Phenomics (2022) 2:194–200
https://doi.org/10.1007/s43657-022-00045-2

ARTICLE

Immunosuppression Induced by Brain-Specific HDAC6 Knockdown Improves Aging Performance in *Drosophila melanogaster*

Yingying Zhao[1] · Hongwen Xuan[2] · Chao Shen[1] · Peiyi Liu[1] · Jing-Dong J. Han[2,3] · Wei Yu[1]

Received: 1 August 2021 / Revised: 15 January 2022 / Accepted: 19 January 2022 / Published online: 23 February 2022

Abstract

HDAC6 is involved in several biological processes related to aging-associated diseases. However, it was unknown whether HDAC6 could directly regulate lifespan and healthspan. We found that HDAC6 knockdown induced transcriptome changes to attenuate the aging changes in the *Drosophila* head, particularly on the inflammation and innate immunity-related genes. Whole-body knockdown of HDAC6 extended lifespan in the fly, furthermore brain-specific knockdown of HDAC6 extended both lifespan and healthspan in the fly. Our results established HDAC6 as a lifespan regulator and provided a potential anti-aging target.

Keywords HDAC6 · Aging · *Drosophila* · Inflammation · Innate immunity · Motor neuron

Introduction

Aging is an inevitable process in nearly all living organisms accompanied by systemic declines in tissue or cellular functions, such as neurodegeneration, immune dysfunction, and reduced mobility (Campisi 2013). Increasing evidence from model animals shows that aging could be delayed with genetic perturbation or drug treatment on conserved signaling pathways (Kenyon 2010) or epigenetic factors (Sen et al. 2016). Blocking histone deacetylases (HDACs) with HDAC inhibitors is a highly promising strategy in anti-aging studies (Pasyukova and Vaiserman 2017). However, the pan-HDAC inhibitors also generate toxic effects due to global influence on unwanted targets (Qin et al. 2017). It is essential to figure out the individual functions of different HDACs in lifespan regulation.

HDAC6 is one of the class IIb HDAC family proteins targeted by the most commonly used HDAC inhibitors (Qin et al. 2017). Unlike other family members, HDAC6 contains a cytoplasmic retention signal, and one of its well-known cellular functions is tubulin deacetylation (Valenzuela-Fernandez et al. 2008). HDAC6 is also involved in several biological processes related to aging-associated diseases. For instance, HDAC6 regulates innate immunity by directly interact with MyD88 and β-Catenin (Moreno-Gonzalo et al. 2018). Recently, the deletion of HDAC6 activates NLRP3 and pyrin's inflammasomes by an aggresome-like mechanism (Magupalli et al. 2020). Inhibiting HDAC6 shows beneficial effects on several common neurodegenerative diseases (Simoes-Pires et al. 2013). Its depletion also shows a protective effect against muscle atrophy (Ratti et al. 2015). Consistently, earlier, we found that among HDACs, only HDAC6 knockout induced global alternation of RNA expression (even in the liver) that mimicked the effect of HDAC inhibitor SAHA treatment and anti-correlated with aging transcriptome in the mammalian brain (Cheng et al. 2018). Interestingly, Weiwei Dang's Group recently used

Yingying Zhao and Hongwen Xuan have contributed equally to this work.

✉ Jing-Dong J. Han
jackie.han@pku.edu.cn

✉ Wei Yu
yuw@fudan.edu.cn

1 State Key Laboratory of Genetic Engineering, School of Life Sciences, Zhongshan Hospital, Fudan University, Shanghai 200438, China

2 CAS Key Laboratory of Computational Biology, Shanghai Institutes for Biological Sciences, CAS-MPG Partner Institute for Computational Biology, Shanghai Institute of Nutrition and Health, Chinese Academy of Sciences, 320 Yue Yang Road, Shanghai 200031, China

3 Peking-Tsinghua Center for Life Sciences, Center for Quantitative Biology (CQB), Academy for Advanced Interdisciplinary Studies, Peking University, Beijing 100871, China

the high-throughput Sequencing-Based Yeast replicative Lifespan screen method to demonstrate that the HAD complex regulates aging (Yu et al. 2021). They also identified that knockdown of the HAD homologs in *C. elegans* and *Drosophila* increases their lifespan (Yu et al. 2021). These observations led us to test whether HDAC6 could directly regulate lifespan and healthspan.

