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EDTP/MTMR14: a novel target for improved survivorship to prolonged anoxia and cellular protein aggregates

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Highlights

- Heterozygous flies carrying hypomorphic *EDTP* allele have improved survivorship to prolonged anoxia
- Downregulation of *EDTP* in motoneurons improves the survivorship to cellular protein aggregates and extends lifespan
- *MTMR14*, a mammalian homolog to *EDTP*, is expressed in the mouse hippocampus and cortex
- Selective downregulation of *EDTP/MTMR14* in motoneurons or glia is promising for the treatment of neurodegeneration

ABSTRACT

Drosophila egg-derived tyrosine phosphatase (*EDTP*), a lipid phosphatase that removes 3-position phosphate at the inositol ring, has dual functions in oogenesis and muscle performance in adults. A mammalian homologous gene *MTMR14*, which encodes the myotubularin-related protein 14, negatively regulates autophagy. Mutation of *EDTP/MTMR14*, however, causes at least three deleterious consequences: (1) the lethality in early embryogenesis in *Drosophila*; (2) a “jumpy” phenotype with apparently impaired motor functions; and (3) an association with a rare genetic disorder called centronuclear myopathy. The potential benefit of *EDTP/MTMR14* downregulation is likely masked by the lethality or severe muscle defects due to ubiquitous loss of this gene. Here we show that flies carrying a heterozygous *EDTP* mutation had increased survivorship to prolonged anoxia; tissue-specific downregulation of *EDTP* in non-muscle tissues, particularly motoneurons, extended lifespan and improved survivorship to beta-amyloid peptides (A β 42) and polyglutamine protein aggregates. These data highlight the significance of selective

downregulation of EDTP in non-muscles for beneficial consequences. MTMR14 expression was evident in the hippocampus and cortex in C57BL/6J and APP/PS1 mice. Compared with C57BL/6J mice, APP/PS1 mice had reduced MTMR14 in the cortex. Hippocampal expression of MTMR14 was increased and plateaued at 9-17 months compared with 2-6 months in C57BL/6J mice. Additionally, MTMR14 was inducible by A β 42 in the rat primarily hippocampal neurons and mouse Neuro2a neuroblasts. We demonstrate a novel approach of tissue-specific downregulation of the disease-associated gene *EDTP/MTMR14* for extended lifespan and improved survivorship to cellular protein aggregates. This approach could be extended from insects to mammals.

Keywords: Egg-derived tyrosine phosphatase; myotubularin-related protein 14; survival; anoxia; polyglutamine protein aggregate; beta-amyloid peptide; tissue-specific expression; motoneuron; *Drosophila melanogaster*

1. Introduction

The egg-derived tyrosine phosphatase (EDTP) is a lipid phosphatase that removes 3-position phosphate at the inositol ring of phosphatidylinositol 3-phosphate and phosphatidylinositol (3,5)-bi-phosphate [1]. EDTP was originally identified in the flesh fly *Sarcophaga peregrina* [2] and then in *Drosophila melanogaster* [3]. EDTP possesses a function opposite to Vps34, a sole class III phosphoinositide 3-kinase [4,5]. The most interesting characteristic of EDTP expression is that there are two peaks, one at oogenesis [3,6], and another at adult stages [7,8]. Transcription of *MTMR14*, a mouse homolog of *EDTP*, also shows a peak at day five of differentiation in C2C12 myoblasts [1]. Human MTMR14 is detectable at the ages between 19 and 69 years in the tissues including heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas [1], and in neurons of the superior temporal gyrus of an 82-year-old woman [9]. *MTMR14* transcription is highly coincident with *Vps34* in the human brain, heart, and skeletal muscles [10], indicating a tightly regulated process associated with phosphatidylinositol 3-phosphate levels.

