A Bioinformatic Investigation of Proteasome And Autophagy Expression in The Central Nervous System

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Abstract

The ubiquitin proteasome system (UPS) and autophagy lysosome pathway (ALP) play major roles in protein quality control. However, data regarding the relative significance of UPS and ALP in the central nervous system (CNS) remain limited. In this study, we reckon the quantitative expression status of UPS- and ALP-related genes and their products in the CNS compared to that in other tissues. We collected human and mouse gene expression datasets from the reference expression dataset (RefEx) and Genevestigator (a tool for handling curated transcriptomic data from public repositories) and human proteomics data from the proteomics database (ProteomicsDB). The expression levels of genes and proteins in four categories—ubiquitin, proteasome, autophagy, and lysosome in cells and tissues were extracted. Perturbation of expression by drugs was also analyzed based on the four categories. Compared to that for the other three categories, proteasome gene expression was consistently low in the CNS of mice, and was more pronounced in humans. Neural stem cells and neurons showed low proteasome gene expressions when compared to non-neuronal stem cells. Proteomic analyses, however, did not show trends similar to those observed in the gene expression analyses. Perturbation analyses revealed that agents such as azithromycin and vitamin D3 upregulated the expression of both the UPS and ALP. Disproportional expression of the UPS and ALP might play a role in the pathophysiology of CNS disorders and this imbalance might be redressed by several therapeutic candidates.

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 neurodegenerative disorders, such as Parkinson's disease (PD) 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 (MSCs) derived from an ALS mice model [5], which expresses mutant 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, in comparison to that in other tissues. We referred to public datasets of mRNA expression and proteomics and then comprehensively compared levels of the UPS and ALP. The results indicated that compared to that in other tissues, the expression of proteasome in the CNS is low, whereas ALP expression is average or relatively high level. We also employed drug perturbation analyses, which demonstrated that the UPS and ALP share several common upstream regulators, revealing the possibility that a single intervention could modulate both UPS and ALP expression.

Results

Gene selection

Ubiquitin, proteasome, and autophagy-related genes were selected based on the commonality of their classification in two datasets, the Kyoto Encyclopedia of Genes and Genomes (KEGG) orthology and that reported by Singh et al [16] (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 Supplementary Table 1.

Cap analysis gene expression (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 (Figure 1A), while the values of the proteasome in the same regions were slightly smaller than 1.0. One-way analysis of variance (ANOVA) 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 (Figure 1B), 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).

Microarray analyses of cells and tissues

The comparisons were performed on mouse tissue (Figure 2A), human tissue (Figure 2B) and human cells (Figure 2C), since there was no heart cell data from mouse microarray. In mouse tissue analyses (Figure 2A), 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 (Figure 2B). 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, 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.

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 aspects of neuronal cells. Desired datasets were only those of human CAGE data, which we used to compare the expression levels (cf. compared to the array data (i.g., Figure 2B), decreased expression value of the proteasome was lower in the CAGE data (Figure 1B)). 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 (Figure 3). In contrast, higher expression of lysosome genes in neural stem cell and neuron was correlated with the low expression of lysosome genes in iPS.

Proteomics comparison

Proteomics data were only obtainable for humans. In contrast to the gene expression data, the levels of proteasome proteins in the brain (Figure 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 (Figure 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.

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 (Supplementary Table 2), 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.

Discussion

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. The ratio values in the brain and spinal cord in CAGE were 0.91 and 0.99 in mouse (Figure 1A), and 0.84 and 0.80 in human (Figure 1B), respectively; meanwhile, those in tissue array were 0.56 and 0.95 in mouse (Figure 2A), while 0.58 and 0.34 in human (Figure 2B), respectively. Further, the CAGE analysis in human revealed that only proteasome genes showed low level expression compared to iPS or ES cells.

Proteasome activity is most commonly measured by quantifying the proteolytic activity on substrates of the UPS. The gold standard for measuring autophagy activity is autophagic flux assessments [17]. Thus, measuring proteasome and autophagy activities in the CNS compared to those in other organs would be extremely challenging, particularly in humans. Thus, we addressed this question using a different approach. Using public bioinformatics data, we attempted to comprehend the basal expression levels of the UPS and ALP in the CNS relative to those in other cells or tissues.