Materials and Methods

Drosophila Stocks and Maintenance

Drosophila stocks were raised on cornmeal-molasses fly food (unless otherwise noted) at 25 °C on a 12 h:12 h light–dark cycle. The *Da-GAL4* and *Elav-GAL4* driver lines were kindly provided by Dr. Zhiqiang Yan of Fudan University who were gifted from YuhNung Jan's Lab, and the *nsyb-GAL80-DD* driver line was kindly provided by Dr. Yufeng Pan of Southeast University who was gifted from Dr. Jing W. Wang (Sethi and Wang 2017). UAS-HDAC6-RNAi line (THU0614/BL34072) was purchased from Tsinghua Fly Center of Tsinghua University (Ni et al. 2009, 2011). A single male fly was backcrossed with w; if/CyO; MKRS,sb/TM6B females to control the potential variance of the genetic background. The backcrossed RNAi males were then crossed with Gal4 virgin females.

Drosophila Lifespan and Behavior Analysis

Lifespan and behavioral analyses were performed using male flies. For the determination of adult offspring frequencies, the number of adult flies eclosing were counted for each genotype. The UAS/Gal4 system with tissue-specific promotors was used to express UAS-HDAC6 RNAi transgenes for assaying longevity conditionally. Adult flies carrying the *Da-Gal4*, *Elva-Gal4*, and *UAS-HDAC6 RNAi* transgenes were crossed. Male flies were collected within 24 h of eclosion and grouped into batches of 20 flies per food vial. For assaying longevity of specific knockdown of HDAC6 in the brain of old flies, *nsyb-GAL80-DD* male flies were crossed with *Elva-Gal4* female flies to collect the *Elav-Gal4*;; *nsyb-GAL80-DD* female flies, and then crossed with *UAS-HDAC6 RNAi* male flies. To inducible knockdown HDAC6 in old flies' brains, flies were collected within 24 h of eclosion and treated with DMSO or the 0.5 mM trimethoprim (TMP) (Apexbio Inc., B2057) containing food every 3 days. From 25-day adult, flies were transferred to new vials without TMP containing food. The number of dead flies was counted every day, and flies were transferred to fresh food vials every two days. At least 200 flies per genotype were used.

Flies for motor performance assays were kept at 25 °C with a 12-h light/dark cycle. Male flies were collected within 24 h after eclosion and divided into groups of 20 individuals. Motor performance of 3- to 42-day-old flies was evaluated. On the assay day, flies were transferred in test tubes without anesthesia and assayed within 15 min under standardized daylight conditions. The test tubes were loaded into a self-made device, which was released from a certain height. The device fell down onto the table, shaking the flies to the bottom of the test tubes and inducing a negative geotaxis climbing response. The whole procedure was videotaped with a camera and repeated four more times. The average climbing time of the top 5 flies to reach 6 cm was determined and compared between genotypes. At least 5 tubes with 20 flies per tube were used for each genotype.

RNA-Seq Samples' Preparation and Data Analysis

Isolated male *Drosophila* heads were lysed in TRIzol (ThermoFisher, Cat. # 15596026). Total RNA was extracted according to TRIzol standard procedures. RNA integrities of samples were measured by Agilent RNA 6000 Nano Kit (Cat. # 5067-1511). Sequencing libraries were constructed using Illumina standard protocols. PolyA plus 150 bp paired-end reads were generated by the Illumina Nova-seq platform.

Reads from RNA-seq were mapped to *Drosophila melanogaster* dmel r6.21 genome using STAR (v2.5.2a) (Dobin et al. 2013) with 2% max mismatch. Cuffdiff (v2.2.1) (Trapnell et al. 2012) with default parameters was used to generate RPKM and define DEGs (p value < 0.05). DAVID (v6.8) (Huang et al. 2009a, b) was used to do Gene Ontology biological process annotations, and Benjamini adjust p value is shown.

Statistical Analysis

GraphPad Prism software (GraphPad) was used for all statistical analysis. Survival curves of different genotypes were analyzed using log-rank test. Unpaired t test was used to analyze behavior data. All data are reported as the mean Standard Error of Mean (SEM).