The decline of *Drosophila* EDTP between early embryogenesis and young adult stages is accompanied by the metamorphosis, a process requiring extensive autophagy and apoptosis for histolysis [11]. These observations suggest a role for EDTP in the regulation of autophagy. This is indeed supported by the findings that phosphatidylinositol 3-phosphate stimulates autophagy in human HT-29 cells [12], that MTMR14 negatively controls the autophagosome formation and maturation in mammalian cells [13], and that an EDTP/MTMR14 inhibitor, AUTEN-99, activates autophagy in human HeLa cells and mouse tissues [9]. Interestingly, the latter group also shows that overexpression of a modified *EDTP* (by skipping the first exon) in the fat body of *Drosophila* antagonizes the effect of AUTEN-67 in inducing autophagy [14].

Despite the negative regulation of autophagy, a deletion of *Drosophila* *EDTP* is lethal during embryogenesis or in the first instar, and germline clones with a null *EDTP* allele fail to produce mature oocytes [3]. Homozygous flies carrying a hypomorphic allele are short-lived with impaired motor functions and reduced fecundity [15]. Additionally, the muscles of the *MTMR14*-deficient mice have decreased force production, prolonged relaxation, and exacerbated fatigue [16]. A human *MTMR14* missense variant (R336Q) is associated with centronuclear myopathy, a rare genetic disorder with muscle weakness and wasting [1]. Therefore, the potential function of autophagy initiation is likely overwhelmed by the lethality or disease-causing effects due to ubiquitous loss of *EDTP/MTMR14*.

There are advantages of autophagy in removing and recycling the disrupted organelles, long-lived proteins, and denatured protein aggregates [12,17]. A strategy to amplify the potential benefit of *EDTP* is to manipulate its downregulation in selected tissues while leaving the expression unaffected in oocytes, and muscles during adult stages. This seems to be feasible by using the *Drosophila* Gal4/UAS system [18]. A study has shown that downregulation of *EDTP* in glial cells improves the survivorship to polyglutamine protein aggregates and extends lifespan in *Drosophila* [19]. Such beneficial effects are not observed by targeting all the neurons, whereas similar results are observable by targeting only motoneurons for *EDTP* knockdown [19]. These findings highlight the tissue-specificity of *EDTP* downregulation for the expected consequences.

Here we extended our studies to three aspects. We first show that a heterozygous mutant *EDTP*^{DJ694}/+ had improved survival to prolonged anoxia. Next, selective downregulation of *EDTP* in motoneurons improved the survivorship to the beta-amyloid peptides (A β 42) or polyglutamine protein aggregates (containing 103 polyQ tracts in each molecule). We further examined the expression of *MTMR14* in the mammalian system and found that the transgenic

mice APP/PS1 had reduced levels of MTMR14 in the cortex and that MTMR14 was inducible in the cultured cells by an acute A β 42 treatment.

2. Methods

2.1. Animals

Fly strains used in this study and their sources were: *EDTP^{DJ694}* and *w¹¹¹⁸* (L. Seroude laboratory, Queen's University), *EDTP^{DJ694}* (Bloomington Drosophila Stock Center (BDSC) #17050), D42-Gal4 (BDSC #8816), UAS-EDTP-RNAi (II) (with an RNAi sequence CAGTAGTGTAATAGTAATCAA targeting the transcripts of exon 3 of *EDTP*, BDSC #41633), UAS-EDTP-RNAi (III) (with an RNAi sequence CAGCTACGACGAAGTCATCAA targeting the transcripts of exon 2, BDSC #36917), UAS-A β 42 (BDSC #32038), UAS-Httex1-Q103-eGFP [20]. Flies were maintained with a standard medium (cornmeal, agar, molasses, and yeast) at 21-23 °C with a 12h:12h photoperiod.

Heterozygous mutants (*EDTP^{DJ694}/+*) and their sibling controls (*w¹¹¹⁸*) were prepared by two consecutive crosses between homozygous *EDTP^{DJ694}* mutants and *w¹¹¹⁸* flies. We chose the virgin female progeny (*EDTP^{DJ694}/+*) from the first mating and crossed them with *w¹¹¹⁸* males. Flies carrying the *EDTP* mutation (red-eyed) and their siblings (white-eyed) were collected for experiments.