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 [18]; it is not just a matter of gene expression. Proteostasis is an equilibrium of protein levels controlled by factors such as the biogenesis, folding, trafficking, and degradation of proteins [19 20]. 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 [21] [22]. 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 [23]. Thus, gene expression is only an indicator of protein abundance and quite a modest predictor of protein functionality.

We tried to minimize the mRNA-protein discrepancy by analyzing as many quintessential genes and their products as possible *en bloc* in each pathway, which we anticipated would help to predict their functionalities. Despite our efforts, we found an enormous discrepancy between gene expression and proteomic analyses in the present study. There is very little quantitative data (for example, immunoblot or functional activity data) comparing activity levels of the UPS and ALP in various tissues. We could obtain only one report that compared proteasome activity *ex vivo* in mouse tissues; however, the spinal cord was not included in this comparison [24]. Moreover, it is quite difficult to discriminate the gray matter 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. We believe that the incompleteness of conventional proteomics techniques for quantification and poor spatial resolution due to sample preparation could have impacted this discrepancy.

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 [25] and proteasome genes [26], and transcription factor EB (TFEB) is a master regulator of lysosome biogenesis and autophagy [23]. Autophagy is upregulated under conditions of UPS deficiency [23], which is the result of activation of the unfolded protein response [27]. Conversely, a compromised state of autophagy results in upregulation of the proteasome through activation of the transcription factor Nrf2 [26]. 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 [28].

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 [29]. In addition, beta-adrenergic receptors regulate cardiac fibroblast autophagy and collagen degradation [30].

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 [31]. In an analysis of UV-exposed human skin biopsies, vitamin D3 was shown to induce an increase in

macrophage autophagy [32]. In addition, vitamin D3 induces an autophagic transcriptional signature in normal mammary glands and luminal breast cancer cells [33].

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 [34]. Further, ATRA induces autophagy through mechanisms that have not been fully elucidated [35].

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 [36]. However, other reports have indicated that AZM causes autophagy in airway smooth muscle cells [37]. At therapeutic concentrations, AZM has been demonstrated to increase the number of autophagosomes in macrophages [38]. 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 [38]. AZM was shown to be effective against brain ischemia [39] and spinal cord injury [40], 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.

Gene expression data provide detailed information with high spatial resolution. As almost all experiments analyzed herein were carried out for different purposes, and they were deemed unbiased data for the current research. In our analyses, we consistently observed reduced expression of the proteasome, and average to relatively high expression of the ALP in the CNS. Increasing evidence [23] indicates that oxidative stress upregulates the UPS and ALP, whereas their expression is reduced with age. Together with the accumulation of environmental burdens and/or genetic susceptibility, the basal expression patterns in the CNS might contribute to neurodegenerative disorders such as PD and ALS. Adjusting this by upregulating the UPS in the CNS, for example, could be a promising option to treat neurodegeneration. Moreover, some methods to upregulate the UPS and ALP could already be available, as their regulatory systems overlap considerably.

Materials And Methods

Selection of genes for analyses. Ubiquitinating enzymes, proteasomal components, and autophagy-associated genes were selected using the pathway maps of the KEGG database (Release 98.1) (https://www.genome.jp/kegg/) and a published report from Singh et al [16]. 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.

Gene expression and proteomics databases. CAGE is an analytical technique to produce a snapshot of the 5' end of the capped (very beginning of) mRNA population in the transcriptome [41]. 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 [41] (http://refex.dbcls.jp/). We also used a commercial data analysis program, Genevestigator (Nebion, Switzerland) [42,43], 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 [44] (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 4 categories of proteins from which expression values in the brain, spinal cord, heart, lung, and liver were available.

Comparison of expression between different cells or tissues. As the CAGE data were expressed as $\log_2$ values and the Genevestigator data and ProteomicsDB data as $\log_{10}$ values, the data were converted to their antilogarithms for subsequent analyses. For CAGE analyses, references were set as the average expression value of that gene across different cells and tissues respectively in human and mouse. In other analyses, references were also set as the average expression value of genes across different cells or tissues. Analytical subjects were neuronal cells (neural stem cells, neurons, and astrocytes), multipotent stem cells (embryonic stem cells and iPS cells), and neural tissues (brain and spinal cord). Also, heart, lung, and liver cells or tissues were used for comparison.