Results

To explore the significant targets of HDAC6 during the aging process, we generated transcriptome profiles of *Drosophila* head from ubiquitously expressed driver HDAC6 knockdown (Da-GAL4>UAS-HDAC6 RNAi) and paired control on the adult day (AD) 2 and AD12. In the WT group, we obtained 169 age up-regulated (Age-Up) and 344 age down-regulated (Age-Down) genes (Fig. 1a, cuffdiff p value < 0.05). The Age-Up genes were enriched with innate immunity functions (Fig. S1), and Age-Down genes were mainly related to mitochondrial functions and

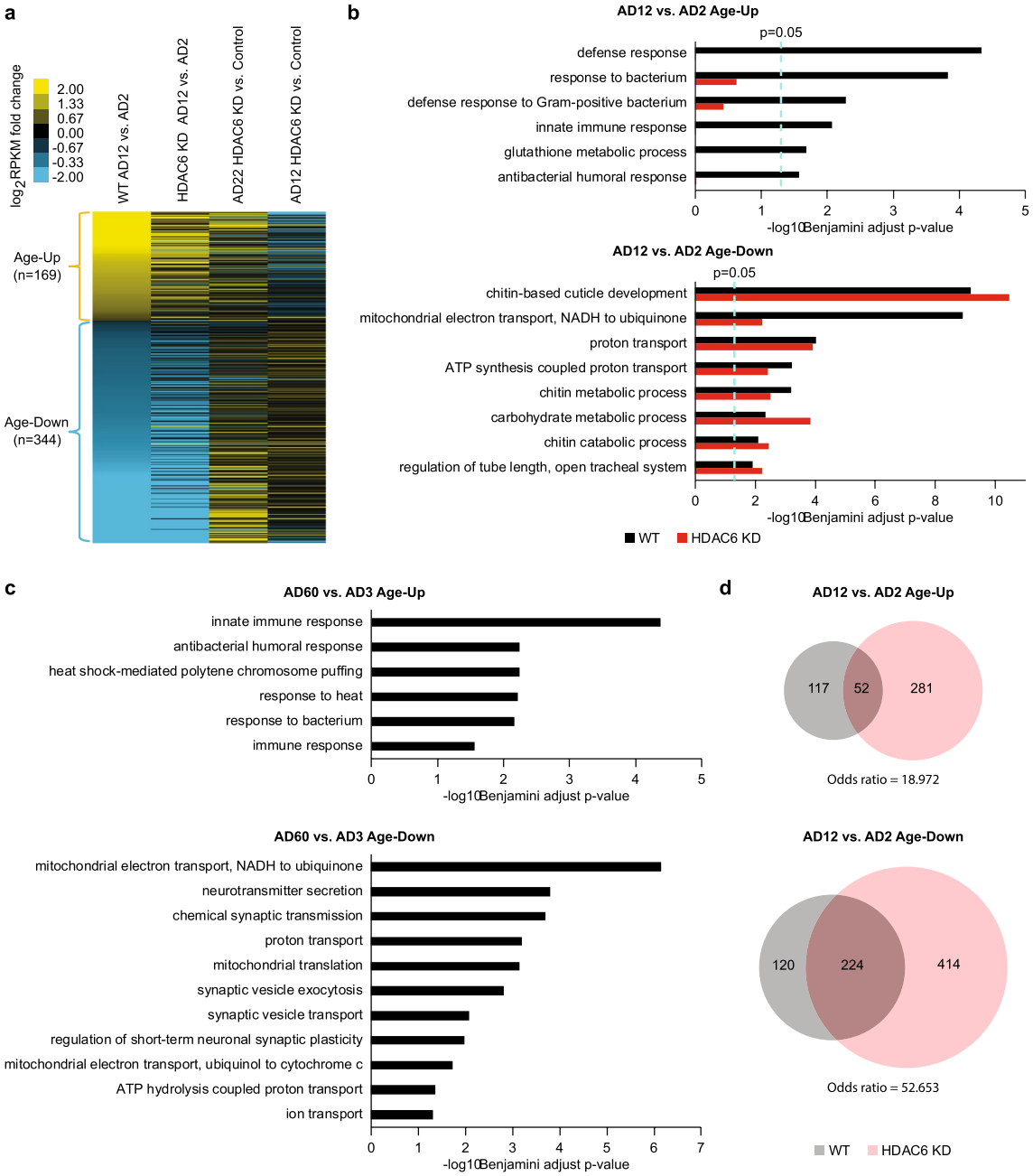
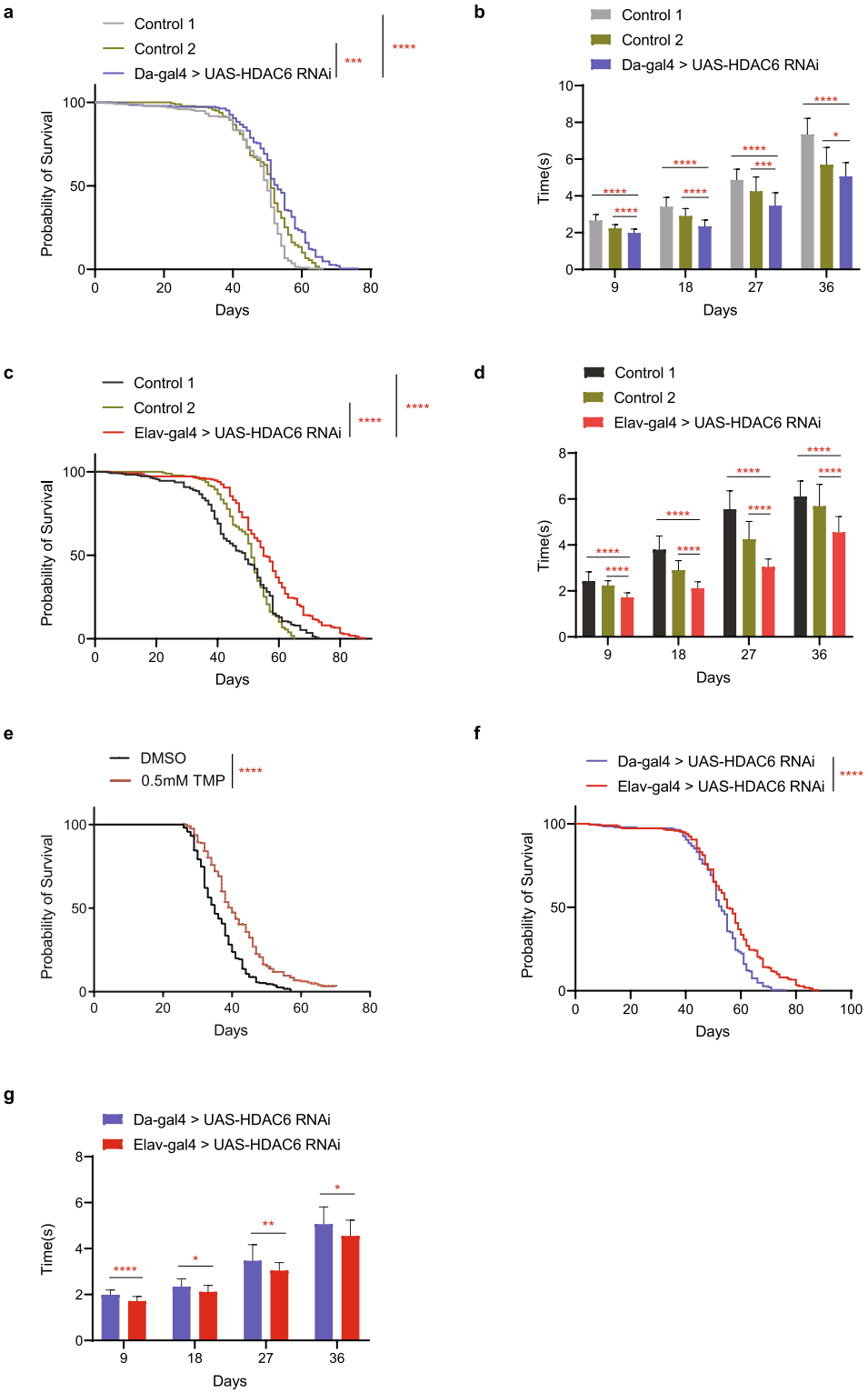


