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Research article

Upregulation of PSMB8 and cathepsins in the human brains of dementia with Lewy bodies

Qunxing Ding*, Haiyan Zhu

Department of Biological Sciences, Kent State University at East Liverpool, East Liverpool, OH 43920, USA

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ABSTRACT

Proteasome and lysosome are responsible for the homeostasis of proteins, lipids and carbohydrates in cells. Numerous reports indicate the proteolytic pathways have altered functions during neurodegeneration and aging. Dementia with Lewy bodies (DLB) is one of the leading forms of dementia, and the proteolytic alteration in DLB has not yet been fully investigated. This study shows that the components of proteasome and lysosome had selectively altered gene expression and enzymatic functions. Specifically, PSMB8, an inducible proteasomal β subunit, had elevated mRNA level and protein level in DLB brain compared with age-matched controls. The proteasomal caspase-like peptidase showed significant decreased activity in DLB brains and the trypsin-like/chemotrypsin-like activities did not reach statistical significance. Lysosomal cathepsin B and D had elevated mRNA levels while only cathepsin B showed elevated enzymatic activity in DLB brains. This data indicate that the alteration of proteolytic pathways is highly selective and comprehensive. Further study to elucidate the correlation between neurodegenerative development and the alteration of proteolytic pathways would be important for therapeutic development.

1. Introduction

Dementia with Lewy bodies (DLB) is a neurodegenerative disorder declined in thinking, reasoning and other cognition functions, and is the second most common dementia after Alzheimer's disease (AD) [1]. The pathological hallmark of DLB is the intracellular protein aggregates mainly formed by α-synuclein (SNCA), called Lewy bodies (LB) and Lewy neurites (LN) [2]. SNCA may associate with the presynaptic vehicle transport but its actual physiological role is not yet fully understood [3,4]. Protein aggregation is a common feature of many neurodegenerative disorders, including DLB, AD, Parkinson's disease (PD) and Huntington disease (HD). The wide presence of the aggregated structures in neurodegenerative disorders suggests that they may play an important role in etiology of neurodegeneration. For example, some reports indicated that aggregated proteins were toxic, other reports showed evidence that they might be protective during pathological development in multiple neurodegenerative processes, due to the fact that protein aggregation is a reversible process and regulated by multiple factors [4–8].

Cells constantly synthesize and degrade proteins to maintain cellular homeostasis, and the degradation of proteins relies on the function of the proteasome and lysosome [6], which are involved in the formation of protein aggregation. The proteasome is an intracellular proteolytic complex composed of multiple subunits, and possesses multiple proteolytic activities. The enzymatic functions of proteasome may be inhibited in pathological conditions to induce protein aggregation and even cell death [6]. For example, the inhibition of proteasome inhibition was identified in multiple neurodegenerative conditions to suggest the involvement of proteasome in neurodegeneration [7,8]. Meanwhile, selective upregulation of the lysosome have been demonstrated in a variety of neurodegenerative conditions, and a specialized form of elevated lysosomal activity, termed autophagy, is known to occur in a variety of neurodegenerative conditions [8,9].

In regarding to DLB, it was believed that proteasome and lysosome were involved in the formation of LB [10], and the SNCA in LB were ubiquitinated [11]. In fact, more than 90 different proteins were in the structure of LB in some co-localization studies, including some proteasome subunits, ubiquitin and ubiquitin-activating enzyme E1, and components of autophagy [12–14]. It was also reported that the inhibition of proteasome lead to the formation of LB [15], and autophagy was coupled with the formation of LB [9,16,17].

In summary, tremendous progress has been made in the understanding of LB formation, as well as the involvement of proteasomal and lysosomal systems in LB formation. However, it is still not clear that how the gene expression pattern as well as the enzymatic functions are altered in DLB, and the correlation between gene expression and

* Corresponding author.
E-mail address: qding@kent.edu (Q. Ding).

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Table 1
Human subjects and related clinic data.

	Age (yrs)	Gender	PMI (hr)	CDR	MMSE
Control	79.5 ± 5.5	3 M/2F	2.9 ± 0.76	0.14 ± 0.26	25.11 ± 1.49
DLB	78.8 ± 4.7	4 M/1F	2.7 ± 0.60	1.75 ± 0.67	12.62 ± 2.51

PMI: postmortem interval; CDR: clinical dementia rating; MMSE: mini-mental state examination. Values are average ± standard deviation.

protein function is not yet fully investigated especially in human specimen. This study reports that the proteasomal subunits PSMB8, and lysosomal cathepsin B and D were upregulated in both RNA and protein levels in DLB brains. Meanwhile, the proteasomal caspase-like activity was decreased and the lysosomal cathepsin B activity was increased in DLB brains. Our finding supports the view that proteasome and lysosome are involved in the pathogenesis of DLB, and modulation of proteasomal and lysosomal systems may become potential therapeutic targets.

2. Methods and materials

2.1. Human brain tissues

Data were obtained from 5 control (3 males and 2 females) and 5 DLB cases (4 males and 1 female). The related clinic data were summarized in Table 1. The brain sections and frozen tissue were from brain region superior middle temporal gyri (SMTG) due to the facts that significant LB and LN are present in SMTG regions. The neuropathology and neurophysiological diagnosis for each subject was conducted according to revised criteria [18] in the Sanders-Brown Center on Aging, University of Kentucky.

2.2. Real-Time RT-RCR

Total RNA was isolated with TriReagent (Sigma-Aldrich, St. Louis, MO, USA) method as described previously [19]. The concentration of each RNA sample was determined by OD_{260} after Qiagen RNA column purification (Cat No.: 74104, Qiagen, Hilden, Germany), and 800 ng total RNA were loaded in each reaction (CYBR® Green system was used). The ABI PRISM® 7000 Sequence Detection System and related reagents were used for real-time quantitative RT-PCR according to manufacturer's manuals (Applied Biosystems, CA) (Table 2).

2.3. Western blotting

Brain tissues were homogenized with lysis buffer (0.3% DTT, 0.8% Triton X-100, 7.5% glycerol, 50 mM HEPES in TBS, pH 7.4). The total proteins were then purified and quantified by OD_{280} method, and 30 µg total proteins were used in analysis for each subject. Briefly, total proteins were separated in 10% SDS-PAGE, and then transferred to nitrocellulose membrane. The membranes were blocked in 10% milk suspended in TBS, and probed with designed antibodies according to the manufacturer's procedure. Primary antibodies against the followed proteins were purchased from Santa Cruz Biotechnology Inc. These proteins are: PSMA1 (antibody cat# sc-166073, used at 1:500 dilution), PSMB3 (antibody cat# MCP102, used at 1:1000 dilution), PSMB8 (antibody cat# sc-100284, used at 1:1000 dilution), cathepsin B (antibody cat# sc-365558, used at 1:1000 dilution), cathepsin D (antibody cat# sc-136282, used at 1:500 dilution) and actin (antibody cat# sc-70319, used at 1:2000 dilution as calibration control). The second antibody (donkey anti-mouse IgG-B: sc-2098, 1:400 dilution) was biotinated and detected by streptavidin-biotin complex according to the manufacturer's procedure (Santa Cruz, CA, USA).

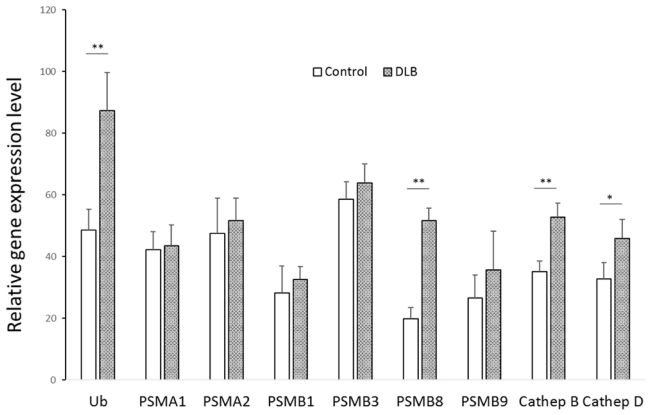

Fig. 1. Altered gene expression level of proteasomal and lysosomal components. Total RNA from DLB and control brain tissues (superior middle temporal gyri (SMTG) region, n = 5) was purified and analyzed by real-time RT-PCR. Ub, PSMB8, cathepsin B and D showed statistical significant difference. Ub: ubiquitin; PSMA1 & 2: proteasome 20S α subunits; PSMB1 & 3: proteasome 20S β subunits; PSMB8 & 9: proteasome inducible β subunits; cathep B: lysosome cathepsin B; cathep D: lysosome cathepsin D. The vertical axis is the relative expression level after calibration with 18S rRNA gene in each subjects. **: $p < 0.01$, *: $p < 0.05$.

2.4. Immunohistochemistry and In-Situ hybridization

Immunostaining of tissue sections (SMTG) was conducted with protocol as described previously [20]. Briefly, tissue sections were deparaffinized and rehydrated, then blocked in TBS with 2% donor horse serum for 5 min, incubated with primary antibody (PSMB8 antibody cat# sc-100284, 1:1000 dilution) overnight at 4 °C. After washing, tissue sections were sequentially incubated with biotinylated secondary antibody (donkey anti-mouse IgG-B: sc-2098, 1:200 dilution) for 1 h, followed incubation with streptavidin-biotin complex for 1 h. Bound antibody complexes were visualized by incubating sections in analyzing solution (0.1% (v/v) Triton X-100, 1.4 mM 3,3′-diaminobenzidine,

Table 2
Primers used in this study.

Gene	Sense primer (5′to 3′)	Antisense primer (5′ to 3′)
Ub	GTC AAA ATG CAG ATC TTC GTG AA	CAC CTC TCA GAC GCA GGA CC
PSMA 1	CGA AAT CAG TAT GAC AAT GAT GTC	ATG TCT CTC CAA GTA AGT AC
PSMA 2	TCG CTG ACT ACA TTC AGC C	TCG AGC TCT GTG CAC AAG CAC TC
PSMB 1	CTC CTG GCA GAG ACT TGG GGA	GTA GAC AGC ATT GCA GCA ATT G
PSMB 3	GAT GGT GAC CAC GGA CTT C	GCA GGT GCC ACT GAC CAC A
PSMB 8	GGG TGA ACA AGG TGA TTG AGA*	TTG ACA ACG CCT CCA GAA TAG
PSMB 9	CAT GGG ATA GAA CTG GAG GAA C	CAC ACC GGC AGC TGT AAT A
Cathep B	ACT TCT ACA ACG TGG ACA TGA G	CCA TGA TGT CCT TCT CGC TAT T
Cathep D	GTG GCA CAG ACT CCA AGT ATT A	ACG GTC AAA CAC AGT GTA GTA G
18S rRNA	GAC AGG ATT GAC AGA TTG ATA G	CAC TTG TCC CTC TAA GAA GTT G

Ub: ubiquitin; *used as probe for *in-situ* mRNA detection after biotin labelling.