Expression with drug intervention. 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.

Statistical analysis. Data analyses were performed using SPSS statistics, version 27 (IBM). One-way analysis of variance (ANOVA) followed by *post hoc* Bonferroni comparison was applied.

Declarations

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Author contributions

Yasuhiro Watanabe conceptualized and designed the study, acquired and analyzed the data, and drafted and revised the manuscript. Harka Takeda and Noato Honda revised the manuscript. Ritsuko Hanajima revised the manuscript. All authors approved the final version of the manuscript.

Competing interests

The authors declare no competing interests.

Abbreviations

UPS, ubiquitin-proteasome system; ALP, autophagy-lysosome pathway; MSCs, mesenchymal stem cells; PD, Parkinson's disease; ALS, amyotrophic lateral sclerosis; CNS, central nervous system; AZM, azithromycin; FTD, frontotemporal dementia

References

1 Collins, G. A. & Goldberg, A. L. The Logic of the 26S Proteasome. *Cell* **169**, 792-806 (2017).

2 Kwon, Y. T. & Ciechanover, A. The Ubiquitin Code in the Ubiquitin-Proteasome System and Autophagy. *Trends Biochem Sci* **42**, 873-886 (2017).

3 Riley, B. E. *et al.* Ubiquitin accumulation in autophagy-deficient mice is dependent on the Nrf2-mediated stress response pathway: a potential role for protein aggregation in autophagic substrate selection. *J Cell Biol* **191**, 537-552 (2010).

4 Watanabe, Y., Taguchi, K. & Tanaka, M. Ubiquitin, Autophagy and Neurodegenerative Diseases. *Cells* **9** (2020).

5 Une, M. *et al.* SOD1-interacting proteins: Roles of aggregation cores and protein degradation systems. *Neurosci Res* (2020).

6 Keskin, I. *et al.* Effects of Cellular Pathway Disturbances on Misfolded Superoxide Dismutase-1 in Fibroblasts Derived from ALS Patients. *PLoS One* **11**, e0150133 (2016).

7 Imamura, K. *et al.* The Src/c-Abl pathway is a potential therapeutic target in amyotrophic lateral sclerosis. *Sci Transl Med* **9** (2017).

8 Fecto, F. & Siddique, T. Making connections: pathology and genetics link amyotrophic lateral sclerosis with frontotemporal lobe dementia. *J Mol Neurosci* **45**, 663-675 (2011).

9 Sasaki, S. Autophagy in spinal cord motor neurons in sporadic amyotrophic lateral sclerosis. *J Neuropathol Exp Neurol* **70**, 349-359 (2011).

10 Majcher, V., Goode, A., James, V. & Layfield, R. Autophagy receptor defects and ALS-FTLD. *Mol Cell Neurosci* **66**, 43-52 (2015).

11 Freischmidt, A. *et al.* Haploinsufficiency of TBK1 causes familial ALS and fronto-temporal dementia. *Nat Neurosci* **18**, 631-636 (2015).

12 Cirulli, E. T. *et al.* Exome sequencing in amyotrophic lateral sclerosis identifies risk genes and pathways. *Science* **347**, 1436-1441 (2015).

13 Hattori, N. *et al.* Importance of familial Parkinson's disease and parkinsonism to the understanding of nigral degeneration in sporadic Parkinson's disease. *Adv Res Neurodegener* **8**, 101-116 (2000).

14 Lucking, C. B. *et al.* Association between early-onset Parkinson's disease and mutations in the parkin gene. *New Engl J Med* **342**, 1560-1567 (2000).

15 Bang, Y., Kim, K. S., Seol, W. & Choi, H. J. LRRK2 interferes with aggresome formation for autophagic clearance. *Molecular and Cellular Neuroscience* **75**, 71-80 (2016).

16 Singh, S. R. *et al.* A high-throughput screening identifies ZNF418 as a novel regulator of the ubiquitin-proteasome system and autophagy-lysosomal pathway. *Autophagy*, 1-16 (2020).

17 Klionsky, D. J. *et al.* Guidelines for the use and interpretation of assays for monitoring autophagy (3rd edition). *Autophagy* **12**, 1-222 (2016).

18 Vogel, C. & Marcotte, E. M. Insights into the regulation of protein abundance from proteomic and transcriptomic analyses. *Nat Rev Genet* **13**, 227-232 (2012).

19 Balch, W. E., Morimoto, R. I., Dillin, A. & Kelly, J. W. Adapting proteostasis for disease intervention. *Science* **319**, 916-919 (2008).

20 Powers, E. T., Morimoto, R. I., Dillin, A., Kelly, J. W. & Balch, W. E. Biological and chemical approaches to diseases of proteostasis deficiency. *Annu Rev Biochem* **78**, 959-991 (2009).

21 Arai, T. *et al.* TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Biochem Bioph Res Co* **351**, 602-611 (2006).

22 Neumann, M. *et al.* Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. *Science* **314**, 130-133 (2006).

23 Sun-Wang, J. L., Ivanova, S. & Zorzano, A. The dialogue between the ubiquitin-proteasome system and autophagy: Implications in ageing. *Ageing Res Rev* **64**, 101203 (2020).

24 Berkers, C. R. *et al.* Profiling proteasome activity in tissue with fluorescent probes. *Mol Pharm* **4**, 739-748 (2007).

25 Pajares, M. *et al.* Transcription factor NFE2L2/NRF2 is a regulator of macroautophagy genes. *Autophagy* **12**, 1902-1916 (2016).

26 Kwak, M. K., Wakabayashi, N., Greenlaw, J. L., Yamamoto, M. & Kensler, T. W. Antioxidants enhance mammalian proteasome expression through the Keap1-Nrf2 signaling pathway. *Mol Cell Biol* **23**, 8786-8794 (2003).

27 Hetz, C., Chevet, E. & Oakes, S. A. Proteostasis control by the unfolded protein response. *Nat Cell Biol* **17**, 829-838 (2015).

28 McMahon, M., Itoh, K., Yamamoto, M. & Hayes, J. D. Keap1-dependent proteasomal degradation of transcription factor Nrf2 contributes to the negative regulation of antioxidant response element-driven gene expression. *J Biol Chem* **278**, 21592-21600 (2003).

29 Glaumann, H., Arborgh, B. & Lindeborg, T. Induction of liver lysosomal enzymes during the autophagic phase following phenobarbital treatment of rat. *Virchows Arch B Cell Pathol* **23**, 17-28 (1977).

30 Aranguiz-Urroz, P. *et al.* Beta(2)-adrenergic receptor regulates cardiac fibroblast autophagy and collagen degradation. *Biochim Biophys Acta* **1812**, 23-31 (2011).

31 Morello, M. *et al.* The Influence of Vitamin D on Neurodegeneration and Neurological Disorders: A Rationale for its Physio-pathological Actions. *Curr Pharm Des* **26**, 2475-2491 (2020).

32 Das, L. M., Binko, A. M., Traylor, Z. P., Peng, H. & Lu, K. Q. Vitamin D improves sunburns by increasing autophagy in M2 macrophages. *Autophagy* **15**, 813-826 (2019).

33 Tavera-Mendoza, L. E. *et al.* Vitamin D receptor regulates autophagy in the normal mammary gland and in luminal breast cancer cells. *Proc Natl Acad Sci U S A* **114**, E2186-E2194 (2017).

34 Maden, M. Retinoic acid in the development, regeneration and maintenance of the nervous system. *Nat Rev Neurosci* **8**, 755-765 (2007).

35 Orfali, N., McKenna, S. L., Cahill, M. R., Gudas, L. J. & Mongan, N. P. Retinoid receptor signaling and autophagy in acute promyelocytic leukemia. *Exp Cell Res* **324**, 1-12 (2014).

36 Tanaka, H. *et al.* Comparison of autophagy inducibility in various tyrosine kinase inhibitors and their enhanced cytotoxicity via inhibition of autophagy in cancer cells in combined treatment with azithromycin. *Biochem Biophys Rep* **22** (2020).