Fig. 1 HDAC6 knockdown suppressed immune-related transcripts. **a** HDAC6 knockdown attenuated age-related transcriptome changes in the fly head. DEGs were sorted by their $\log_2$ fold change of expression between WT AD12 vs AD2, HDAC6 KD vs AD2, AD22 HDAC6 KD vs AD22 control, and AD12HDAC6 KD vs AD12 control. **b** Significantly enriched GO biological processes among WT fly head AD12 vs. AD2 age-up and age-down genes determined by DAVID. **c** Significantly enriched GO biological processes among WT fly brain AD60 vs. AD3 age-up and age-down genes determined by DAVID. The major terms are shared with terms in **b**. **d** Age-down genes shared between WT and HDAC6 KD (lower panel) are much more than age-up genes (upper panel). The data are representative of three independent experiments

chitin metabolism (Fig. 1b). We also analyzed a published microarray data from AD60 fly neuron (Liu et al. 2012), which shows that AD12 flies already have similar aging transcriptional changes to very old flies (Fig. 1c). After HDAC6 KD (Fig. S2), age-related differential expressed genes (DEGs) were globally attenuated on AD12 (Fig. 1a, fourth column, Pearson Correlation Coefficient = − 0.32). However, only the innate immunity-related terms were significantly blocked (no longer enriched) in Age-Up genes compared with WT, and terms enriched in Age-Down



genes were largely unaffected (Fig. 1b). Consistent with GO terms results, Age-Down genes were more similar between WT and HDAC6 KD fly head than Age-Up genes (Fig. 1d). Together, HDAC6 positively regulated immune-related genes in the brain during *Drosophila* aging, and knockdown of HDAC6 could obviously suppress the over-activation of innate immunity.