The mice (C57BL/6J and APP/PS1) and rats (Sprague/Dawley) were purchased from the Experimental Animal Centre of Wuhan University. C57BL/6J mice are wild-type whereas APP/PS1 (stock number 004462, Jackson Laboratory) are the transgenic models of Alzheimer's disease. APP/PS1 mice express a chimeric mouse/human amyloid precursor protein

(Mo/HuAPP695swe) and a mutant human presenilin 1 (PS1-dE9) both directed to CNS neurons. Mice were housed in a light/dark (12h:12h) cycle in standard cages (4-5 per cage) with the accessible food and water *ad libitum*. Sprague/Dawley rats were used for the isolation of primary hippocampal neurons. Experimental procedures with mice and rats were approved by the Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology.

2.2. Post-anoxia survival

Newly emerged flies were collected and raised to 2-7 days in regular conditions and exposed to anoxia (generated by pure argon or nitrogen gas) during the light phase of the photoperiod. The percent survival at 12 h (% 12-h survival) after anoxia was examined. Replicated groups (15-25 flies per group, $n = 3 \sim 7$) of flies were examined. We chose 1, 3, or 6 h exposure because flies are highly tolerant to anoxia for hours [21].

Recovered flies were further examined for the post-anoxia lifespan. The dead flies were scored daily during the first week of recovery, and twice a week thereafter until all the flies were counted. Throughout the lifespan experiments, alive flies were transferred to fresh food vials twice a week.

2.3. Lifespan experiments

Newly emerged flies were collected and raised at a density of 20-25 flies per vial. They were transferred to fresh culture media twice a week. The lifespan was examined by counting dead flies during each transfer until all the flies were counted. This procedure was also used for the lifespan analysis of flies expressing beta-amyloid peptides N-42 ($A\beta_{42}$) or polyglutamine protein

aggregates in motoneurons, and of flies with simultaneous expression of A β 42 or polyglutamine aggregates and RNAi knockdown of *EDTP* in the same cells.

2.4. Cell Culture

Rat primary hippocampal neurons were prepared by following a reported method [22]. Briefly, hippocampal neurons from the 18-day-old embryonic (E18) Sprague/Dawley rats were isolated and seeded at 30,000 - 40,000 cells per well on six-well plates coated with Poly-D-Lysine/Laminin (Bioscience) in the neurobasal medium (Invitrogen), which was supplemented with 2% B27/0.5 mM glutamine/25 mM glutamate. Half of the culture medium was changed every 3 days with neurobasal medium supplemented with 2% B27 and 0.5 mM glutamine. All cultures were kept at 37 °C in a humidified 5% CO₂ condition. The primary cultures were treated with A β 42 (5 or 10 μ M) or vehicle control for 72 h before harvested for the protein analysis.

Neuro2a cells were cultured at 37 °C in a 1:1 mixture of DMEM (Dulbecco's modified Eagle's medium) and OPTI-MEM supplemented with 15% FBS (fetal bovine serum, Gibco), 100 units/ml penicillin, and 100 mg/ml streptomycin. A humidified atmosphere containing 5 % CO₂ was provided. The cells were cultured on the six-well plates overnight and then treated with 5 μ M A β 42 or vehicle control (DMSO) for 24 h before harvest.

2.5. Immunohistochemistry

Mice were anesthetized and perfused through aorta with 0.9% NaCl followed by phosphate buffer containing 4% paraformaldehyde. Brain tissues were isolated and post-fixed in perfusate overnight. Tissue sectioning (20 μ m) was performed with a vibratome (Leica Biosystems). The

sections were fixed with 0.3% H₂O₂ in the absolute methanol for 30 min and saturated with bovine serum albumin (BSA) for 30 min at room temperature. Tissues were then incubated with polyclonal anti-MTMR14 (mouse-specific polyclonal antibody produced by Aviva System Biology) overnight at 4 °C. After washes, tissues were incubated with appropriate secondary antibody. Immunoreaction was developed with diaminobenzidine using Histostain-SP Kits (Zymed, CA, USA). Images were taken using a light microscope (Olympus BX60, Tokyo, Japan).

2.6. Western Blotting

Western blotting was performed as described previously [23]. The hippocampal region and cortex were quickly removed and homogenized in the sample buffer (Tris-HCl 50mM (pH 7.4), NaCl 150 mM, NaF 10mM, Na₃VO₄ 1mM, EDTA 5 mM, benzamidine 2 mM, and phenylmethylsulphonyl fluoride 1 mM). The extracts were mixed with sample buffer (3:1, v/v, containing 200 mM Tris-HCl (pH 7.6), 8% SDS, 40% glycerol, 40mM dithiothreitol) and incubated with boiled water for 10 min. The proteins were separated by 10% SDS-polyacrylamide gel electrophoresis and transferred to nitrocellulose membrane. The membrane was blocked for 1 h with 5% skim milk (dissolved in TBSTween-20 containing 50 mM Tris-HCl (pH 7.6), 150 mM NaCl, 0.2% Tween-20), and probed with primary antibodies (anti α -tubulin: DM1A clone, T9026, Sigma-Aldrich; polyclonal anti-MTMR14, synthesized by Aviva System Biology) at 4 °C overnight. Blots were incubated with anti-rabbit or anti-mouse IgG conjugated to IRDye (800 CW) at room temperature and visualized using the Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, NE, USA). Protein bands were analyzed using ImageJ [24].

2.7. Statistics

Statistical analysis was conducted using R [25] and the following packages: gdata, survival, survminer, and ggplot2. Two-way ANOVA with Tukey's test was applied for the analysis of 12-h survival of different genotypes of flies which were subject to anoxia with varying durations. Log-rank test was performed for the comparison of two survival curves. Log-rank test with the Benjamini-Hochberg (BH) adjustment was used for the comparison of three survival curves. One-way ANOVA with Bonferroni's multiple comparisons was performed to analyze the MTMR14 expression among three groups of cells. Student *t*-Test was conducted to compare the levels of MTMR14 between two groups of cells. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Improved 12-h survival to prolonged anoxia in male flies of *EDTP* mutant

The *EDTP*^{DJ694} strain is an enhancer-trap line carrying a transposable element *P{GawB}* in the first intron with the orientation opposite to *EDTP* gene [7,8]. Flies homozygous for the *EDTP*^{DJ694} allele are hypomorphic (i.e. reduced *EDTP* transcription, short-lived, motor defective, and reduced fecundity) whereas flies heterozygous for *EDTP*^{DJ694} have unaffected lifespan [15]. To avoid the potential confounding effect, we examined flies heterozygous for *EDTP*^{DJ694}.

EDTP^{DJ694}/+ flies and their sibling controls (*w*¹¹¹⁸) were raised to 6-8 days and exposed to anoxia (generated by pure argon gas), a condition mimicking severe hypoxia that induces autophagy in cultured cells [26]. After 1 h anoxia, the average percentage of survival at 12 h (%)

12-h survival) of male flies was 100.0 ± 0.0 % (Mean $\pm$ SD) in both *EDTP^{DJ694/+}* and controls (Fig. 1a). With 3 h anoxia, *EDTP^{DJ694/+}* males showed % 12-h survival (83.3 ± 10.8 %) higher than controls (67.9 ± 8.4 %) ($P = 0.0097$). After 6 h anoxia, *EDTP^{DJ694/+}* males had % 12-h survival (63.4 ± 6.7 %) remarkably higher than controls (28.4 ± 5.2 %) ($P < 0.0001$). Female *EDTP^{DJ694/+}* flies had % 12-h survival statistically the same as *w¹¹¹⁸* flies with any of the 1, 3, or 6 h exposure. Thus, males but not females of *EDTP^{DJ694/+}* showed improved 12-h survival to the prolonged anoxia (i.e. 3 - 6 h duration).

3.2. Increased post-anoxia lifespan in *EDTP* mutant

We next examined whether *EDTP* mutants had increased post-anoxia lifespan. Young flies (7 days old) were exposed to 6 h anoxia. *EDTP^{DJ694/+}* males had the post-anoxia lifespan (median 36 d, 95 % confidence interval (CI) 1 - 72 d, $n = 167$) markedly longer than controls (median 1 d, CI 1 - 1 d, $n = 163$) ($P = 0.0033$, Log-rank test) (Fig. 1b). *EDTP^{DJ694/+}* females also showed post-anoxia lifespan (median 63.5 d, CI 37 - 79 d, $n = 150$) remarkably longer than controls (median 11 d, CI 8 - 37 d, $n = 148$) ($P = 0.0001$, Log-rank test). Without anoxia, *EDTP^{DJ694/+}* males had post-anoxia lifespan (median 69 d, CI 65 - 69 d, $n = 102$) similar as controls (median 69 d, CI 65 - 69 d, $n = 96$) ($P = 0.945$). Similarly, *EDTP^{DJ694/+}* females had post-anoxia lifespan (median 74 d, CI 69 - 79 d, $n = 107$) the same as controls (median 74 d, CI 69 - 79 d, $n = 97$) ($P = 0.869$). Therefore, both male and female flies of *EDTP^{DJ694/+}* had increased post-anoxia survivorship to 6 h anoxia. Without anoxia, the lifespan of *EDTP^{DJ694/+}* was unaffected. The increased post-anoxia lifespan was also observed in heterozygous females of *EDTP^{EP2390}*, a mutant carrying the *P{EP}* transposon (~8 Kb) in the first intron (Fig. S1). Male flies carrying

heterozygous *EDTP*^{EP2390} had statistically unaffected post-anoxia lifespan. This might be due to differential *EDTP* downregulation compared with mutant *EDTP*^{DJ694}.

3.3. RNAi knockdown of *EDTP* in motoneurons extended lifespan

The mammalian homolog *MTMR14* negatively regulates autophagic removal of protein aggregates [13]. Tissue-specific downregulation of *EDTP* in glial cells suppresses polyglutamine protein aggregates and extends lifespan [19]. Here we targeted motoneurons for *EDTP* downregulation using the Gal4/UAS system [18]. The rationale for targeting motoneurons was to avoid affecting the normally abundant expression of *EDTP* in muscles, ovaries, spermatheca, and oocytes. The motoneuron-specific driver D42-Gal4 [27] was applied for RNAi knockdown.

We used the UAS-*EDTP*-RNAi (III) line with a short interference sequence targeting the transcripts of exon 2. Flies with *EDTP* knockdown in motoneurons (D42-Gal4/UAS-*EDTP*-RNAi (III)) had a median lifespan of 113 d (CI 107 – 118 d, n = 132), which was longer than UAS controls (UAS-*EDTP*-RNAi (III)/+, median 89 d, CI 89 – 98 d, n = 63) ($P < 0.0001$), and also longer than Gal4 controls (D42-Gal4/+, median 73 d, CI 73 – 77 d, n = 137) ($P < 0.0001$) (Fig. 2a). To confirm the results, we used a different RNAi line (UAS-*EDTP*-RNAi (II) with an interference sequence targeting the transcripts of exon 3). Flies with *EDTP* knockdown in motoneurons (UAS-*EDTP*-RNAi (II)/+; D42-Gal4/+) had the lifespan (median 74.5 d, CI 69 – 84 d, n = 114) significantly longer than UAS controls (median 58 d, CI 51 – 58 d, n = 79) or Gal4 controls (median 51 d, CI 51 – 54 d, n = 87) ($P < 0.0001$) (Fig. 2b). Therefore, downregulation of *EDTP* in motoneurons extended lifespan.

3.4. *EDTP* knockdown in motoneurons improved the survivorship to cellular protein aggregates

We examined the survivorship of flies with simultaneous *EDTP* downregulation and the expression of the toxic β amyloid peptides N-42 (A β 42) [28]. The median lifespan in flies expressing A β 42 in motoneurons (UAS-A β 42/Y;; D42-Gal4/+) was 64 d (CI 64 - 64 d, n = 111). Median lifespan was increased to 94 d (CI 94 - 98 d, n = 86) in flies with A β 42 expression and RNAi knockdown of *EDTP* in motoneurons (UAS-A β 42/Y;; D42-Gal4/UAS-*EDTP*-RNAi) ($P < 0.0001$) (Fig. 3a).

We also examined the survivorship of flies with *EDTP* downregulation and simultaneous expression of polyglutamine protein aggregates (containing 103 tandem repeats of glutamine) in the motoneurons. The median lifespan was 38 d (CI 38 - 38 d, n = 45) in flies (UAS-Httex1-Q103-eGFP/Y;; D42-Gal4/+) expressing protein aggregates. Median lifespan was significantly increased to 44.5 d (CI 41 - 48 d, n = 30) in flies (UAS-Httex1-Q103-eGFP/Y;; D42-Gal4/UAS-*EDTP*-RNAi) with both aggregate expression and *EDTP* downregulation in motoneurons ($P < 0.0001$) (Fig. 3b).

It has been shown that the co-existence of two independent UAS constructs has no dilution effect on the Gal4 availability [19]. Therefore, *EDTP* downregulation in motoneurons improved the survivorship to A β 42 or polyglutamine protein aggregates.

3.5. Expression of *MTMR14* in the hippocampus and cortex in C57BL/6J and APP/PS1 mice

Human *MTMR14* is transcribed ubiquitously at relatively low levels in the brain [29]. Mouse *MTMR14* is expressed in muscles, liver, and fat [30], but there is little evidence for brain

expression. We examined MTMR14 in the hippocampus and cortex in C57BL/6J and APP/PS1 mice. C57BL/6J is a wild-type, whereas APP/PS1 is a transgenic strain with constitutive expression of a chimeric mouse/human amyloid precursor protein (Mo/HuAPP695swe) and a mutant human presenilin 1 (PS1-dE9) in CNS neurons [31].

MTMR14 expression was observable in the pyramidal neurons (CA3 region) and dentate gyrus (DG) in the hippocampus of a 3-month-old C57BL/6J mouse (Fig. 4a). MTMR14 was detectable in the hippocampus in both C57BL/6J and APP/PS1 mice. Levels of MTMR14 between two strains were similar with no significant difference (Fig. 4b). MTMR14 expression was also detectable in the cortex in both strains. However, APP/PS1 mice had reduced MTMR14 compared with C57BL/6J mice ($P < 0.05$, t -Test).

Levels of MTMR14 increased and plateaued at 9, 12 and 17 months compared with the levels at 2 and 6 months in C57BL/6J hippocampus (Fig. 4c), suggesting an age-dependent manner of MTMR14 expression.

3.6. A β 42 treatment induced MTMR14 expression in cultured cells

A β 42 causes the deregulation of microRNA in the primary hippocampal neurons in C57BL/6J mice [32] and inhibits the viability of mouse neuroblastoma Neuro2a cells [33]. We explored the effect of A β 42 treatment on MTMR14 expression in the cultured cells.

Primary hippocampal neurons from 18-day-old embryonic (E18) Sprague/Dawley rats were isolated and treated with A β 42. After 72-h incubation with 10 μ M A β 42, relative levels of MTMR14 increased compared with controls ($P < 0.01$) (Fig. 4d). There was no significant change of MTMR14 levels in the cultures treated with 5 μ M A β 42 for 72 h. Data indicated a

dosage-dependent induction of MTMR14 by A β 42 treatment. Additionally, MTMR14 expression was increased in Neuro2a cells treated with A β 42 at 5 μ M for 24 h compared with controls ($P < 0.01$) (Fig. 4e). Thus, A β 42 treatment induced MTMR14 expression in both types of cultured cells.

4. Discussion

We report that flies carrying a heterozygous *EDTP*^{DJ694} allele have improved survivorship to prolonged anoxia and that selective downregulation of EDTP in motoneurons extends lifespan and improves survivorship to cellular protein aggregates. These findings add strong evidence to support a proposal that downregulation of EDTP in non-muscle cells circumvents the deleterious consequences of ubiquitous loss of *EDTP* and extends lifespan. We expand our study to the mammalian system and find that a mouse homolog gene *MTMR14* is expressed in the hippocampus and cortex, and its expression is age-dependent in the hippocampus. MTMR14 is reduced in the cortex of APP/PS1 mice whereas MTMR14 is inducible by A β 42 in the cultured neurons. These data promise an application through tissue-specific downregulation of *EDTP/MTMR14* in both insects and the mammalian system for the treatment of neurodegeneration.

The proposed tissue-specific downregulation of *EDTP/MTMR14* for beneficial consequences is formulated largely from the preliminary observation that flies heterozygous for *EDTP*^{DJ694} have increased survivorship to prolonged anoxia. Without anoxia, heterozygous flies have unaffected lifespan whereas homozygous flies display a deleterious “jumpy” phenotype – impaired motor function associated with shortened lifespan and reduced fecundity [15]. These

findings indicate a conditional benefit of partial/moderate downregulation of *EDTP*. This is in line with the observation that motoneuronal downregulation of *EDTP* improves survivorship to cellular protein aggregates and extends lifespan. A recent study has shown that downregulation of *EDTP* in glial cells improves the survivorship to polyglutamine protein aggregates and extends lifespan [19]. Notably, lifespan is reduced in flies homozygous for the *EDTP*^{DJ694} allele, unaffected in flies heterozygous for this allele, and extended in flies with selective downregulation of *EDTP* in non-muscles. Such a gradient effect highlights the significance of partial and tissue-specific manipulation of *EDTP/MTMR14* expression for expected consequences.

EDTP/MTMR14 has two main functions: the negative regulation of autophagy and the disease-causing effect if deficient ubiquitously [1,9,13–16]. *Drosophila EDTP* has two expression peaks, one at oogenesis and another at adult stages around 20-30 days [3,6–8]. Both male and female flies have abundant *EDTP* in muscles and additionally, female flies express rich amounts of *EDTP* in ovaries [3]. Different expression pattern of *EDTP* between sexes might be responsible for the differential short-term survival (i.e. % 12-h survival) to anoxia or post-anoxia lifespan. The dual functions, multiple expression peaks throughout life, broad expression in many tissues, and sexual dimorphism of expression together with the lethality in *EDTP* null mutants suggest that *EDTP* has essential functions and a complete loss of *EDTP/MTMR14* is impractical for beneficial consequences. A subtle control of *EDTP/MTMR14* downregulation in the favorable cells would be critical for such benefits. The current study presents a strategy that cell-specific downregulation of *EDTP* in non-muscles has advantageous effects. This sharply contrasts to the generally deleterious effects observed in *EDTP/MTMR14* mutants. Thus, we

demonstrate a novel approach of fine-tuning a disease associated gene for improved survivorship to cellular wastes and extended lifespan.

Motoneurons are highly specialized and terminally differentiated with reduced repairing abilities [34,35]. Furthermore, neuronal cells utilize autophagy as an essential process for the turnover of cytoplasmic contents [36]. In *Drosophila*, motoneuronal expression of a human Cu-Zn superoxide dismutase (SOD1) results in a marked extension of lifespan by up to 40% [37]. Motoneuronal overexpression of the heat shock protein 70 causes the structural plasticity of axonal terminals which is associated with increased larval thermotolerance [38]. Thus, motoneurons are a potential candidate for testing the effects of *EDTP* downregulation. This is evident in the current study that motoneuronal downregulation of *EDTP* extends lifespan and improves survivorship to A β 42 or polyQ protein aggregates.

We have expanded our study from flies to the mammalian system. The evident expression of MTMR14 in the mouse hippocampus and cortex provides a foundation from which the cell-specific downregulation of *EDTP/MTMR14* could be applied in mammals. Interestingly, transgenic expression of the amyloid precursor protein (Mo/HuAPP695swe) in APP/PS1 mice is associated with MTMR14 downregulation in the cortex but not in the hippocampus. These findings suggest an altered MTMR14 expression in response to amyloid peptides and this association is tissue-specific. MTMR14 is actively expressed in mouse embryonic fibroblasts [39] and its expression is increased and plateaued in the hippocampus during 9-17 months, mimicking the multiple expression peaks of *Drosophila EDTP*. Furthermore, we show that MTMR14 is inducible, instead of down-regulated, by A β 42 treatment in cultured neurons. Perhaps the increase of MTMR14 in the *in vitro* cultures represents a short-term response to acute A β 42 treatment, whereas MTMR14 downregulation in the *in vivo* studies reflects an

adaptive response to amyloid peptides that are constitutively expressed. Nevertheless, our findings highlight a promising application in mammals through targeting EDTP/MTMR14 for lifespan extension and survival improvement to cellular protein aggregates.

Declaration of interest

None

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Figure Legends

Figure 1. Improved survivorship to prolonged anoxia in flies heterozygous for *EDTP*^{DJ694}.

(a) Percent survival at 12 h after anoxia. Flies at seven days were exposed to 1, 3, or 6 h anoxia.

** $P < 0.01$, *** $P < 0.001$, two-way ANOVA with Tukey's multiple comparison. (b) Post-

anoxia lifespan in flies with 6 h anoxia or without anoxia. The genotype, sample size, and sex are indicated. P values are from Log-rank tests.

Figure 2. Downregulation of *EDTP* in motoneurons extended lifespan. (a) The survival curves of flies with *EDTP* knockdown in motoneurons. An RNAi line, UAS-EDTP-RNAi (III, BDSC #36917), was used for *EDTP* knockdown. (b) The survival curves of flies with *EDTP* knockdown in motoneurons. An RNAi line, UAS-EDTP-RNAi (II), was used. Data of male flies were present. *** $P < 0.001$ from Log-rank test with BH adjustment.

Figure 3. Downregulation of *EDTP* in motoneurons improved the survivorship to the cellular protein aggregates. (a) The survival curves of flies expressing A β 42 (red) and flies with both A β 42 expression and *EDTP* knockdown in motoneurons (green). (b) The survival curves of flies expressing polyglutamine protein aggregates (red) and flies with both protein aggregates and *EDTP* knockdown in the motoneurons (green). *P* values are from Log-rank tests.

Figure 4. Expression of the mammalian MTMR14 in the brains and cultured cells. (a)

MTMR14 expression in the hippocampus of a C57BL/6J mouse. CA3, CA3 pyramidal neurons; DG, dentate gyrus. **(b)** Expression of MTMR14 in the hippocampus and cortex in C57BL/6J and APP/PS1 mice. α -Tubulin, loading control; * $P < 0.05$, Student's t -test. **(c)** Age-dependent expression of MTMR14 in the hippocampus in C57BL/6J mice. Numbers in the bars indicate the

ages of mice. **(d)** MTMR14 induction by the A β 42 treatment in the rat primary hippocampal neurons. ** $P < 0.01$, Student's t -test. **(e)** MTMR14 induction by A β 42 in Neuro2a cells. ** $P < 0.01$, Student's t -test.