37 Stamatiou, R. *et al.* Azithromycin has an antiproliferative and autophagic effect on airway smooth muscle cells. *Eur Respir J* **34**, 721-730 (2009).

38 Renna, M. *et al.* Azithromycin blocks autophagy and may predispose cystic fibrosis patients to mycobacterial infection. *J Clin Invest* **121**, 3554-3563 (2011).

39 Amantea, D. *et al.* Azithromycin Affords Neuroprotection in Rat Undergone Transient Focal Cerebral Ischemia. *Front Neurosci* **13**, 1256 (2019).

40 Kopper, T. J. *et al.* Delayed Azithromycin Treatment Improves Recovery After Mouse Spinal Cord Injury. *Front Cell Neurosci* **13**, 490 (2019).

41 Ono, H., Ogasawara, O., Okubo, K. & Bono, H. RefEx, a reference gene expression dataset as a web tool for the functional analysis of genes. *Sci Data* **4** (2017).

42 Hruz, T. *et al.* Genevestigator v3: a reference expression database for the meta-analysis of transcriptomes. *Adv Bioinformatics* **2008**, 420747 (2008).

43 Hruz, T. *et al.* RefGenes: identification of reliable and condition specific reference genes for RT-qPCR data normalization. *BMC Genomics* **12**, 156 (2011).

44 Samaras, P. *et al.* ProteomicsDB: a multi-omics and multi-organism resource for life science research. *Nucleic Acids Res* **48**, D1153-D1163 (2020).

Tables

Table 1 Protein selection in analyses

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

Table 2 Drug effect on protein groups (human)

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 apatient 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 xenograftsin 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 cellline derived from skin of 54 years old female patient with malignant amelanotic melanoma.