To investigate whether HDAC6 knockdown could affect flies' lifespan, we next conducted the lifespan analysis of global HDAC6-RNAi flies. HDAC6-RNAi flies showed a

◂**Fig. 2** HDAC6 knockdown improved lifespan and healthspan of *Drosophila*. **a** ubiquitous knockdown of HDAC6 extends lifespan in *Drosophila*. Control groups genotypes: Da-Gal4/+;+/+ (Control 1) and +/+;UAS-HDAC6RNAi/+ (Control 2). Ubiquitous knockdown of HDAC6 group genotype: Da-Gal4/+;UAS-HDAC6RNAi/+. ****$p < 0.0001$ (log-rank test). $n = 200$ male flies for each genotype in survival experiments. **b** Climbing ability analysis of ubiquitous knockdown of HDAC6 male flies and two control groups. *T* test was used for statistics. $n = 100$ flies for each genotype in experiments. **c** Brain-specific knockdown of HDAC6 extends lifespan in *Drosophila*. Control groups genotypes: Elav-Gal4/+;+/+ (Control 1) and +/+;UAS-HDAC6RNAi/+ (Control 2). Brain-specific knockdown of HDAC6 group genotype: Elav-Gal4/+;UAS-HDAC6RNAi/+. ****$p < 0.0001$ (log-rank test). $n = 200$ male flies for each genotype in survival experiments. **d** Climbing ability analysis of brain-specific knockdown of HDAC6 male flies and two control groups. *T* test was used for statistics. $n = 100$ flies for each genotype in experiments. **e** Brain-specific knockdown of HDAC6 using GAL80-DD system in old flies extends lifespan. TMP removed from the fly food on day 25 to active GAL4-mediated RNAi. Log-rank test was used for statistics: ****$p < 0.0001$, $n > 180$ flies. **f** Brain-specific knockdown of HDAC6 improves more lifespan in flies compared with ubiquitous knockdown of HDAC6. Survival of brain-specific (red) and ubiquitous (blue) knockdown of HDAC6 male flies. ****$p < 0.0001$ (log-rank test). $n = 200$ flies for each genotype in survival experiments. **g** Brain-specific knockdown of HDAC6 increases more motor performance in flies compared with ubiquitous knockdown of HDAC6. Climbing ability analysis of brain-specific (red) and ubiquitous (blue) knockdown of HDAC6 male flies. *T* test was used for statistics. $n = 100$ flies for each genotype in experiments. The data are representative of three independent experiments

significantly longer lifespan than control flies of Da-gal4 and UAS-HDAC6 RNAi flies (Fig. 2a and Table S1, log-rank test *p* value < 0.0001). One of the significant biomarkers of aging in flies is the progressive decline in motor function (Grotewiel et al. 2005). To determine whether the knockdown of HDAC6 might affect the motor performance in flies, we next assessed HDAC6 knockdown flies' motor behavior using tube climbing assays. Consistent with previous reports, control flies showed progressively longer climbing times to reach the target during aging. In addition, we found that ubiquitously knockdown HDAC6 increased the climbing tube's ability compared with age-matched Da-gal4 and UAS-HDAC6 RNAi controls (Fig. 2b).

