Heliyon 9 (2023) e18188

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

A bioinformatic investigation of proteasome and autophagy expression in the central nervous system

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ARTICLE INFO

Keywords:
Microarray
Neurodegeneration
Protein quality control
Proteomics
Spinal cord

ABSTRACT

The ubiquitin proteasome system (UPS) and autophagy lysosome pathway (ALP) are crucial in the control of protein quality. However, data regarding the relative significance of UPS and ALP in the central nervous system (CNS) are limited. In the present study, using publicly available data, we computed the quantitative expression status of UPS- and ALP-related genes and their products in the CNS as compared with that in other tissues and cells. We obtained human and mouse gene expression datasets from the reference expression dataset (RefEx) and Genevestigator (a tool for handling curated transcriptomic data from public repositories) as well as human proteomics data from the proteomics database (ProteomicsDB). The expression levels of genes and proteins in four categories—ubiquitin, proteasome, autophagy, and lysosome—in the cells and tissues were assessed. Perturbation of the gene expression by drugs was also analyzed for the four categories. Compared with that for ubiquitin, autophagy, and lysosome, gene expression for proteasome was consistently lower in the CNS of mice but was more pronounced in humans. Neural stem cells and neurons showed low proteasome gene expression as compared with embryonic stem cells. Proteomic analyses, however, did not show trends similar to those observed in the gene expression analyses. Perturbation analyses revealed that azithromycin and vitamin D3 upregulated the expression of both UPS and ALP. Gene and proteomic expression data could offer a fresh perspective on CNS pathophysiology. Our results indicate that disproportional expression of UPS and ALP might affect CNS disorders and that this imbalance might be redressed by several therapeutic candidates.

1. Introduction

The ubiquitin-proteasome system (UPS) targets the majority of cellular proteins to the proteasome for degradation [1], whereas small aggregated or insoluble proteins are preferentially degraded via the autophagy-lysosome pathway (ALP) [2,3]. Many

Abbreviations: UPS, ubiquitin proteasome system; ALP, autophagy lysosome pathway; PD, Parkinson's disease; AD, Alzheimer's disease; ALS, amyotrophic lateral sclerosis; SOD1, Cu/Zn superoxide dismutase; iPS, induced pluripotent stem; FTD, frontotemporal dementia; TBK1, TANK-binding kinase 1; LRRK2, leucine rich-repeat kinase 2; KEGG, Kyoto Encyclopedia of Genes and Genomes; CAGE, cap analysis of gene expression; RefEx, reference expression dataset; AZM, azithromycin; ATRA, all-trans retinoic acid; Aβ, amyloid β; TDP-43, TAR-DNA binding protein-43; PTEN, phosphatase and tensin homolog deleted on chromosome 10; MTOR, mammalian target of rapamycin; Nrf2, nuclear factor erythroid-2-like 2; TFEB, transcription factor EB.

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https://doi.org/10.1016/j.heliyon.2023.e18188
Received 22 May 2023; Received in revised form 26 June 2023; Accepted 11 July 2023
Available online 17 July 2023
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neurodegenerative disorders, such as Parkinson's disease (PD), Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS), involve the accumulation of harmful and aggregation-prone proteins [4]. Although these proteins are immediately degraded by proteolytic systems in a healthy individual, the dysregulation of proteostasis caused by aging, environmental factors, or genetic mutation results in the accumulation of protein aggregates, leading to neuronal cell death.

The relative significance of and level of overlap between UPS and ALP functions in the central nervous system (CNS) are currently poorly understood. Our previous study using mesenchymal stem cells derived from an ALS mouse model [5], which expresses mutant Cu/Zn superoxide dismutase (mSOD1), revealed that proteasome inhibition increases mSOD1 protein levels, whereas no significant differences were observed after autophagy inhibition. This suggests that mSOD1 degradation predominantly occurs via the UPS and that deficiencies in the ALP can be compensated for by the UPS. This result is consistent with a previous study using fibroblasts [6], which also concluded that the UPS is the principal determinant of mSOD1 levels.

However, Imamura et al. [7] reported that boosting autophagy by targeting Src/c-Abl signaling could reduce mSOD1 levels in motor neurons generated from induced pluripotent stem (iPS) cells. Furthermore, bosutinib (a Src/c-Abl inhibitor) delays disease onset and extends the survival of mSOD1 transgenic mice [7]. Furthermore, increasing evidence suggests that autophagy might be disturbed in both ALS and frontotemporal dementia (FTD), two disorders with similar pathological and genetic characteristics. Postmortem analyses of patient tissues revealed increased numbers of autophagosomes [8,9], and several autophagy-associated gene mutations have been reported in ALS-FTD families, including UBQLN2/ubiquilin-2, OPTN/optineurin, SQSTM1/p62, and more recently, TBK1 (TANK-binding kinase 1) [10–12]. This complexity regarding the roles of the UPS and ALP in neurodegeneration is not only relevant to ALS/FTD. Mutations in the E3 ubiquitin ligase parkin cause early onset PD with an autosomal recessive inheritance pattern (PARK2) [13,14], whereas autosomal dominant PD (PARK8) is linked to leucine rich-repeat kinase 2 (LRRK2), mutations of which are related to autophagy dysregulation [15].

This contrariety led us to examine the state of ubiquitin, proteasome, autophagy, and lysosome in CNS cells and tissues as compared with that in the other tissues. Proteasome activity is primarily measured by quantifying the proteolytic activity on substrates of UPS, whereas autophagic flux assessment is the gold standard for measuring autophagy activity [16]. As far as current technology is concerned, measuring proteasome and autophagy activities in the CNS and comparing them with those in other organs is practically impossible, particularly in humans in vivo. Thus, we addressed this point through a different approach, i.e., by using bioinformatics data. We accessed publicly available datasets of mRNA expression and proteomics data and comprehensively compared the levels expressed through UPS and ALP. The results indicated that the expression of proteasome is low whereas the expression of ALP is average or relatively high in the CNS as compared to that in other tissues. We also performed drug perturbation analyses, which demonstrated that UPS and ALP share several common upstream regulators, indicating that a single intervention may modulate both UPS and ALP expression.

2. Materials and methods

2.1. Gene selection of genes

Ubiquitinating enzymes, proteasomal components, and autophagy-associated genes were selected using the pathway maps of the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Release 98.1) (https://www.genome.jp/kegg/) and a published report from Singh et al. [17]. Lysosome-related genes were selected using the KEGG database and PathCards (pathway unification database) at the Weizmann Institute of Science (Version 5.2.396.0) (https://pathcards.genecards.org/card/lysosome). Genes with common pathway classes in the two databases were selected. As we aimed to focus on mainstream players in each respective pathway, we further selected genes with common pathway descriptors in both human and mouse. We did not use any tissue samples, experimental animals or human participants in this report. All bioinformatic analyses were carried out in accordance with relevant guidelines and regulations.

2.2. Gene expression and proteomic datasets

Cap analysis of gene expression (CAGE) is an analytical technique to produce a snapshot of the 5′ end of the capped (very beginning of) mRNA population in the transcriptome [18]. The CAGE expression datasets for human (RefEx_expression_CAGE_all_human_PRJDB1099.tsv.zip) and mouse (RefEx_expression_CAGE_all_mouse_PRJDB1100.tsv.zip) were obtained from RefEx [18] (http://refex.dbcls.jp/). We also used a commercial data analysis program, Genevestigator (Nebion, Switzerland) [19,20], which enables the analysis of deeply curated bulk tissue and cell transcriptomic data from public repositories. For *Homo sapiens*, we analyzed data from the Affymetrix Human Genome U133 Plus 2.0 Array and the mRNA-Seq Gene Level *Homo sapiens* (ref: Ensembl 97, GRCh38.p12) datasets, using the "Anatomy" filter for both (cell lines, neoplasms, and unspecified organs, tissues, or cells were excluded). For *Mus musculus*, the Affymetrix Mouse Genome 430 2.0 Array and the mRNA-Seq Gene Level *Mus musculus* (ref. Ensembl 88, GRCm38.p5) datasets were filtered for both "Anatomy" and "wild-type genetic background only".

Protein expression data sets were obtained from ProteomicsDB [21] (https://www.proteomicsdb.org/). As we could not obtain a comprehensive data set from the website, we obtained each protein expression profile and then merged these to form a single data file. Analyses were performed on the four categories of proteins from which expression values in the brain, spinal cord, heart, lung, and liver were available.

2.3. Comparison of expression between different cells or tissues

Because the CAGE data were expressed as $\log_2$ values and the Genevestigator and ProteomicsDB data were expressed as $\log_{10}$ values, the data were converted to their antilogarithms for subsequent analyses. In all the analyses, references were set as the average expression value of each gene across different cells or tissues, respectively, in human and mouse. The expression levels relative to the references were obtained for all respective genes. The analytical subjects included neuronal cells (neural stem cells, neurons, and astrocytes), multipotent stem cells (embryonic stem cells and iPS cells), and neural tissues (brain and spinal cord). Moreover, cells or tissue from the heart, lung, and liver were used for comparison. Other than bar graph, data were presented as heatmaps, scatter plots, or box plots.

2.4. Perturbation analysis

Using the perturbation setting of Genevestigator, the aforementioned human and mouse array and mRNA-seq data sets were subjected to the filters "Anatomy" and "cell lines" (neoplasms and unspecified organs, tissues, or cells were excluded). In the mouse analyses, the "wild-type genetic background only" filter was not applied. Using the selection filter, the data were further filtered for "drug classification only". The top 10 candidates from each analysis were selected.

2.5. Statistical analysis

Data handling, arithmetic operation and graphing were performed using Microsoft Excel (Microsoft Corp., USA). Data analyses were performed using SPSS statistics, version 27 (IBM). One-way analysis of variance (ANOVA) followed by *post hoc* Bonferroni comparison was applied.

3. Results

3.1. Gene selection

Ubiquitin, proteasome, and autophagy-related genes were selected based on the commonality of their classification in two datasets, the KEGG orthology and that reported by Singh et al. [17] (Table 1). Lysosome genes were obtained in the same way using the KEGG orthology and PathCards databases. Genes that were common to both datasets in each case were then screened further for common classifications between humans and mice, eventually resulting in 70 genes for ubiquitin, 38 for the proteasome, 64 for autophagy, and 81 for the lysosome (Table 1).

Quantitative proteomic data were extracted for genes within these datasets for which protein expression values of brain, spinal cord, heart, lung, and liver were available (29 proteins for ubiquitin, 37 for the proteasome, 33 for autophagy, and 51 for the lysosome) (Table 1). All gene (protein) sets are listed in S1 Table.

3.2. CAGE analysis

Mouse CAGE analysis showed that ubiquitin, autophagy, and lysosome expression ratios in the cortex and spinal cord were significantly greater than 1.0 (Fig. 1a and b), while the values of the proteasome in the same regions were slightly smaller than 1.0. ANOVA analysis showed no significant differences in ubiquitin, autophagy, or lysosome levels in the cortex, spinal cord, heart, lung, and liver. Meanwhile, the significant differences were observed in the proteasome, while *post hoc* analysis revealed the proteasome genes expression in the brain was lower than that of the lung ($p < 0.001$) and heart ($p < 0.05$), and proteasome expression in the spinal cord and liver were lower than that in the lung ($p < 0.05$). Similar but more distinct trends were observed in human CAGE (Fig. 1c and d), with the mean values of proteasome genes expressions in the cortex and spinal cord being significantly less than 1.0. *Post hoc* analyses showed that the proteasome genes expression in the brain, spinal cord, and lung were significantly lower than that in the liver ($p < 0.001$) and heart ($p < 0.01$ in the brain and spinal cord, and $p < 0.01$ in the lung).

Table 1
Protein selection in analyses.

	Ubiquitin	Proteasome	Autophagy	Lysosome
KEGG orthology	131	49	109	105
Singh SR, et al.	430	46	232	N/A
PathCards	N/A	N/A	N/A	128
Common genes (human & mouse)*	70	38	64	81
Proteomic analysis	29	37	33	51

*: Genes selected based on commonality (between databases and between species) are used in gene expression analyses. The datailed information of the genes are listed in Supplementary Table 1. N/A: not available.

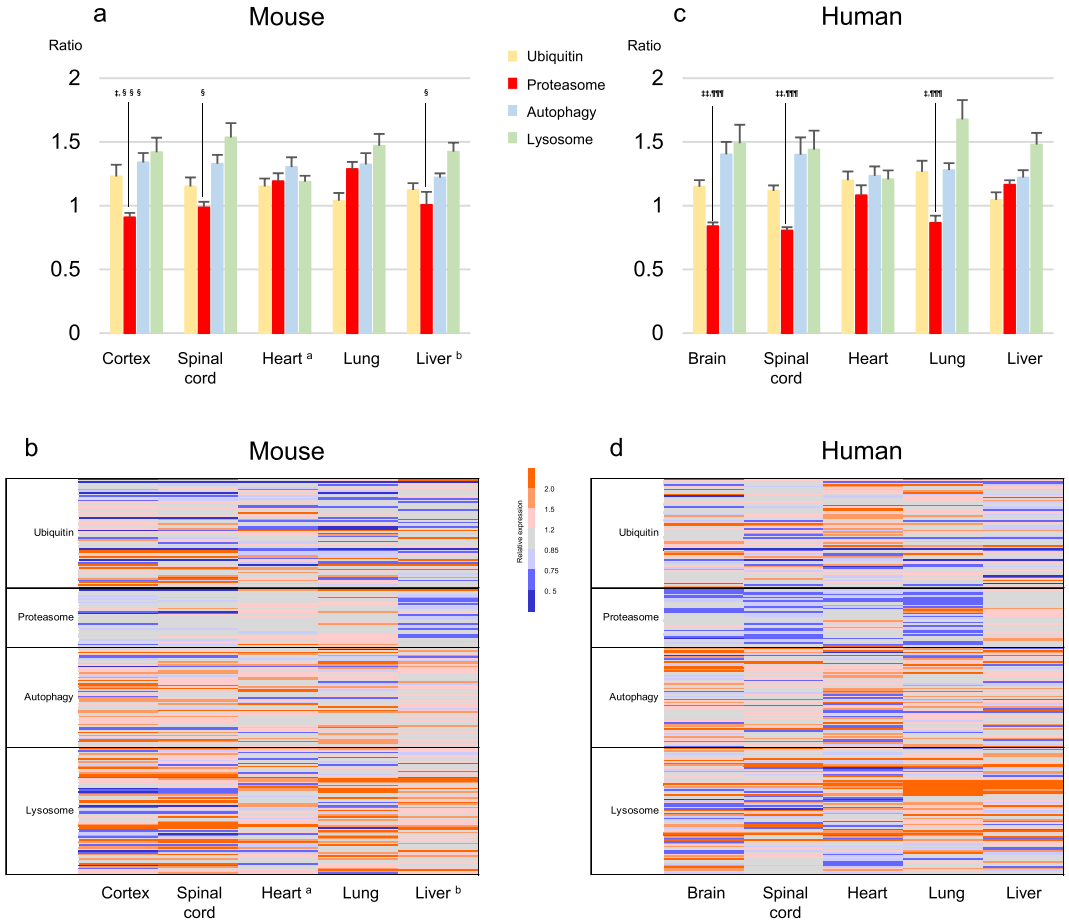

Fig. 1. Cap analysis gene expression (CAGE) analyses. Relative gene expression values were obtained for ubiquitin, proteasome, autophagy, and lysosome in the CNS tissues as well as the heart, lung, and liver from the CAGE datasets of (**a,b**) mouse and (**c,d**) human. The results are presented in heatmaps; genes are ordered in the same succession as that in S1 Table. (**a,b**) Low expression levels in the cortex compared with that in the heart ($p < 0.05$) and lung ($p < 0.001$); in the spinal cord and liver compared to that in the lung ($p < 0.05$) were observed. (**c,d**) In humans, significantly low expression of the proteasome genes in the brain, spinal cord, and lung were observed. ‡: $p < 0.05$, ‡‡: $p < 0.01$ compared to heart; §: $p < 0.05$, §§§: $p < 0.001$ compared to lung; ¶¶¶: $p < 0.001$ compared to liver (Bonferroni *post hoc* comparison). a, derived from neonate 30; b, derived from adult pregnant day 1.

3.3. Microarray analyses of cells and tissues

The comparisons were performed on mouse tissue (Fig. 2a and b), human tissue (Fig. 2c and d) and human cells (Fig. 2e and f), since there was no heart cell data from mouse microarray. In mouse tissue analyses (Fig. 2a and b), the one-way ANOVA showed significant differences in the proteasome and lysosome genes expression in tissues. *Post hoc* analyses revealed that proteasome expression in the frontal lobe was significantly lower than that in other tissues followed by lung and spinal cord. Lysosome expressions of the frontal lobe and lung were also significantly lower.

Overall, more distinct trends were observed while comparing the human array dataset (Fig. 2c and d). Markedly low expression of proteasome genes in the frontal lobe and spinal cord, and lysosome genes in the frontal lobe, spinal cord, and heart was observed, along with low expression levels of ubiquitin (against other four tissues) and autophagy (against other four tissues) in the spinal cord.

In human cell analyses (Fig. 2e and f), ubiquitin and proteasome genes expression were low in the CNS cells (brain and spinal cord); proteasome genes expression levels in the brain and spinal cord neurons were significantly less than 0.5. In the cases of the spinal cord neurons and heart, lysosome genes expression showed low trends. The high-level proteasome expression in liver, heat, and lung cells indicated relatively low expression of proteasome genes. The lysosome gene expression in the spinal cord and heart cells were significantly low.

3.4. Between cell lineage comparison in the human array

As the previous results suggested that the CNS tissues had less proteasome expression, we further assessed the developmental

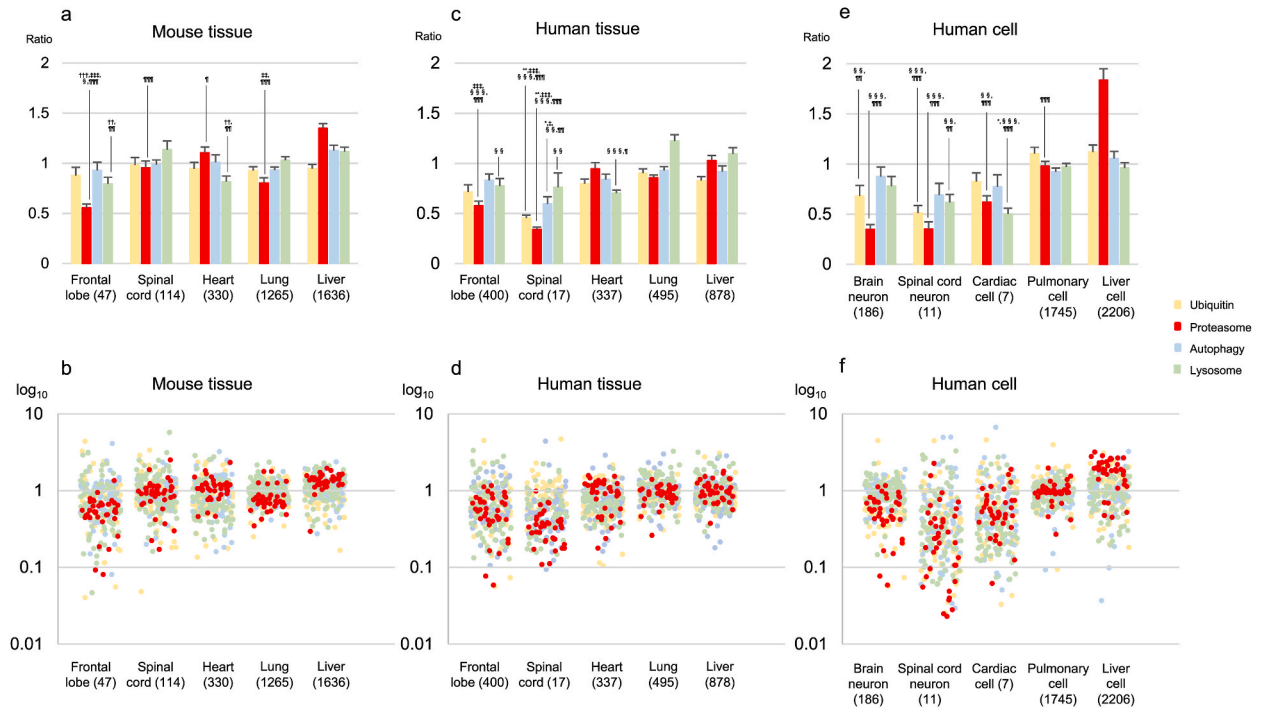

Fig. 2. Microarray analyses of tissues and cells. Relative expression levels of ubiquitin, proteasome, autophagy, and lysosome genes are presented for (**a,b**) mouse tissue, (**c,d**) human tissue, and (**e,f**) human cells. The results are presented as scatter plots. (**a,b**) In mouse, one-way ANOVA revealed statistically significant differences in the proteasome and lysosome categories. Proteasome gene expression was lower in the frontal lobe as compared with that in all other tissues, followed by that in the lung and spinal cord. Lysosome gene expression in the brain and heart were low as compared with that in the spinal cord and liver. (**c,d**) In human tissue analysis, the expression of the genes expressed through UPS and ALP in the spinal cord was markedly lower than that expressed by at least one part of other tissues. Proteasome gene expression in the brain and lysosome gene expression in the brain and heart were low. (**e, f**) In human cells, the low expression level of proteasome in the brain and spinal cord was significant, followed by the expression level of ubiquitin. In the spinal cord and heart, lysosome gene expression was low. Parentheses indicate number of array measurements. *: $p < 0.05$, **: $p < 0.01$ compared to brain; ††p < 0.01, †††p < 0.001 compared to spinal cord; ‡: $p < 0.05$, ‡‡: $p < 0.01$, ‡‡‡: $p <$ 0.001 compared to heart; §: $p < 0.05$, §§: $p < 0.01$, §§§: $p < 0.001$ compared to lung; ¶: $p < 0.05$, ¶¶: $p < 0.01$, ¶¶¶: $p < 0.001$ compared to liver.

aspects of neuronal cells. Desired datasets were only obtained from those of human CAGE data. Setting ES cell as 100%, the expression levels of the UPS and ALP are analyzed with iPS cells, neural stem cells, neurons, and astrocytes. As predicted, neural stem cell and neuron tended to show low level proteasome expressions compared to iPS or ES cells (Fig. 3a and b). In contrast, higher expression of lysosome genes in neural stem cell and neuron was correlated with the low expression of lysosome genes in iPS.

3.5. Proteomics comparison

Proteomics data were only obtainable for humans. In contrast to the gene expression data, the levels of proteasome proteins in the brain (Fig. 4) were high, following ubiquitin and followed by autophagy and lysosome proteins, which protruded other tissues thereby lowing the expression level ($p < 0.001$). In the spinal cord (Fig. 4), the overall protein expressions of the UPS and ALP was significantly low compared to those of brain ($p < 0.001$), consistent with the level of expression in the spinal cord based on the results of the human microarray analysis. However, the reason behind these differences remains to be determined.

3.6. Perturbation by agents

In the human array dataset, azithromycin (AZM) remarkably increased the expression levels of the UPS and ALP genes, up to a maximum of 47.7-, 33.5-, 53.0-, and 85.6-fold for ubiquitin, proteasome, autophagy, and lysosome genes, respectively (Table 2). The results of mRNA-Seq showed that the active form of vitamin D3 (1,25(OH)2D3 or calcitoriol) and all-trans retinoic acid (ATRA) were also inducers of UPS and ALP gene expression (Table 2). In the mouse datasets (S2 Table), phenobarbital and beta-adrenergic modulators (isoprenaline, a non-selective beta-adrenergic receptor agonist, and atenolol, a beta-adrenoreceptor antagonist) regulated the expression of both the UPS and ALP.

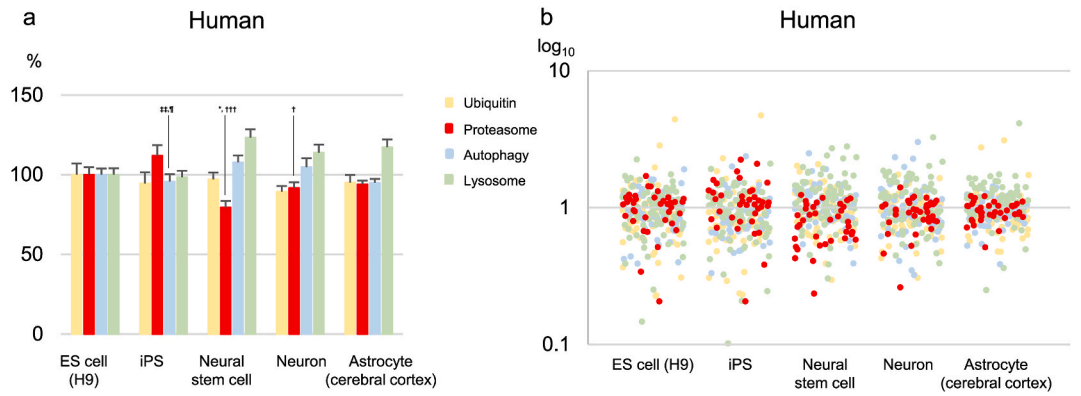

Fig. 3. The UPS and ALP expression from immature cells to neuronal cells (CAGE). (**a, b**) The relative expression levels of ubiquitin, proteasome, autophagy, and lysosome genes from immature cells to neuronal cells were presented with each value of ES cells as 100%. In only proteasome comparison, the expression levels of those in neural stem cells and neurons were significantly low compared to iPS cells ($p < 0.001$ in neural stem cells and $p < 0.05$ in neurons). Comparison between ES and neural stem cells differences in proteasome expression were also significant ($p < 0.05$). Lysosome expression in iPS cells was low compared to neural stem cells and astrocytes. *: $p < 0.05$ compared to ES cell; †: $p < 0.05$, †††: $p < 0.001$ compared to iPS; ‡‡: $p < 0.01$ compared to neural stem cell; ¶: $p < 0.05$ compared to astrocyte.

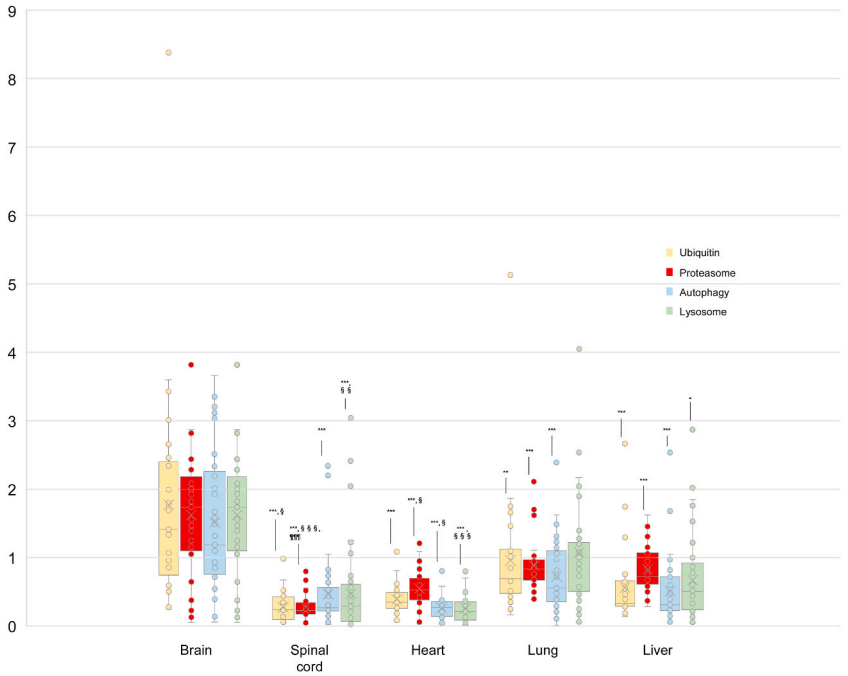

Fig. 4. Proteomics comparison. High levels of expression of the UPS and ALP in the brain were significant, so were the low levels of expression of the four categories in the spinal cord compared to other regions. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$ compared to brain, §: $p < 0.05$, §§: $p < 0.01$, §§§: $p < 0.001$ compared to lung, ¶¶¶: $p < 0.001$ compared to liver.

4. Discussion

Any perturbation of proteolytic systems results in the accumulation of harmful protein aggregates [4]. Perturbations are caused not only by genetic variation but also by aging or environmental factors. Regarding sporadic PD, α-synuclein (α-syn) is the major protein in Lewy bodies, and its rare mutations can cause early-onset PD, PARK1 [22] and PARK4 [23]. α-syn is degraded by the proteasome and autophagy [24]. Similarly, the activities of UPS and autophagy pathways are impaired, preventing adequate removal of amyloid β (Aβ) plaques and phosphorylated tau aggregates in AD [25]. Excessive amounts of Aβ42 block proteasome activity, promoting neurodegeneration and the accumulation of immature autophagosomes, which cannot clear the vast deposits of Aβ [25]. Additionally, lysosomal dysfunction reportedly enhanced neurofibrillary tangle accumulation and neurotoxicity [26]. Thus, the proteolytic systems are important in proteostasis. However, the current knowledge of the UPS and ALP status in the CNS is limited.

Table 2
Drug effect on protein groups (human).

Affymetrix					mRNA-Seq				
Sum of fold change	Drug	Sample	GEO accession	Study and description	Sum of fold change	Drug	Sample	GEO accession	Study and description
Ubiquitin					Ubiquitin				
47.7	azithromycin	bronchial epithelium cell	GSE10592	azithromycin study 1 (48h / 6h)	129.9	CaCl2	immortalized keratinocyte	GSE59275	keratinocyte differentiation study 1 (CaCl2; 5d / 2d)
39.2				azithromycin study 1 (48h / 24h)	50.8	IL-4			keratinocyte differentiation study 1 (CaCl2 + IL-4; + / -)
31.3				azithromycin study 1 (24h / 6h)	93.5	vitD3 (calcitoriol)	monocyte differentiated from HL-60	GSE79044	myeloid differentiation study 1 (96h / 6h)
42.9	IFN-a 2b + ribavirin	liver-infiltrating lymphocyte	GSE17183	hepatitis C study 6 (non-responder / responder)	85.4				myeloid differentiation study 1 (96h / 48h)
30.1		liver		hepatitis C study 4 (non-responder / responder)	78.5				myeloid differentiation study 1 (96h / 24h)
33.4	acetylsalicylic acid	whole blood	GSE38511	aspirin study 7 (≥ 550 ARU / < 500 ARU)	63.0				myeloid differentiation study 1 (12h / 6h)
20.9				aspirin study 7 (500-549 ARU / < 500 ARU)	73.7	ATRA			myeloid differentiation study 1 (96h / 12h)
32.9	TNF	mesenchymal stem cell	GSE24422	insulin study 2 (stromal; TNF + insulin / insulin)	62.0				myeloid differentiation study 1 (96h / 3h)
21.6	canakinumab	peripheral blood mononuclear cell	GSE68049	plasma stimulation study 6 (canakinumab; 12 m / baseline)	55.8				myeloid differentiation study 1 (96h / 24h)
9.2	VTX-2337 / 3M-055	peripheral blood monocyte	GSE64480	VTX-2337 study 1 / 3M-055 study 1	49.0				myeloid differentiation study 1 (48h / 12h)
Proteasome					Proteasome				
53.1	VTX-2337 / 3M-055	peripheral blood monocyte	GSE64480	VTX-2337 study 1 / 3M-055 study 1	63.0	PMA	monocyte differentiated from HL-60	GSE79044	myeloid differentiation study 1 (vitD3 + PMA; 12h / 6h)
46.2	TNF	mesenchymal stem cell	GSE24422	insulin study 2 (stromal; TNF + insulin / insulin)	53.7				myeloid differentiation study 1 (vitD3 + PMA; 24h / 6h)
36.7				insulin study 2 (mixed; TNF + insulin / insulin)	36.5				myeloid differentiation study 1 (vitD3 + PMA; 12h / 3h)
35.7				insulin study 2 (adipocyte; TNF + insulin / insulin)	35.4	vitD3 (calcitoriol)			myeloid differentiation study 1 (96h / 48h)
33.5	azithromycin	bronchial epithelium cell	GSE10592	azithromycin study 1 (48h / 24h)	34.2				myeloid differentiation study 1 (96h / 24h)
33.1	acetylsalicylic acid	whole blood	GSE38511	aspirin study 7 (500-549 ARU / < 500 ARU)	26.4				myeloid differentiation study 1 (12h / 6h)
18.3				aspirin study 7 (≥ 550 ARU / < 500 ARU)	44.8	R1881 + darolutamide	VCaP	GSE148397	darolutamide (0.5uM); R1881 (1nM) study 1 (22h / 8h)
31.7	IFN-a2b and ribavirin	liver	GSE17183	hepatitis C study 4 (non-responder / responder)	43.6				darolutamide (2uM); R1881 (1nM) study 1 (22h / 8h)

(*continued on next page*)

Table 2 (*continued*)

Affymetrix					mRNA-Seq				
Sum of fold change	Drug	Sample	GEO accession	Study and description	Sum of fold change	Drug	Sample	GEO accession	Study and description
21.4	vit D3 (calcitriol) / ATRA	peripheral blood monocyte	GSE46268	calcitriol study 7 (calcitriol) / vitamin A study 3 (ATRA)	36.6	BAY-155	MCF-7	GSE136272	BAY-155 + estradiol study 1 / estradiol study 35
18.3	IL-4	monocyte derived macrophage	GSE16385	IL-4 + rosiglitazone study 1 / rosiglitazone study 8	34.9	LPS	Neutrophil from HL-60	GSE79044	myeloid differentiation study 1 (ATRA + LPS + / -)
Autophagy					Autophagy				
53.0	azithromycin	bronchial epithelium cell	GSE10592	azithromycin study 1 (48h / 6h)	122.9	vitD3 (calcitoriol)	monocyte (HL-60)	GSE79044	myeloid differentiation study 1 (96h / 6h)
47.3				azithromycin study 1 (24h / 6h)	110.5				myeloid differentiation study 1 (96h / 24h)
23.5				azithromycin study 1 (48h / 24h)	90.0				myeloid differentiation study 1 (96h / 48h)
28.5	canakinumab	peripheral blood mononuclear cell	GSE68049	plasma stimulation study 6 (canakinumab; 9 m / baseline)	69.1				myeloid differentiation study 1 (96h / 12h)
20.9				plasma stimulation study 6 (canakinumab; 12 m / baseline)	55.6				myeloid differentiation study 1 (12h / 6h)
28.4	IFN-a2b and ribavirin	liver-infiltrating lymphocyte	GSE17183	hepatitis C study 6 (non-responder / responder)	95.5	ATRA			myeloid differentiation study 1 (96h / 3h)
22.7		liver		hepatitis C study 4 (non-responder / responder)	83.7				myeloid differentiation study 1 (96h / 12h)
14.4		hepatocyte		hepatitis C study 5 (non-responder / responder)	77.0				myeloid differentiation study 1 (96h / 24h)
13.5	acetylsalicylic acid	whole blood	GSE38511	aspirin study 7 (500-549 ARU / < 500 ARU)	62.7				myeloid differentiation study 1 (96h / 48h)
8.8	vit D3 (calcitriol)	RWPE-1 cells	GSE15947	calcitriol study 12 (100nM; 48h / 24h)	42.1	IL-4	immortalized keratinocyte	GSE59275	keratinocyte differentiation study 1 (CaCl2 + IL-4; + / -)
Lysosome					Lysosome				
85.6	azithromycin	bronchial epithelium cell	GSE10592	azithromycin study 1 (48h / 6h)	207.9	vitD3 (calcitoriol)	monocyte (HL-60)	GSE79044	myeloid differentiation study 1 (96h / 24h)
66.0				azithromycin study 1 (24h / 6h)	203.9				myeloid differentiation study 1 (96h / 6h)
60.8				azithromycin study 1 (48h / 24h)	173.7				myeloid differentiation study 1 (96h / 48h)
44.1	IFN-a2b and ribavirin	liver	GSE17183	hepatitis C study 4 (non-responder / responder)	137.0				myeloid differentiation study 1 (96h / 12h)
32.7	vit D3 (calcitriol)	RWPE-1 cells	GSE15947	calcitriol study 12 (100nM; 48h / 6h)	146.7	ATRA			myeloid differentiation study 1 (96h / 3h)
26.2				calcitriol study 12 (100nM; 24h / 6h)	114.3				myeloid differentiation study 1 (96h / 6h)
24.2	acetylsalicylic acid	whole blood	GSE38511	aspirin study 7 (≥ 550 ARU / < 500 ARU)	110.0				myeloid differentiation study 1 (96h / 12h)
21.7				aspirin study 7 (500-549 ARU / < 500 ARU)	99.8				myeloid differentiation study 1 (48h / 3h)

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Table 2 (*continued*)

Affymetrix					mRNA-Seq				
Sum of fold change	Drug	Sample	GEO accession	Study and description	Sum of fold change	Drug	Sample	GEO accession	Study and description
23.3	TNF	mesenchymal stem cell	GSE24422	insulin study 2 (stromal; TNF + insulin / insulin)	93.2				myeloid differentiation study 1 (96h / 24h)
22.8	GSK256066	immortalized bronchial epithelial cell line	GSE106710	GSK25606 + indacaterol study 2 / indacaterol study 1	94.1	vemurafenib	A-375	GSE64741	vemurafenib study 2 (48h / 6h)

IFN-a 2b: interferon-alpha 2b, ARU: aspirin reaction units, TNF: tumor necrosis factor, VTX-2337: toll-like receptor 8 agonist, 3M-055: toll-like receptor 7 agonist, vit D3; vitamin D3, ATRA; all-trans retinoic acid, biologically active form of vitamin A, IL-4: interleukin 4, RWPE-1: human HPV-18-transformed normal prostate epithelial cell line derived from a 54 years old male Caucasian, GSK256066: inhibitor of phosphodiesterase 4, HL-60: human primary cancer cell line derived from the peripheral blood of a patient with acute myeloid leukemia, PMA: phorbol myristate acetate, R1881: synthetic androgen, VCaP cell: human xenograft derived metastatic cancer cell line derived from the bone of a patient with carcinoma of the prostate and passaged as xenografts in mice, BAY-155: menin-MLL tool inhibitor, MCF-7: human metastatic cancer cell line derived from the pleural effusion of a patient (69 years old, caucasian) with adenocarcinoma of the breast, LPS: lipopolysaccharide, A-375: human primary cancer cell line derived from skin of 54 years old female patient with malignant amelanotic melanoma.

4.1. mRNA-protein correlation and rationale for the analyses

In the present bioinformatics analyses, the proteasome exhibited consistently low expression levels in the datasets regardless of CAGE or microarray compared to the heart, lung, or liver. The trends were more prominent in the human than mouse analyses. Further, the CAGE analysis in human revealed that only proteasome genes showed low level expression compared to iPS or ES cells.

Proteostasis is an equilibrium of protein levels controlled by factors such as the biogenesis, folding, trafficking, and degradation of proteins [27,28]. In terms of gene versus protein expression levels, mRNA transcript abundance is considered to correlate only partially with protein abundance, typically explaining one-to two-thirds of the variance in steady-state protein levels [29]. Furthermore, there are several instances wherein protein localization and/or status has been demonstrated to be more important than protein abundance. For example, the mislocalization of TAR-DNA binding protein-43 (TDP-43) from the nucleus to the cytoplasm and the formation of aggregates causes ALS and FTD [30,31]. In the case of p62, existing in either an oligomeric or non-oligomeric state, the fates of substrate/bound proteins are determined by this state; specifically, non-oligomeric p62 with bound proteins are processed via proteasomal degradation, whereas oligomeric p62 is preferentially an autophagy receptor that delivers substrates to the ALP [32]. Thus, gene expression is only an indicator of protein abundance and quite a modest predictor of protein functionality.

We attempted to analyze as many quintessential genes and their products as possible *en bloc* in each pathway, for example, the genes involved in autophagy positively (such as phosphatase and tensin homolog deleted on chromosome 10 or PTEN) and negatively (such as mammalian target of rapamycin or MTOR) were both included. Through this process, we anticipated to elucidate their functional size (or significance) within cells or tissues. We also anticipated that the process would minimize the mRNA-protein expression inconsistency. Despite our efforts, we found quite a discrepancy between gene expression and proteomic analyses in the present study. It is particularly difficult to discriminate the gray from the white matter in the mouse spinal cord, and it is too crude to analyze the brain without dividing it into gray and white matter in humans. This poor spatial resolution due to sample preparation, accompanied with the incompleteness of conventional proteomics techniques for quantification, as well as the small number of proteomics data reposited might have impacted this discrepancy.