Figures

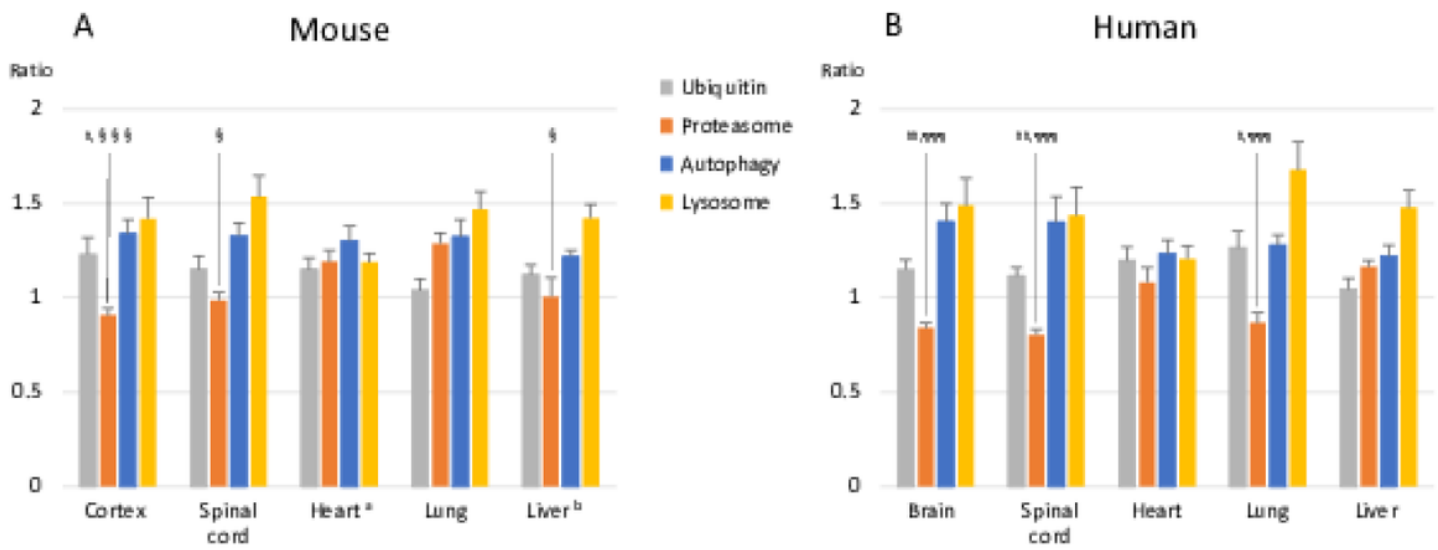

Figure 1

Cap analysis gene expression (CAGE) analyses Relative gene expression values were obtained for ubiquitin (gray), proteasome (orange), autophagy (blue), and lysosome (yellow) components in the CNS tissues as well as heart, lung, and liver from the CAGE datasets of (A) mouse and (B) human. (A) Low expression levels in the cortex compared to heart ($p < 0.05$) and lung ($p < 0.001$); in the spinal cord and liver compared to lung ($p < 0.05$) were observed. (B) In humans, significantly low expression of the proteasome genes in 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.

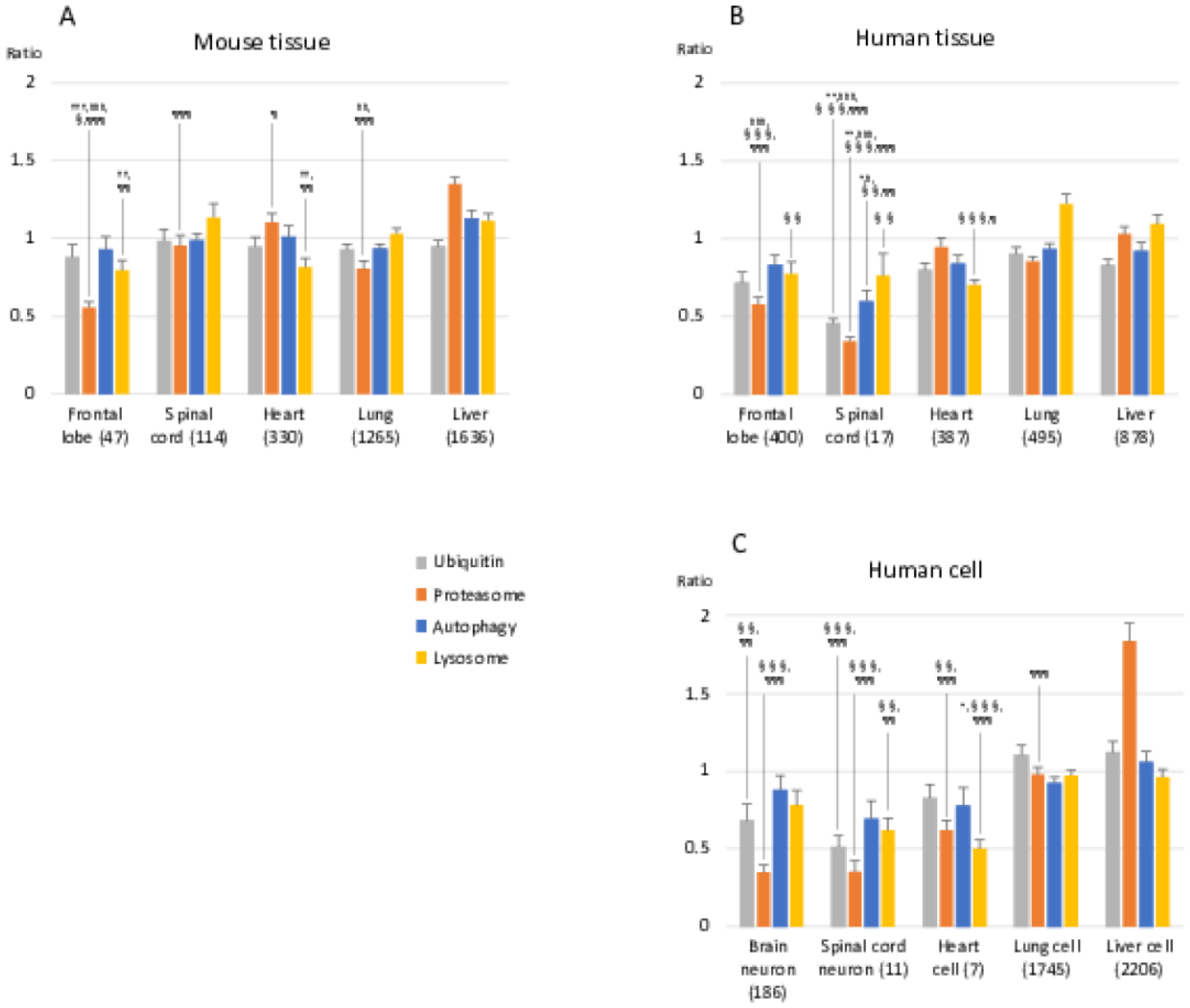

Figure 2

Microarray analyses of tissues and cells (A-C) Relative expression levels of ubiquitin, proteasome, autophagy, and lysosome genes are presented for (A) mouse tissues, (B) human tissues, and (C) human cells. In mouse (A), one way ANOVA test revealed statistically significant differences in proteasome and lysosome categories. Proteasome gene expression in frontal lobe was low compared to all other tissues followed by the lung and spinal cord. Lysosome gene expressions in brain and heart were low compared to the spinal cord and liver. In human tissue analysis (B), the expression of the UPS and ALP genes in the spinal cord were markedly low compared to at least one part of other tissues. Proteasome gene expression in brain and lysosome gene expression in brain and heart were low. In human cell (C), the low expression level of proteasome in brain and spinal cord were significant, followed by those of ubiquitin. In the spinal cord and heart, lysosome gene expression was also 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.

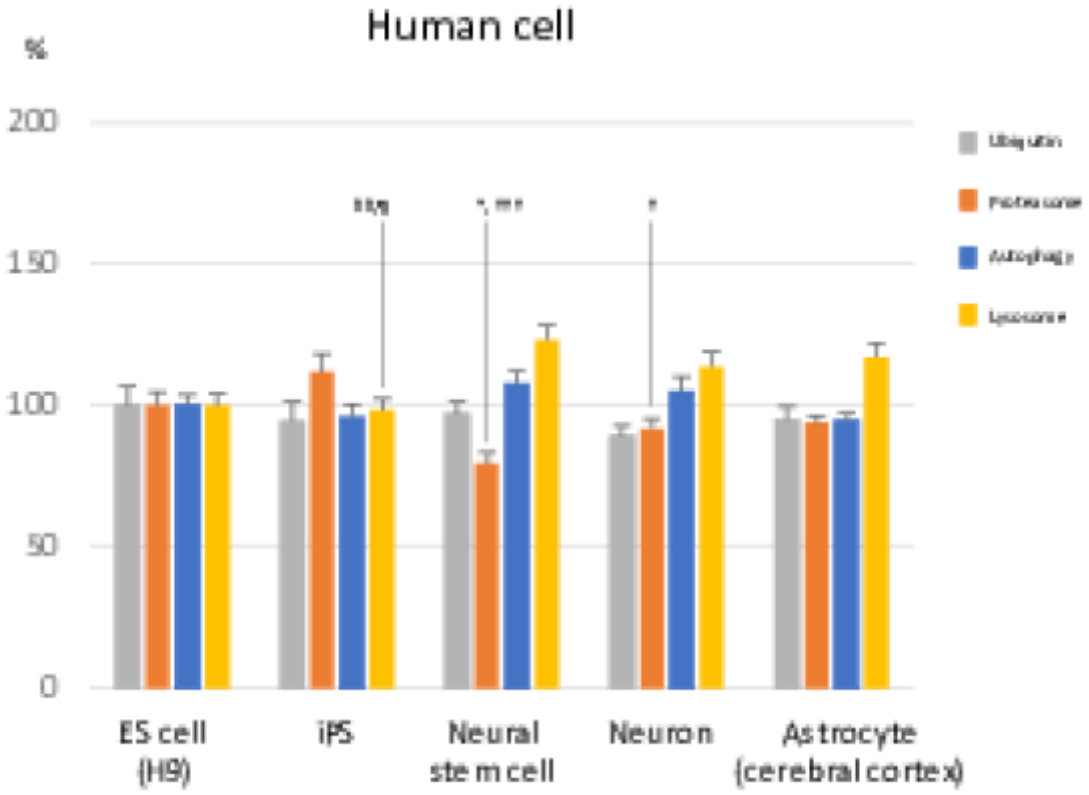

Figure 3

The UPS and ALP expression from immature cells to neuronal cells (CAGE). 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.

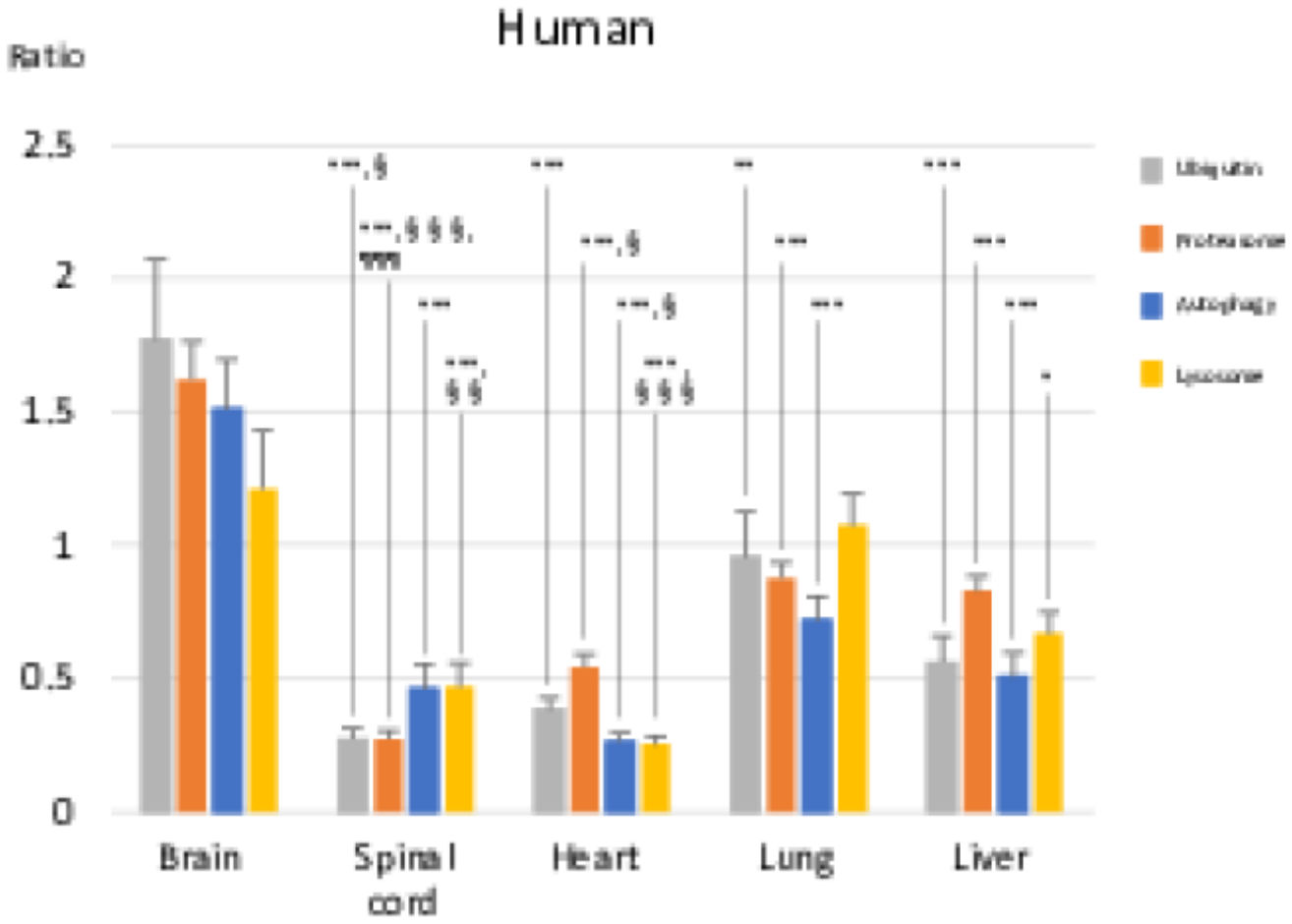

Figure 4

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.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TablesSupple.pdf