Since, targeting the expression or deacetylation activity of HDAC6 or SIRT2 has been implied to be beneficial in aging-related neurodegenerative diseases such as Alzheimer's disease (AD) (Govindarajan et al. 2013; Zhang et al. 2014), Charcot-Marie-Tooth neuropathy (CMT) (d'Ydewalle et al. 2011; Zhao et al. 2021), and amyotrophic lateral sclerosis (ALS) (Guo et al. 2017; Taes et al. 2013). We performed the lifespan analysis on brain-specific HDAC6 knockdown flies using the pan-neuronal elva-GAL4 driver. We found that knocking down of HDAC6 in the brain also significantly extended lifespan in flies compared with Da-gal4 and UAS-HDAC6 RNAi (Fig. 2c and Table S1, log-rank test *p* value < 0.0001). We also performed the tube climbing assays in brain-specific HDAC6 knockdown flies. The brain-specific knockdown of HDAC6 increased the ability of climbing tube compared with control Da-gal4 and UAS-HDAC6 RNAi (Fig. 2d). To specifically investigate the HDAC6 function in the brain of old flies, we generated a chemically inducible HDAC6 RNAi system in the brain of old flies using GAL80-DD (Sethi and Wang 2017). TMP was removed from the fly food on day 25 to active GAL4-mediated RNAi. Knocking down HDAC6 in old flies' brains also significantly extended the lifespan compared with the control flies (Fig. 2e and Table S1, log-rank test *p* value < 0.0001). Moreover, the lifespan and the climbing time of brain-specific knockdown of HDAC6 even significantly were improved compared with the whole-body HDAC6 knockdown (Fig. 2f–g), indicating the improvement of motor ability almost entirely contributed by neuron, consistent with the brain being more critical in controlling the age-related motor neuron function decline than other tissues and a more critical impact of HDAC6 in the brain on healthspan. Taken together, these results suggest that inhibition of HDAC6 in the brain could rescue the motor performance deficits of aging and extends the lifespan in the *Drosophila* model.

Although HDAC6 can directly regulate immune response and neural function through deacetylates non-histone targets in both cytoplasm and nucleus (Seidel et al. 2015), we cannot exclude histone deacetylase function as HDAC6 can be recruited to the genome by physically interacting with other factors (Cheng et al. 2014a, b). As the deacetylase activity of HDAC6 on histone has been detected (Wang et al. 2009), histone acetylation may function as a scaffold to support HDAC6 binding on histones.

Discussion

In conclusion, our data supported HDAC6 in the neural system and other tissues as an anti-aging target, whose knockdown at middle age elicited similar lifespan extension to constitutive knockdown. Mechanically, the down-regulation of inflammation and innate immunity-related genes after HDAC6 knockdown implies attenuated inflammaging (Franceschi et al. 2000) involved in its anti-aging effects, consistent with recent research of HDAC6 mediating the activation of NLRP3 and pyrin inflammasomes (Magupalli et al. 2020). Interestingly, recent work from the Weiwei Dang lab showed that the knockdown of the yeast HDA homologs in *C. elegans* and *Drosophila* increases their lifespan and delays aging-associated physical declines in adult flies (Yu et al. 2021). Together, all these studies conclude that suppression of HDAC6 could benefit the healthspan and lifespan in diverse species and suggest that inhibitors of HDAC6 could be a promising intervening strategy in aging.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s43657-022-00045-2.

Acknowledgements This work was supported by grants from the National Natural Science Foundation of China (31771545, 92049301, 31821002, 91329302, and 31210103916), China Ministry of Science and Technology (2015CB964803, 2016YFE0108700, and 2016YFA0500600), and Max Planck fellowship to J.D.J.H.

Authors' Contributions JDJH, WY, and HX conceived the project, YZ, PL, and CS performed the flies experiment, HX performed data analysis, and HX, JDJH, and WY wrote the manuscript.

Data and Code Availability Not applicable.

Declarations

Conflicts of Interest The authors declare no competing or conflicts of interest.

Ethics Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publication Not applicable.

References

Campisi J (2013) Aging, cellular senescence, and cancer. Annu Rev Physiol 75:685–705. https://doi.org/10.1146/annurev-physiol-030212-183653

Cheng F, Lienlaf M, Perez-Villarroel P, Wang HW, Lee C, Woan K, Woods D, Knox T, Bergman J, Pinilla-Ibarz J, Kozikowski A, Seto E, Sotomayor EM, Villagra A (2014a) Divergent roles of histone deacetylase 6 (HDAC6) and histone deacetylase 11 (HDAC11) on the transcriptional regulation of IL10 in antigen presenting cells. Mol Immunol 60:44–53. https://doi.org/10.1016/j.molimm.2014.02.019

Cheng F, Lienlaf M, Wang HW, Perez-Villarroel P, Lee C, Woan K, Rock-Klotz J, Sahakian E, Woods D, Pinilla-Ibarz J, Kalin J, Tao J, Hancock W, Kozikowski A, Seto E, Villagra A, Sotomayor EM (2014b) A novel role for histone deacetylase 6 in the regulation of the tolerogenic STAT3/IL-10 pathway in APCs. J Immunol 193:2850–2862. https://doi.org/10.4049/jimmunol.1302778

Cheng H, Xuan H, Green CD, Han Y, Sun N, Shen H, McDermott J, Bennett DA, Lan F, Han JJ (2018) Repression of human and mouse brain inflammaging transcriptome by broad gene-body histone hyperacetylation. Proc Natl Acad Sci USA 115:7611–7616. https://doi.org/10.1073/pnas.1800656115

Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, Batut P, Chaisson M, Gingeras TR (2013) STAR: ultrafast universal RNA-seq aligner. Bioinformatics 29:15–21. https://doi.org/10.1093/bioinformatics/bts635

d'Ydewalle C, Krishnan J, Chiheb DM, Van Damme P, Irobi J, Kozikowski AP, Vanden Berghe P, Timmerman V, Robberecht W, Van Den Bosch L (2011) HDAC6 inhibitors reverse axonal loss in a mouse model of mutant HSPB1-induced Charcot-Marie-Tooth disease. Nat Med 17:968–974. https://doi.org/10.1038/nm.2396

Franceschi C, Bonafe M, Valensin S, Olivieri F, De Luca M, Ottaviani E, De Benedictis G (2000) Inflamm-aging. An evolutionary perspective on immunosenescence. Ann N Y Acad Sci 908:244–254. https://doi.org/10.1111/j.1749-6632.2000.tb06651.x

Govindarajan N, Rao P, Burkhardt S, Sananbenesi F, Schluter OM, Bradke F, Lu J, Fischer A (2013) Reducing HDAC6 ameliorates cognitive deficits in a mouse model for Alzheimer's disease. EMBO Mol Med 5:52–63. https://doi.org/10.1002/emmm.201201923

Grotewiel MS, Martin I, Bhandari P, Cook-Wiens E (2005) Functional senescence in *Drosophila melanogaster*. Ageing Res Rev 4:372–397. https://doi.org/10.1016/j.arr.2005.04.001

Guo W, Naujock M, Fumagalli L, Vandoorne T, Baatsen P, Boon R, Ordovas L, Patel A, Welters M, Vanwelden T, Geens N, Tricot T, Benoy V, Steyaert J, Lefebvre-Omar C, Boesmans W, Jarpe M, Sterneckert J, Wegner F, Petri S, Bohl D, Vanden Berghe P, Robberecht W, Van Damme P, Verfaillie C, Van Den Bosch L (2017) HDAC6 inhibition reverses axonal transport defects in motor neurons derived from FUS-ALS patients. Nat Commun 8:861. https://doi.org/10.1038/s41467-017-00911-y

Huang DW, Sherman BT, Lempicki RA (2009a) Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. Nucleic Acids Res 37:1–13. https://doi.org/10.1093/nar/gkn923 (**Epub 2008 Nov 25**)

Huang DW, Sherman BT, Lempicki RA (2009b) Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 4:44–57. https://doi.org/10.1038/nprot.2008.211

Kenyon CJ (2010) The genetics of ageing. Nature 464:504–512. https://doi.org/10.1038/nature08980

Liu N, Landreh M, Cao K, Abe M, Hendriks GJ, Kennerdell JR, Zhu Y, Wang LS, Bonini NM (2012) The microRNA miR-34 modulates ageing and neurodegeneration in *Drosophila*. Nature 482:519–523. https://doi.org/10.1038/nature10810

Magupalli VG, Negro R, Tian YZ, Hauenstein AV, Di Caprio G, Skillern W, Deng QF, Orning P, Alam HB, Maliga Z, Sharif H, Hu JJ, Evavold CL, Kagan JC, Schmidt FI, Fitzgerald KA, Kirchhausen T, Li YQ, Wu H (2020) HDAC6 mediates an aggresome-like mechanism for NLRP3 and pyrin inflammasome activation. Science 369:1448. https://doi.org/10.1126/science.aas8995

Moreno-Gonzalo O, Mayor F, Sanchez-Madrid F (2018) HDAC6 at crossroads of infection and innate immunity. Trends Immunol 39:591–595. https://doi.org/10.1016/j.it.2018.05.004

Ni JQ, Liu LP, Binari R, Hardy R, Shim HS, Cavallaro A, Booker M, Pfeiffer BD, Markstein M, Wang H, Villalta C, Laverty TR, Perkins LA, Perrimon N (2009) A *Drosophila* resource of transgenic RNAi lines for neurogenetics. Genetics 182:1089–1100. https://doi.org/10.1534/genetics.109.103630

Ni JQ, Zhou R, Czech B, Liu LP, Holderbaum L, Yang-Zhou DH, Shim HS, Tao R, Handler D, Karpowicz P, Binari R, Booker M, Brennecke J, Perkins LA, Hannon GJ, Perrimon N (2011) A genome-scale shRNA resource for transgenic RNAi in *Drosophila*. Nat Methods 8:405-U446. https://doi.org/10.1038/nmeth.1592

Pasyukova EG, Vaiserman AM (2017) HDAC inhibitors: a new promising drug class in anti-aging research. Mech Ageing Dev 166:6–15. https://doi.org/10.1016/j.mad.2017.08.008

Qin HT, Li HQ, Liu F (2017) Selective histone deacetylase small molecule inhibitors: recent progress and perspectives. Expert Opin Ther Pat 27:621–636. https://doi.org/10.1080/13543776.2017.1276565

Ratti F, Ramond F, Moncollin V, Simonet T, Milan G, Mejat A, Thomas JL, Streichenberger N, Gilquin B, Matthias P, Khochbin S, Sandri M, Schaeffer L (2015) Histone deacetylase 6 Is a FoxO transcription factor-dependent effector in skeletal muscle atrophy.

J Biol Chem 290:4215–4224. https://doi.org/10.1074/jbc.M114.600916

Seidel C, Schnekenburger M, Dicato M, Diederich M (2015) Histone deacetylase 6 in health and disease. Epigenomics 7:103–118. https://doi.org/10.2217/epi.14.69

Sen P, Shah PP, Nativio R, Berger SL (2016) Epigenetic mechanisms of longevity and aging. Cell 166:822–839. https://doi.org/10.1016/j.cell.2016.07.050

Sethi S, Wang JW (2017) A versatile genetic tool for post-translational control of gene expression in *Drosophila melanogaster*. Elife. https://doi.org/10.7554/eLife.30327

Simoes-Pires C, Zwick V, Nurisso A, Schenker E, Carrupt PA, Cuendet M (2013) HDAC6 as a target for neurodegenerative diseases: what makes it different from the other HDACs? Mol Neurodegener. https://doi.org/10.1186/1750-1326-8-7

Taes I, Timmers M, Hersmus N, Bento-Abreu A, Van Den Bosch L, Van Damme P, Auwerx J, Robberecht W (2013) Hdac6 deletion delays disease progression in the SOD1G93A mouse model of ALS. Hum Mol Genet 22:1783–1790. https://doi.org/10.1093/hmg/ddt028

Trapnell C, Roberts A, Goff L, Pertea G, Kim D, Kelley DR, Pimentel H, Salzberg SL, Rinn JL, Pachter L (2012) Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nat Protoc 7:562–578. https://doi.org/10.1038/nprot.2012.016

Valenzuela-Fernandez A, Cabrero JR, Serrador JM, Sanchez-Madrid F (2008) HDAC6: a key regulator of cytoskeleton, cell migration and cell-cell interactions. Trends Cell Biol 18:291–297. https://doi.org/10.1016/j.tcb.2008.04.003

Wang Z, Zang C, Cui K, Schones DE, Barski A, Peng W, Zhao K (2009) Genome-wide mapping of HATs and HDACs reveals distinct functions in active and inactive genes. Cell 138:1019–1031. https://doi.org/10.1016/j.cell.2009.06.049

Yu R, Cao X, Sun L, Zhu JY, Wasko BM, Liu W, Crutcher E, Liu H, Jo MC, Qin L, Kaeberlein M, Han Z, Dang W (2021) Inactivating histone deacetylase HDA promotes longevity by mobilizing trehalose metabolism. Nat Commun 12:1981. https://doi.org/10.1038/s41467-021-22257-2

Zhang L, Liu C, Wu J, Tao JJ, Sui XL, Yao ZG, Xu YF, Huang L, Zhu H, Sheng SL, Qin C (2014) Tubastatin A/ACY-1215 improves cognition in Alzheimer's disease transgenic mice. J Alzheimers Dis 41:1193–1205. https://doi.org/10.3233/JAD-140066

Zhao Y, Xie L, Shen C, Qi Q, Qin Y, Xing J, Zhou D, Qi Y, Yan Z, Lin X, Dai R, Lin J, Yu W (2021) SIRT2-knockdown rescues GARS-induced Charcot-Marie-Tooth neuropathy. Aging Cell 20:e13391. https://doi.org/10.1111/acel.13391