4.2. UPS and ALP crosstalk and agents for intervention

The UPS and ALP share several regulators of expression. For example, nuclear factor erythroid-2-like 2 (Nrf2) is a dual activator of autophagy genes, including p62 [33] and proteasome genes [34], and transcription factor EB (TFEB) is a master regulator of lysosome biogenesis and autophagy [32]. Autophagy is upregulated under conditions of UPS deficiency [32], which is the result of activation of the unfolded protein response [35]. Conversely, a compromised state of autophagy results in upregulation of the proteasome through activation of the transcription factor Nrf2 [34]. This was shown to occur via increased p62 levels during autophagy deficiency, as p62 binds competitively to Nrf2, which is normally kept inactive by binding to Keap1 [36].

Surprisingly, drug intervention changed UPS and ALP expression dramatically, sometimes by several hundred orders of magnitude. Furthermore, agents that upregulated the UPS had a strong ability to upregulate the ALP. Mouse analyses have revealed that these agents are often used daily in the clinic. In addition to comprehensive gene expression experiments, there are examples of the effects of these agents on protein degradation systems. Phenobarbital, a widely used anticonvulsant, induces liver lysosomal enzymes during the autophagic phase in rats [37]. In addition, beta-adrenergic receptors regulate cardiac fibroblast autophagy and collagen degradation [38].

It is intriguing that the two vitamin species were found to be inducers of both the UPS and ALP. Vitamin D3 is implicated in the regulation of neuronal integrity among many other functions in the brain. Its influence on the physiopathology of neurodegenerative diseases has been continuously emphasized [39]. In an analysis of UV-exposed human skin biopsies, vitamin D3 was shown to induce an increase in macrophage autophagy [40]. In addition, vitamin D3 induces an autophagic transcriptional signature in normal mammary glands and luminal breast cancer cells [41].

ATRA, an active metabolite of vitamin A, is involved in the induction of neural differentiation, motor axon outgrowth, and neural patterning. Elevated signaling in adults triggers axon outgrowth, nerve regeneration, and maintenance of the differentiated state of adult neurons [42]. Further, ATRA induces autophagy through mechanisms that have not been fully elucidated [43].

Seemingly, the most effective agent, the antibiotic AZM, has been studied in relation to autophagy, but its effects remain controversial. It has been reported to inhibit autophagy flux in several human cancer cell lines [44]. However, other reports have indicated that AZM causes autophagy in airway smooth muscle cells [45]. At therapeutic concentrations, AZM has been demonstrated to increase the number of autophagosomes in macrophages [46]. In contrast to the results of the present study, molecular studies revealed that this increase is due to AZM inhibiting autophagosome degradation, rather than increasing its synthesis [46]. AZM was shown to be effective against brain ischemia [47] and spinal cord injury [48], although the proposed underlying therapeutic mechanism is an effect on macrophages or immunomodulation, not particularly on proteolytic modulation. To date, there seems to be no data regarding the therapeutic effects of AZM on neurodegenerative disorders. Notably, these findings related to perturbation were obtained using specific cell types or mice under certain conditions, with specific concentrations of agents. Further scientific verification is therefore crucial for repositioning these drugs.

This study has several limitations. First, there is no absolute standard in the expression levels of each protein when compared across cells and tissues. Therefore, it should be a relative value. Second, the results credibility is solely depending on the quality and quantity of the repository data. Third, the discrepancy between gene and proteomics expression levels, which we discussed, should be further investigated.

5. Conclusions

Repository bioinformatics data could be a powerful method offering a different approach for observing the normal and disease states of the CNS. Gene expression data provide detailed information with high spatial resolution. In our analyses, we consistently observed reduced proteasome expression and average to relatively high expression of ALP in the CNS. Accumulating evidence [32] indicates that oxidative stress upregulates UPS and ALP, whereas their expression reduces with age. The accumulation of environmental burdens and/or genetic susceptibility with basal expression patterns in the CNS may contribute to neurodegenerative disorders such as PD, AD and ALS. Adjusting this, for example, by upregulating UPS in the CNS, could be a promising treatment option for neurodegeneration. Moreover, because their regulatory systems overlap considerably, some methods for upregulating UPS and ALP may already be available.

Author contribution statement

Yasuhiro Watanabe: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Haruka Takeda; Naoto Honda: Contributed reagents, materials, analysis tools or data.

Ritsuko Hanajima: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

The authors do not have permission to share data.

Additional information

Supplementary content related to this article has been published online at [URL].

Ethical approval and consent

All bioinformatic analyses were carried out in accordance with relevant guidelines and regulations.

Availability of data and material

The CAGE expression datasets for human (RefEx_expression_CAGE_all_human_PRJDB1099.tsv.zip, https://doi.org/10.6084/m9.figshare.4028613) and mouse (RefEx_expression_CAGE_all_mouse_PRJDB1100.tsv.zip, https://doi.org/10.6084/m9.figshare.4028616) were obtained from RefEx (http://refex.dbcls.jp/). Each protein expression profile was obtained from ProteomicsDB (https://www.proteomicsdb.org/). Through Genevestigator (Nebion, Switzerland), we obtained data from the Affymetrix Human Genome U133 Plus 2.0 Array and the mRNA-Seq Gene Level *Homo sapiens* (ref: Ensembl 97, GRCh38.p12) datasets for *Homo sapiens*; and the Affymetrix Mouse Genome 430 2.0 Array and the mRNA-Seq Gene Level *Mus musculus* (ref. Ensembl 88, GRCm38.p5) datasets for *Mus musculus*.

Funding

This work was supported by Grants-in-Aid from the Research Committee of CNS Degenerative Diseases, Research on Policy Planning and Evaluation for Rare and Intractable Diseases, Health, Labour and Welfare Sciences Research Grants, the Ministry of Health, Labour and Welfare, Japan, and by JSPS KAKENHI (Grant Number JP20k07867-00).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank all the authors for their time and effort in this study, and all the institutions for their financial support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e18188.

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