Biochemical and Biophysical Research Communications 462 (2015) 112–118

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

# Essential role of POLDIP2 in Tau aggregation and neurotoxicity via autophagy/proteasome inhibition

YoungDoo Kim, Hyejin Park, Jihoon Nah, Seowon Moon, WonJae Lee, Se-hoon Hong, Tae-In Kam, Yong-Keun Jung*

*Global Research Laboratory, School of Biological Sciences, Seoul National University, Seoul 151-747, Republic of Korea*

ARTICLE INFO

*Article history:*
Received 4 April 2015
Available online 27 April 2015

*Keywords:*
POLDIP2
Tau
Proteasome
Autophagy
Lifespan

ABSTRACT

In Alzheimer's disease and other tauopathy, abnormal Tau proteins form intracellular aggregates and Tau filaments. However, the mechanisms that regulate Tau aggregation are not fully understood. In this paper, we show that POLDIP2 is a novel regulator of Tau aggregation. From a cell-based screening using cDNA expression library, we isolated POLDIP2 which increased Tau aggregation. Expression of POLDIP2 was increased in neuronal cells by the multiple stresses, including Aβ, TNF-α and $H_2O_2$. Accordingly, ectopic expression of POLDIP2 enhanced the formation of Tau aggregates without affecting Tau phosphorylation, while down-regulation of POLDIP2 alleviated ROS-induced Tau aggregation. Interestingly, we found that POLDIP2 overexpression induced impairments of autophagy activity and partially proteasome activity and this activities were retained in DUF525 domain of POLDIP2. In a drosophila model of human tauopathy, knockdown of the drosophila *POLDIP2* homolog, CG12162, attenuated rough eye phenotype induced by Tau overexpression. Further, the lifespan of neural-Tau$^{R406W}$ transgenic files were recovered by CG12162 knockdown. Together, these observations indicate that POLDIP2 plays a crucial role in Tau aggregation via the impairment of autophagy activity, providing insight into Tau aggregation in Tau pathology.



## 1. Introduction

Tau is a microtubule-associated protein that localizes mainly in the axon of neurons. Tau protein binds to and stabilizes microtubules and is highly soluble in normal condition [1]. On the contrary, Tau is hyperphosphorylated in AD and other tauopathies, during aging and under stress condition, leading to the formation of insoluble aggregates, known as neurofibrillary tangles (NFTs) in the affected neurons [2]. In familiar type AD, several genes that are involved in generating Aβ (*APP*, *PSEN1* and *PSEN2*) [3] or in frontotemporal dementia with parkinsonism 17, Tau itself (*TAU* P301L, R406W, V337M etc.) are closely related to onset of the disease [4]. In most case (>95%) of chronic AD and tauopathy, however, no single gene induces these diseases, and both age-related memory loss and increases rate of disease occurs with aging.

In general, the accumulation of Tau aggregates is critical for the pathogenesis in tauopathy. There seems to be a linkage between Tau aggregation and protein degradation machineries, the ubiquitin-proteasome system (UPS) and autophagy degradation pathway. UPS is the major mechanism for protein quality control through selective degradation of damaged/unfolded proteins [5]. Autophagy also degrades cytosolic materials from proteins to organelles, especially insoluble protein aggregates [6]. Accordingly, altered UPS function and autophagy degradation are frequently found in chronic neurodegenerative diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD) and amyotrophic lateral sclerosis (ALS) [7,8]. In these diseases, reduced activity of the degradation systems is accompanied by accumulation of the aggregation-prone proteins, such as Tau in tauopathy, including AD. However, cellular signal(s) and molecular determinant(s) responsible for the regulation of the degradation system and neuronal degeneration remain unknown.

*Abbreviations:* AD, Alzheimer's disease; ROS, reactive oxygen species; $H_2O_2$, hydrogen peroxide; GFP-Tau, Green fluorescent protein-Tau fusion protein; UPS, ubiquitin proteasome system.

* Corresponding author. School of Biological Sciences, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 151-747, Republic of Korea. Fax: +82 2 873 7524.
*E-mail address:* ykjung@snu.ac.kr (Y.-K. Jung).

http://dx.doi.org/10.1016/j.bbrc.2015.04.084
0006-291X/

Reactive oxygen species (ROS) often contributes to the development of neurodegenerative diseases [9]. Levels of ROSs are elevated during aging and increased in Alzheimer's disease [10]. Mutant superoxide dismutase 1 (mSOD1) hetero knockout mice crossed with AD model mice show increased ROS and early impairment of learning/memory deficit [11]. Scavenging ROS suppresses neurotoxicity and reduces Tau aggregation, showing positive effects in animal models of neuronal diseases [12]. Despite the proposed contribution of ROS to neurodegeneration *in vitro* and *in vivo*, little is known about the molecular mechanism that ROS regulates the accumulation of aggregation-prone proteins underlying the neuropathology. POLDIP2 (also known as PDIP2, PDIP38) was first recognized as a protein that binds to DNA polymerase delta [13]. POLDIP2 is predicted to interact with PCNA [14] and regulates NOX4 function in muscle cells [15]. However, the role of POLDIP2 in neurodegenerative disease is not understood.

In this paper, we identified POLDIP2 as an important modulator of Tau aggregation. We showed that POLDIP2 regulated Tau aggregation via the inhibition of autophagy and mediated neurotoxicity in a fly model expressing Tau.

## 2. Materials and methods

### 2.1. Cell culture and DNA transfection

Cell culture was previously described [16]. HEK293T, HeLa and SH-SY5Y cells were cultured in DMEM (Hyclone) supplemented with 10% (v/v) fetal bovine serum (Hyclone). Primary cortical neurons were prepared from mouse brains at embryonic day 16. The neurons were seeded on poly-L-lysine (0.01% in 100 mM borate buffer, pH 8.5)-coated plates and maintained in neurobasal medium containing 2% B-27 supplement (Invitrogen) and 0.5 mM L-glutamine. Half of the medium was exchanged every 3 days. HEK293T and SH-SY5Y cells were transfected using the Polyfect reagent (Qiagen).

### 2.2. Western blotting and antibodies

The lysates were prepared as described previously [17]. The blots were blocked for 1 h and incubated with following antibodies: PHF-1, TG5 (a generous gift from Dr. P. Davies, Albert Einstein College of Medicine, USA), P3R and P8R (a gift from Dr. E. Klaile, Karolinska Institute, Sweden), FLAG (Sigma), α-tubulin, β-actin and GFP (Santa Cruz Biotechnology), p62, ULK1, and p-ULK1 (Cell signaling) antibodies. Membranes were rinsed and incubated for 1 h with peroxidase-conjugated anti-mouse or rabbit antibody and visualized using the ECL detection system.

### 2.3. Filter trap assay for Tau aggregates

SH-SY5Y cells transfected with pcDNA3-HA and pPOLDIP2-HA with GFP-Tau cDNA and The lysates were prepared as described previously [16]. After brief sonication, equal amounts of cell lysates were passed through a nitrocellulose filter (0.2 mm). The membrane was washed three times with 1% SDS and blocked in the 5% non-fat milk for 1 h followed by immunoblotting.

### 2.4. DNA construction

To construct serial deletion mutants of human POLDIP2 (BC000655.2, polymerase delta-interacting protein 2 isoform 2), POLDIP2-WT (amino acids 1–368), POLDIP2-A (amino acids 201–368), POLDIP2-B (1–200), and POLDIP2-C (1–72) were amplified by PCR using gene-specific synthetic DNA oligonucleotides and subcloned into p3xFLAG-CMV-14.

### 2.5. Small interfering RNA (siRNA) transfection

The siRNA oligonucleotides against POLDIP2 (#1, 1119184; #2, 1119188; #3, 1119189) and scrambled control were purchased (Bioneer) and RNA interference assay was done according to the manufacturer's instruction.

### 2.6. Transgenic drosophila

*Drosophila melanogaster* was raised in the dark at 25 °C. WT ($w^{1118}$) and POLDIP2 mutant ($CG12162^{EY08866}$) strains used in this study were obtained from the Bloomington Drosophila Stock Center at Indiana University. The gl-$tau^{2.1}$ line was kindly provided by Dr. D. Geschwind (University of California, USA) [18]. UAS-$tau^{R406W}$ fly line for the generation of elav-$tau^{406W}$ was from Dr. M. Feany (Harvard Medical School, USA) [19].

### 2.7. Longevity assay

More than 200 files of each genotype were collected, divided into tubes of 10 flies and incubated at 29 °C on the standard food. The culture medium was changed every 2 days and the numbers of dead files were recorded.

## 3. Results

### 3.1. Identification of POLDIP2 as an inducer of Tau aggregation

To find out new regulator(s) of Tau protein aggregation, we screened 5000 cDNAs encoding protein kinases, membrane proteins and other proteins that are expressed in the brain and are associated with human diseases [20]. With the cell-based assay using GFP-Tau, GFP allowed the detection and formation of Tau aggregates under a fluorescence microscope. During the screening, transfection efficiency was normalized by cotransfection with monomeric RFP. GFP-positive dots were counted as aggregates of the Tau. From screening using the expression library, we could isolate putative positive cDNA clones (Fig. 1A and B). Ectopic expression of the putative positive clones enhanced the aggregation of GFP-Tau in the transfected cells (Fig. 1B). In particular, POLDIP2 overexpression exhibited strong effects on the aggregation of GFP-Tau (Fig. 1C) and induced neuronal cell death (Fig. 1D) in a dose-dependent manner.

### 3.2. The expression of POLDIP2 is increased by reactive oxygen stress for Tau aggregation

We then investigated the signal that might regulate the expression of POLDIP2. Interestingly, we found that treatment with amyloid $beta_{1-42}$ (Aβ), a pathogenic factor of AD [17], tumor necrosis factor alpha (TNF-α) or $H_2O_2$ increased the expression of POLDIP2 in mouse primary hippocampal neurons, while other signals did not affect it (Fig. 2A). All these signals are believed to be crucial in many neurodegenerative disease [21]. Among these signals, we focused on ROS because this signal is highly associated with sporadic type of neurodegenerative disease but its mechanism is not known yet [22]. Treatment with $H_2O_2$ also increased POLDIP2 expression in SH-SY5Y human neuroblastoma cells (Fig. 2B).

We next addressed whether ROS could affect Tau aggregation. The results from cell-based GFP-Tau dot formation assay showed that treatment with $H_2O_2$ increased the numbers of GFP-Tau aggregates in SH-SY5Y cells (Fig. 2C). In addition, $H_2O_2$ increased the amounts of detergent-insoluble tau aggregates on filter-trap assay (Fig. 2D, upper panel). With the notion that POLDIP2 expression was induced by ROS, we examined whether POLDIP2 was involved

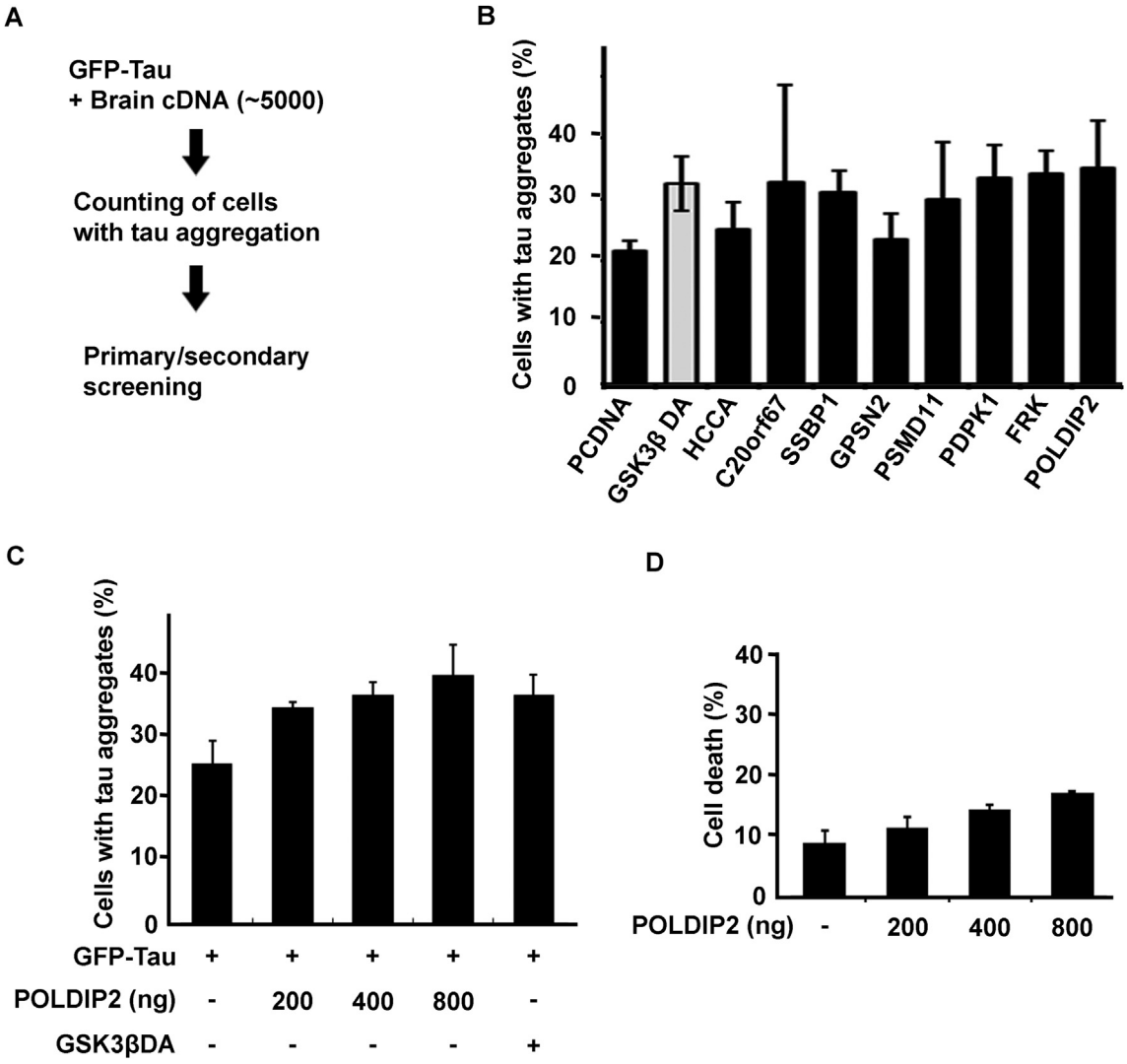


**Fig. 1.** Isolation of POLDIP2 as a regulator of Tau aggregation. The 5000 cDNAs in mammalian expression vector were screened by a cell-based assay using GFP-Tau, as shown in scheme (A). SH-SY5Y cells were transfected with GFP-Tau alone or together with GSK3β or putative positive clones for 24 h. Cells showing Tau aggregates were counted under fluorescence microscope (B). SH-SY5Ycells were transfected with GFP-Tau and the increasing amounts of POLDIP2 for 24 h and GFP-Tau aggregation was then analyzed under microscope (C). SH-SY5Ycells were transfected with POLDIP2 for 24 h and cell death was analyzed (D). Data are means ± S.D. ($p < 0.05$).

with the aggregation of Tau. We found that POLDIP2 expression was significantly reduced by POLDIP2 siRNA#1 and 2, and marginally by POLDIP2 siRNA#3 (Fig. 2E). Interestingly, knockdown of POLDIP2 expression by POLDIP2 siRNA#1 or 2, but not POLDIP2 siRNA#3, inhibited ROS-induced Tau aggregates (Fig. 2F). These results indicate that POLDIP2 is critical in Tau aggregation induced by ROS.

### 3.3. Increased POLDIP2 impairs autophagy activity

Many studies have shown that the aggregation of Tau protein is accompanied by Tau hyperphosphorylation, a typical pathophysiologic modification of Tau [16]. When we examined Tau phosphorylation with western blot analysis, however, ROS or overexpression of POLDIP2 did not much affect the phosphorylation status of Tau protein (Fig. 2D, lower panels; data not shown). These results led us to address a possibility that POLDIP2 might interfere the clearance of Tau protein by affecting autophagy and UPS. In addition, it was shown that multiple stresses, including ROS and Aβ, impair the activity of affect autophagy flux [23] and proteasome complex [24]. Especially, aggregation-prone proteins, including Tau aggregates, are believed to be clarified by autophagy [25].

Thus, we examined whether POLDIP2 affected autophagy activity using mCherry-GFP-LC3. In the mCherry-GFP-LC3 assay, inhibition of lysosomal activity displays $mCherry^{+}/GFP^{+}$ double-positive puncta because of the localization of acid-labile GFP-LC3 in autolysosomes, while acid-sensitive GFP signals normally disappear in autolysosomes. Interestingly, we found that overexpression of POLDIP2 drastically increased the formation of colocalized mCherry-GFP dots and reduced the numbers of mCherry-only cells (Fig. 2G), indicating an inhibition in late autophagosome/lysosome function [26]. Accordingly, we found that overexpressed POLDIP2 greatly increased the levels of LC3 II and p62 (Fig. 2H), an ubiquitin-binding autophagy receptor [27]. Consistent to the recent report [28], knockdown of POLDIP2 expression enhanced autophagy activity (Fig. 2I). These results strongly suggest that increased POLDIP2 impairs autophagy. We further assessed proteasome activity and found that ROS inhibited enzymatic activity of proteasome in SH-SY5Y cells (Fig. S1A). Knockdown of POLDIP2 expression partially but not much rescued the

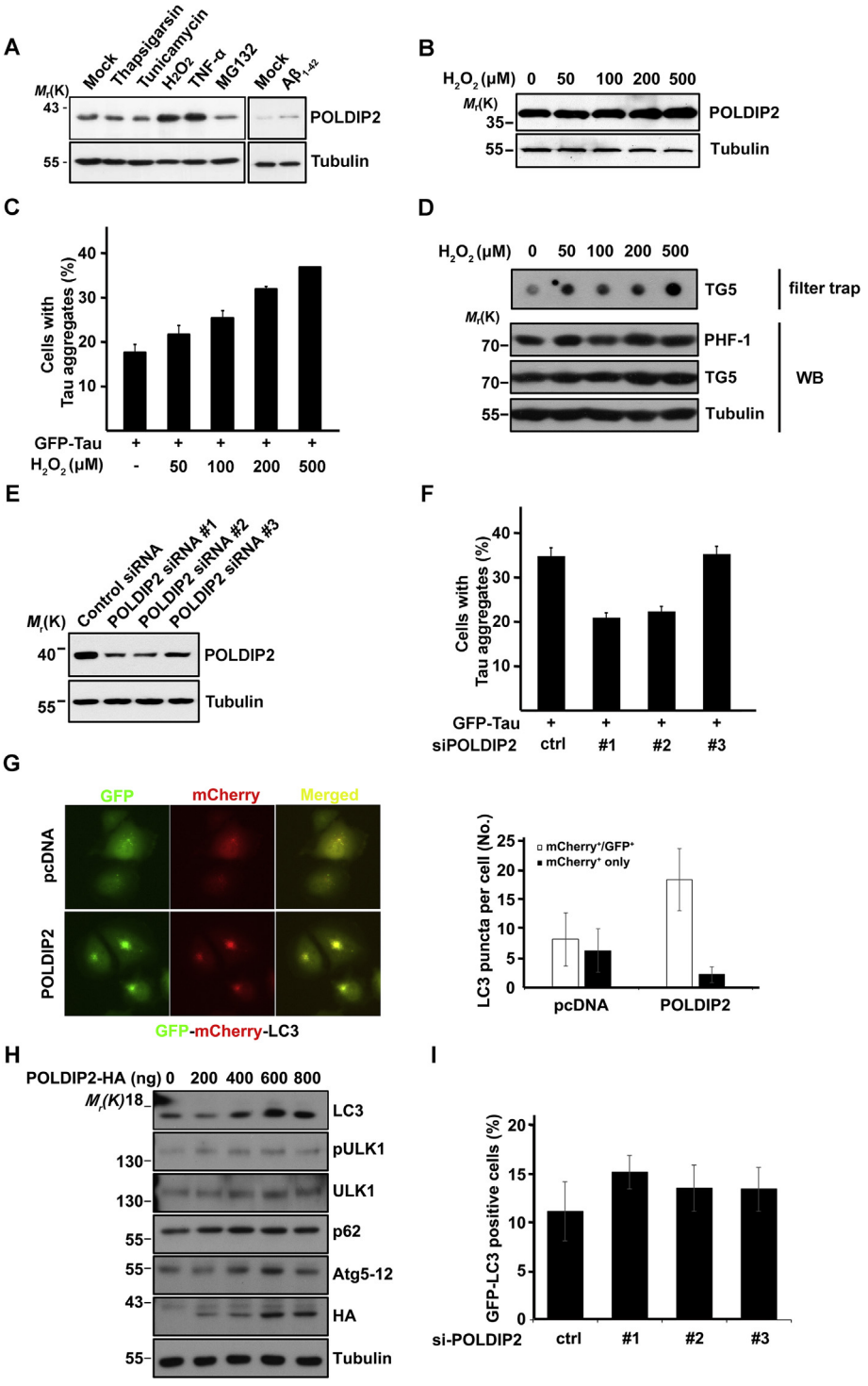


**Fig. 2.** POLDIP2 expression is increased by oxidative stress to enhance Tau aggregation and to impair autophagy flux. Primary cortical neurons were treated with 50 ng/ml TNF-α, 200 μM $H_2O_2$ or 5 μM $A\beta_{1-42}$ and then analyzed by western blotting (A). SH-SY5Y cells were incubated with $H_2O_2$ for 24 h and analyzed by western blotting (B). SH-SY5Y cells were transfected with GFP-Tau and then incubated with 200 μM $H_2O_2$ for 24 h. Tau aggregates were then observed under fluorescence microscope (C) and analyzed by filter-trap assay (D). SH-SY5Y cells were transfected with POLDIP siRNA (#1, 2, and 3) alone (E) or together with GFP-Tau (F). After 48 h, cells were analyzed for POLDIP2 knockdown by western blotting (E) and Tau aggregation (F). HeLa cells were cotransfected with POLDIP2 and GFP-mCherry-LC3 for 24 h (G, left panel) and the numbers of RFP only-positive or GFP/RFP-colocalized cells were counted and represented as bar graph (G, right panel). SH-SY5Ycells were transfected with POLDIP2 and cell lysates were analyzed with western blotting (H). SH-SY5Y cells were cotransfected with GFP-LC3 and POLDIP2 siRNA (#1, 2, and 3) and GFP-LC3 dot formation was examined under fluorescence microscope (I). Data are means ± S.D. ($n > 3$).

impairment of proteasome activity by ROS (Fig. S1B). In addition, ectopic expression of POLDIP2 marginally increased the accumulation of unstable degron (Fig. S1C) and suppressed proteasome activity (Fig. S1D).

### 3.4. The POLDIP2 regulates Tau aggregation and autophagy via DUF525 domain

The POLDIP2 has 2 distinct domains; Yccv-like domain and DUF525 domain [28]. To define the domain(s) responsible for Tau aggregation, several deletion mutants of POLDIP2 were generated (Fig. 3A). From western blot analysis, we observed similar levels of expression among POLDIP2 full-length and two deletion mutants except POLDIP2-C mutant which was not expressed in the transfected cells (Fig. 3B). Like POLDIP2, POLDIP2-A mutant containing the DUF525 domain only effectively increased Tau aggregation, while POLDIP2-B mutant containing the Yccv-like domain and POLDIP2-C mutant lacking both Yccv-like domain and DUF525 domain did not stimulate Tau aggregation (Fig. 3C).

Interestingly, we found that POLDIP2-A containing the DUF525 domain only, but not POLDIP2-B mutant, increased colocalization of mCherry and GFP dots and reduced the numbers of mCherry-only cells as much as POLDIP2 did (Fig. 3D). These results indicate that the DUF525 domain is crucial for autophagy inhibitory function of POLDIP2 and suggest a strong correlation between Tau aggregation and autophagy inhibition exhibited by POLDIP2. Unlike their effects on autophagy inhibition, we could not find any clear correlation between Tau aggregation by the POLDIP2 mutants and their effects on proteasome inhibition (data not shown).

### 3.5. POLDIP2 knockout rescues human Tau-mediated neural degeneration in drosophila

To assess an *in vivo* role of POLDIP2 in Tau pathology, we used a drosophila model expressing Tau in the eye [18] and whole-neurons [19]. The flies with the retinal overexpression of human Tau (gl-tau$^{2.1}$) showed moderate toxicity, characterized by the loss of photoreceptor neurons, leading to observation in drosophila eye as a rough surface (Fig. 4A). The *CG12162* gene has been identified as a fly homolog of the POLDIP2 and 1 p-element insertion mutant line (Bloomington stock No.17500, CG12162$^{EY08866}$) showed reduced level of *CG12162* mRNA (Fig. S2A). We then reconstituted the knockout flies (CG12162$^{\Delta EY08866}$) by ejecting p-element from CG12162$^{EY08866}$ flies. Compared with that in control flies ($w^{1118}$), there was no detectible difference in the eye morphology of *CG12162* knockout flies or CG12162$^{\Delta EY08866}$ reconstituted flies (Fig. 4A, left 3 panels).

We then crossed CG12162$^{EY08866}$ mutant or CG12162$^{\Delta EY08866}$ reconstitution flies with gl-tau$^{2.1}$ Tau flies. Interestingly, knockdown of fly POLDIP2 homologue in the retina of Tau transgenic flies (gl-tau$^{2.1}$/CG12162$^{EY08866}$) markedly rescued Tau-induced retinal degeneration. On the contrary, reconstitution of CG12162$^{EY08866}$ fly in Tau flies (gl-tau$^{2.1}$/CG12162$^{\Delta EY08866}$) again caused rough eye phenotype (Fig. 4A, right 3 panels). These observations indicate that POLDIP2 level is critical in Tau-induced neurotoxicity in the flies. Compared with Tau transgenic flies, the results from western blot analysis revealed that Tau phosphorylation at PHF-1 epitope (Ser 396/404) was not affected by knockdown of fly POLDIP2 homologue in the fly eye expressing Tau (gl-tau$^{2.1}$/CG12162$^{EY08866}$)

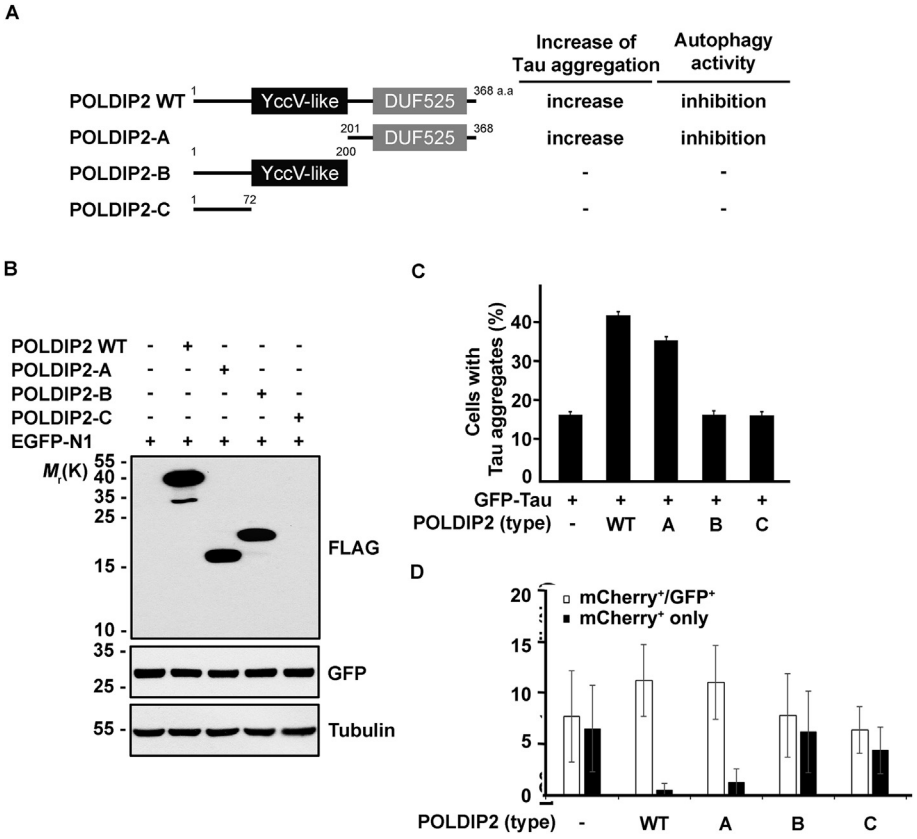


**Fig. 3.** POLDIP2 regulates Tau aggregation and autophagy flux through its DUF525 domain. Schematic diagram of POLDIP2 domains and its deletion mutants. Numbers indicate amino-acid residues of human POLDIP2 (A). Western blot analysis showing expression of the transfected POLDIP2 wild-type (WT) and 3 deletion mutants in HEK293T cells (B). SH-SY5Y cells were cotransfected with POLDIP2 deletion mutants and GFP-Tau (C) or GFP-mCherry-LC3 (D). After 24 h, Tau aggregation (C) and LC3 puncta formation (D) were examined under microscope. The effects of POLDIP2 mutants on Tau aggregation and autophagy activity are summarized in (A). Data are means ± S.D. ($n > 3$).

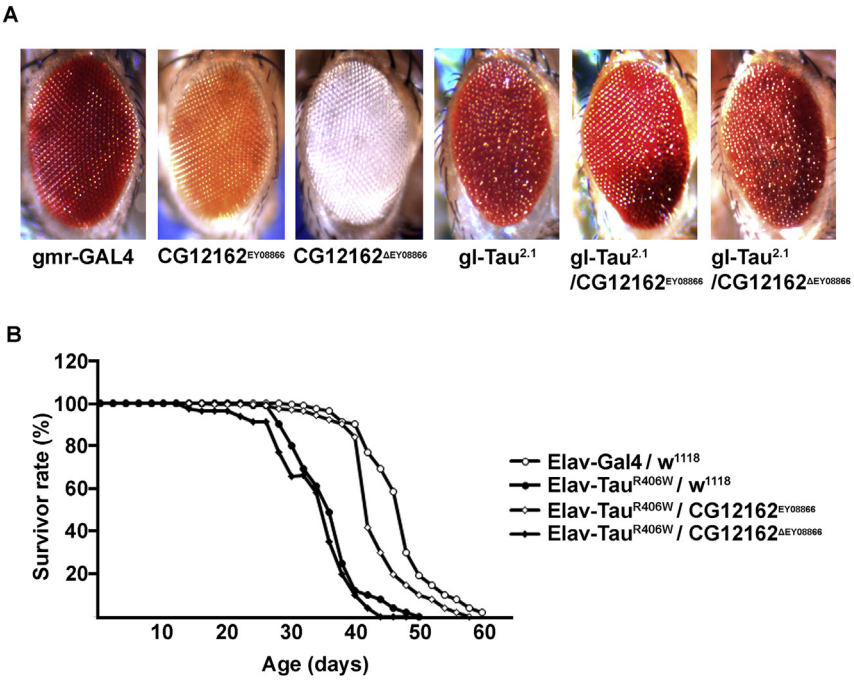


**Fig. 4.** Knockdown of POLDIP2 expression rescues Tau toxicity in a Drosophila model. WT ($w^{1118}$) and POLDIP2 knockdown mutant ($CG12162^{EY08866}$), and POLDIP2-reconstituted ($CG12162^{\Delta EY08866}$) flies were crossed with flies showing tau rough eye (gl-$tau^{2.1}$), generating double transgenic fly lines (gl-$tau^{2.1}$/$CG12162^{EY08866}$ and gl-$tau^{2.1}$/$CG12162^{\Delta EY08866}$). Eye morphology of each line was examined (A). Flies expressing $Tau^{R406W}$ in whole body neurons (elav-gal4; UAS-$Tau^{R406W}$) were crossed with POLDIP2 knockdown mutant flies, generating double transgenic flies (elav-gal4; UAS-$Tau^{R406W}$/$CG12162^{EY08866}$) and with POLDIP2-reconstituted flies, generating double transgenic flies (elav-gal4; UAS-$Tau^{R406W}$/$CG12162^{\Delta EY08866}$). Flies were collected at 1 day after eclosion and lifespan was measured at 29 °C ($n > 200$) (B).

(Fig. S2B), consistent to those of our cell models. Similarly, other Drosophila tau model also showed that ROS exacerbates tau toxicity without altering tau phosphorylation [29].

Further, we crossed wild-type, CG12162 mutant or CG12162 reconstitution flies with $Tau^{R406W}$ flies which express $Tau^{R406W}$ in whole neurons [19], and compared their lifespan. Tau flies (elav-gal4; UAS-$Tau^{R406W}$) exhibited shorter lifespan (average 35 days) than control flies (elav-gal4/+, average 45 days). When Tau flies were crossed with POLDIP2 mutant flies ($CG12162^{EY08866}$) or reconstituted flies ($CG12162^{\Delta EY08866}$), the lifespan was significantly extended by POLDIP2 deficiency (average 42 days in elav-gal4; UAS-$Tau^{R406W}$/$CG12162^{EY08866}$) but not in the reconstituted line (average 35 days in elav-gal4; UAS-$Tau^{R406W}$/$CG12162^{\Delta EY08866}$) (Fig. 4B). Thus, these observations further support that POLDIP2 plays an important role in Tau-mediated neurodegeneration in a model organism.

## 4. Discussion

Despite the discrepancy between Tau protein aggregation and neurodegeneration, NFTs are considered as a major pathogenic factor in tauopathy. Intracellular accumulation of Tau and neurofibrillary degeneration are hallmark of tauopathies, including AD. In this study employing genome-wide screening and subsequent characterization, we found that POLDIP2 is an important player in Tau aggregation and Tau-associated neuropathology. Unlike other pathogenic mediators, such as GSK3β and Cdk5 [30], in Tau aggregation, POLDIP2 does not alter Tau phosphorylation but regulates Tau degradation/clearance system. Especially, it is interesting to note that the POLDIP2 regulates autophagy flux through DUF525 domain which consists of two subdomains, ApaG and F-box c-terminal like subdomain and is predicted to be involved in protein–protein interactions [31].

Though POLDIP2 was reported as a polymerase delta interaction protein [13], it seems to be a multifunctional protein. POLDIP2 is also known as a regulator of NOX4; in smooth muscle cells, POLDIP2 induces RhoA via NOX4 activation [15] and RhoA – ROCK (Rho kinase) increases Tau-mediated neurodegeneration and RhoA and Rock inhibition by Rock inhibitor reduces Tau protein level [32]. Also, RhoA and Rock inhibition stimulates protein degradation system [33]. Thus, POLDIP2 might form a positive feedback loop under ROS stimulation and inhibits protein degradation pathways. Although the previous studies showed that autophagy is upregulated in POLDIP2 knockout mouse embryo fibroblasts [28], the mechanism by which POLDIP2 regulates autophagy remains to be addressed.

Autophagy and proteasome activities are declined during aging, while ROS are increased. Thus, dysregulation of these factors affects longevity and accelerates aging [34,35]. In addition to its effect on aging, global autophagy impairment also causes the aggregation of misfolded proteins, which is associated with perturbation of cellular functions and various human disorders. Moreover, ROS is an important pathophysiological feature of many neurodegenerative diseases and exacerbates the progression of neurodegenerative diseases [36]. Consistently, neurodegenerative model mice with SOD mutation show neurodegeneration with multiple protein aggregation [11]. Most studies, however, have focused on the degradation systems of the oxidized proteins or ROS-damaged organelles, such as mitochondria [37]. On the other hand, our study illustrates an important mediator, POLDIP2, of ROS to impair autophagy pathway. Thus, enhancement of autophagy activity by the inhibition of POLDIP2 may provide an insight into manipulation of Tau-associated neuronal degeneration, as evidenced by the Tau fly model with POLDIP2 deficiency.

In conclusion, we propose that POLDIP2 plays an important role in a tauopathy model through the regulation of autophagy activity,

linking the ROS signal to neuronal degeneration possibly in sporadic neurodegenerative diseases.

## Conflict of interest

The authors declare no conflict of interest.

## Acknowledgments

The authors thank Dr. P. Davies (Albert Einstein College of Medicine, NY, USA) for TG5 and PHF-1 antibodies and Dr. E. Klaile (Karolinska Institute, Sweden) for P3R and P8R POLDIP2 antibodies. This work was supported by Global Research Laboratory (NRF-2010-00341) and a CRI grant (NRF-2013R1A2A1A01016896) funded by the Ministry of Education, Science and Technology in Korea.

## Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.04.084.

## Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.04.084.

## References

[1] O. Schweers, E. Schonbrunn-Hanebeck, A. Marx, E. Mandelkow, Structural studies of tau protein and Alzheimer paired helical filaments show no evidence for beta-structure, J. Biol. Chem. 269 (1994) 24290–24297.
[2] J. Gotz, A. Probst, M.G. Spillantini, T. Schafer, R. Jakes, K. Burki, M. Goedert, Somatodendritic localization and hyperphosphorylation of tau protein in transgenic mice expressing the longest human brain tau isoform, EMBO J. 14 (1995) 1304–1313.
[3] D. Scheuner, C. Eckman, M. Jensen, X. Song, M. Citron, N. Suzuki, T.D. Bird, J. Hardy, M. Hutton, W. Kukull, E. Larson, E. Levy-Lahad, M. Viitanen, E. Peskind, P. Poorkaj, G. Schellenberg, R. Tanzi, W. Wasco, L. Lannfelt, D. Selkoe, S. Younkin, Secreted amyloid beta-protein similar to that in the senile plaques of Alzheimer's disease is increased in vivo by the presenilin 1 and 2 and APP mutations linked to familial Alzheimer's disease, Nat. Med. 2 (1996) 864–870.
[4] M. Hong, V. Zhukareva, V. Vogelsberg-Ragaglia, Z. Wszolek, L. Reed, B.I. Miller, D.H. Geschwind, T.D. Bird, D. McKeel, A. Goate, J.C. Morris, K.C. Wilhelmsen, G.D. Schellenberg, J.Q. Trojanowski, V.M. Lee, Mutation-specific functional impairments in distinct tau isoforms of hereditary FTDP-17, Science 282 (1998) 1914–1917.
[5] A. Ciechanover, Intracellular protein degradation: from a vague idea thru the lysosome and the ubiquitin-proteasome system and onto human diseases and drug targeting, Cell Death Differ. 12 (2005) 1178–1190.
[6] A.M. Cuervo, Autophagy and aging: keeping that old broom working, Trends Genet. – TIG 24 (2008) 604–612.
[7] A. Ciechanover, P. Brundin, The ubiquitin proteasome system in neurodegenerative diseases: sometimes the chicken, sometimes the egg, Neuron 40 (2003) 427–446.
[8] E.H. Baehrecke, Autophagy: dual roles in life and death? Nat. Rev. Mol. Cell. Biol. 6 (2005) 505–510.
[9] K.J. Barnham, C.L. Masters, A.I. Bush, Neurodegenerative diseases and oxidative stress, Nat. Rev. Drug Discov. 3 (2004) 205–214.
[10] Y. Christen, Oxidative stress and Alzheimer disease, Am. J. Clin. Nutr. 71 (2000) 621s–629s.
[11] U. Bodendorf, S. Danner, F. Fischer, M. Stefani, C. Sturchler-Pierrat, K.H. Wiederhold, M. Staufenbiel, P. Paganetti, Expression of human beta-secretase in the mouse brain increases the steady-state level of beta-amyloid, J. