 protein. Further studies will be needed to verify the level of acetylated HSP90, another HDAC6 substrate, in the brains of these patients when a specific antibody becomes available.

In conclusion, we have provided evidence that the level of HDAC6 is increased in the brains of patients with FTLD-TDP and MSA compared with controls. In contrast, the activity of HDAC6 does not parallel the induced level of the protein.

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References

[1] T. Kouzarides, Chromatin modifications and their function, Cell 128 (2007) 693–705.
[2] A. Basu, K.L. Rose, J. Zhang, R.C. Beavis, B. Ueberheide, B.A. Garcia, B. Chait, Y. Zhao, D.F. Hunt, E. Segal, C.D. Allis, S.B. Hake, Proteome-wide prediction of acetylation substrates, Proc. Natl. Acad. Sci. USA 106 (2009) 13785–13790.
[3] C. Choudhary, C. Kumar, F. Gnad, M.L. Nielsen, M. Rehman, T.C. Walther, J.V. Olsen, M. Mann, Lysine acetylation targets protein complexes and co-regulates major cellular functions, Science 325 (2009) 834–840.
[4] N. Mischerikow, A.J. Heck, Targeted large-scale analysis of protein acetylation, Proteomics 11 (2011) 571–589.
[5] T. Kouzarides, Histone acetylases and deacetylases in cell proliferation, Curr. Opin. Genet. Dev. 9 (1999) 40–48.
[6] B. Polevoda, F. Sherman, The diversity of acetylated proteins, Genome Biol. 3 (2002). reviews0006.
[7] J.M. Serrador, J.R. Cabrero, D. Sancho, M. Mittelbrunn, A. Urzainqui, F. Sanchez-Madrid, HDAC6 deacetylase activity links the tubulin cytoskeleton with immune synapse organization, Immunity 20 (2004) 417–428.
[8] S. Zhao, W. Xu, W.Q. Jiang, W. Yu, Y. Lin, T.F. Zhang, J. Yao, L. Zhou, Y.X. Zeng, H. Li, Y.X. Li, J. Shi, W.L. An, S.M. Hancock, F.C. He, L.X. Qin, J. Chin, P.Y. Yang, X. Chen, Q.Y. Lei, Y. Xiong, K.L. Guan, Regulation of cellular metabolism by protein lysine acetylation, Science 327 (2010) 1000–1004.
[9] C. Hubbert, A. Guardiola, R. Shao, Y. Kawaguchi, A. Ito, A. Nixon, M. Yoshida, X.F. Wang, T.P. Yao, HDAC6 is a microtubule-associated deacetylase, Nature 417 (2002) 455–458.
[10] Y. Kawaguchi, J.J. Kovacs, A. McLaurin, J.M. Vance, A. Ito, T.P. Yao, The deacetylase HDAC6 regulates aggresome formation and cell viability in response to misfolded protein stress, Cell 115 (2003) 727–738.
[11] S.S. Hook, A. Orian, S.M. Cowley, R.N. Eisenman, Histone deacetylase 6 binds polyubiquitin through its zinc finger (PAZ domain) and copurifies with deubiquitinating enzymes, Proc. Natl. Acad. Sci. USA 99 (2002) 13425–13430.
[12] J.Y. Lee, H. Koga, Y. Kawaguchi, W. Tang, E. Wong, Y.S. Gao, U.B. Pandey, S. Kaushik, E. Tresse, J. Lu, J.P. Taylor, A.M. Cuervo, T.P. Yao, HDAC6 controls autophagosome maturation essential for ubiquitin-selective quality-control autophagy, EMBO J. 29 (2010) 969–980.
[13] A. Iwata, B.E. Riley, J.A. Johnston, R.R. Kopito, HDAC6 and microtubules are required for autophagic degradation of aggregated huntingtin, J. Biol. Chem. 280 (2005) 40282–40292.
[14] G. Du, X. Liu, X. Chen, M. Song, Y. Yan, R. Jiao, C.C. Wang, Drosophila histone deacetylase 6 protects dopaminergic neurons against α-synuclein toxicity by promoting inclusion formation, Mol. Biol. Cell 21 (2010) 2128–2137.
[15] Y. Miki, F. Mori, K. Tanji, A. Kakita, H. Takahashi, K. Wakabayashi, Accumulation of histone deacetylase 6, an aggresome-related protein, is specific to Lewy bodies and glial cytoplasmic inclusions, Neuropathology 31 (2011) 561–568.
[16] Y. Chiba, S. Takei, N. Kawamura, Y. Kawaguchi, K. Sasaki, S. Hasegawa-Ishii, A. Furukawa, M. Hosokawa, A. Shimada, Immunohistochemical localization of aggresomal proteins in glial cytoplasmic inclusions in multiple system atrophy, Neuropathol. Appl. Neurobiol. 38 (2012) 559–571.
[17] I.G. McKeith, D.W. Dickson, J. Lowe, M. Emre, J.T. O'Brien, H. Feldman, J. Cummings, J.E. Duda, C. Lippa, E.K. Perry, D. Aarsland, H. Arai, C.G. Ballard, B. Boeve, D.J. Burn, D. Costa, T. Del Ser, B. Dubois, D. Galasko, S. Gauthier, C.G. Goetz, E. Gomez-Tortosa, G. Halliday, L.A. Hansen, J. Hardy, T. Iwatsubo, R.N. Kalaria, D. Kaufer, R.A. Kenny, A. Korczyn, K. Kosaka, V.M. Lee, A. Lees, I. Litvan, E. Londos, O.L. Lopez, S. Minoshima, Y. Mizuno, J.A. Molina, E.B. Mukaetova-Ladinska, F. Pasquier, R.H. Perry, J.B. Schulz, J.Q. Trojanowski, M. Yamada, Consortium on DLB, Diagnosis and management of dementia with Lewy bodies: third report of the DLB consortium, Neurology 65 (2005) 1863–1872.
[18] I.R. Mackenzie, M. Neumann, A. Baborie, D.M. Sampathu, D. Du Plessis, E. Jaros, R.H. Perry, J.Q. Trojanowski, D.M. Mann, V.M. Lee, A harmonized classification system for FTLD-TDP pathology, Acta Neuropathol. 122 (2011) 111–113.
[19] J.Q. Trojanowski, T. Revesz, Neuropathology Working Group on MSA, Proposed neuropathological criteria for the post mortem diagnosis of multiple system atrophy, Neuropathol. Appl. Neurobiol. 33 (2007) 615–620.
[20] K. Tanji, H.X. Zhang, F. Mori, A. Kakita, H. Takahashi, K. Wakabayashi, P62/sequestosome 1 binds to TDP-43 in brains with frontotemporal lobar degeneration with TDP-43 inclusions, J. Neurosci. Res. 90 (2012) 2034–2042.

[21] H. Braak, E. Braak, Neuropathological stageing of Alzheimer-related changes, Acta Neuropathol. 82 (1991) 239–259.

[22] C.M. Southwood, M. Peppi, S. Dryden, M.A. Tainsky, A. Gow, Microtubule deacetylase, SirT2 and HDAC6, in the nervous system, Neurochem. Res. 32 (2007) 187–195.

[23] U.B. Pandey, Z. Nie, Y. Batlevi, B.A. McCray, G.P. Ritson, N.B. Nedelsky, S.L. Schwartz, N.A. DiProspero, M.A. Knight, O. Schuldiner, R. Padmanabhan, M. Hild, D.L. Berry, D. Garza, C.C. Hubbert, T.P. Yao, E.H. Baehrecke, J.P. Taylor, HDAC6 rescues neurodegeneration and provides an essential link between autophagy and the UPS, Nature 447 (2007) 859–863.

[24] L. Alvarez-Erviti, M.C. Rodriguez-Oroz, J.M. Cooper, C. Caballero, I. Ferrer, J.A. Obeso, A.H. Schapira, Chaperone-mediated autophagy markers in Parkinson disease brains, Arch. Neurol. 67 (2010) 1464–1472.

[25] L. Crews, B. Spencer, P. Desplats, C. Patrick, A. Paulino, E. Rockenstein, L. Hansen, A. Adame, D. Galasko, E. Masliah, Selective molecular alterations in the autophagy pathway in patients with Lewy body disease and in models of α-synucleinopathy, PLoS One 5 (2010) e9313.

[26] B. Dehay, J. Bove, N. Rodriguez-Muela, C. Perier, A. Recasens, P. Boya, M. Vila, Pathogenic lysosomal depletion in Parkinson's disease, J. Neurosci. 30 (2010) 12535–12544.

[27] S. Higashi, D.J. Moore, M. Minegishi, K. Kasanuki, H. Fujishiro, T. Kabuta, T. Togo, O. Katsuse, H. Uchikado, Y. Furukawa, H. Hino, K. Kosaka, K. Sato, H. Arai, K. Wada, E. Iseki, Localization of MAP1-LC3 in vulnerable neurons and Lewy bodies in brains of patients with dementia with Lewy bodies, J. Neuropathol. Exp. Neurol. 70 (2011) 264–280.

[28] K. Tanji, F. Mori, A. Kakita, H. Takahashi, K. Wakabayashi, Alteration of autophagosomal proteins (LC3, GABARAP and GATE-16) in Lewy body disease, Neurobiol. Dis. 43 (2011) 690–697.

[29] L. Schwarz, O. Goldbaum, M. Bergmann, S. Probst-Cousin, C. Richter-Landsberg, Involvement of macroautophagy in multiple system atrophy and protein aggregate formation in oligodendrocytes, J. Mol. Neurosci. 47 (2012) 256–266.

[30] H. Ding, P.J. Dolan, G.V. Johnson, Histone deacetylase 6 interacts with the microtubule-associated protein tau, J. Neurochem. 106 (2008) 2119–2130.

[31] S.H. Kim, N.P. Shanware, M.J. Bowler, R.S. Tibbetts, Amyotrophic lateral sclerosis-associated proteins TDP-43 and FUS/TLS function in a common biochemical complex to co-regulate HDAC6 mRNA, J. Biol. Chem. 285 (2010) 34097–34105.

[32] F.C. Fiesel, C. Schurr, S.S. Weber, P.J. Kahle, TDP-43 knockdown impairs neurite outgrowth dependent on its target histone deacetylase 6, Mol. Neurodegener. 6 (2011) 64.

[33] P.M. Chiang, J. Ling, Y.H. Jeong, D.L. Price, S.M. Aja, P.C. Wong, Deletion of TDP-43 down-regulates Tbc1d1, a gene linked to obesity, and alters body fat metabolism, Proc. Natl. Acad. Sci. USA 107 (2010) 16320–16324.

[34] Y.F. Xu, T.F. Gendron, Y.J. Zhang, W.L. Lin, S. D'Alton, H. Sheng, M.C. Casey, J. Tong, J. Knight, X. Yu, R. Rademakers, K. Boylan, M. Hutton, E. McGowan, D.W. Dickson, J. Lewis, L. Petrucelli, Wild-type human TDP-43 expression causes TDP-43 phosphorylation, mitochondrial aggregation, motor deficits, and early mortality in transgenic mice, J. Neurosci. 30 (2010) 10851–10859.

[35] L.M. Igaz, L.K. Kwong, E.B. Lee, A. Chen-Plotkin, E. Swanson, T. Unger, J. Malunda, Y. Xu, M.J. Winton, J.Q. Trojanowski, V.M. Lee, Dysregulation of the ALS-associated gene TDP-43 leads to neuronal death and degeneration in mice, J. Clin. Invest. 121 (2011) 726–738.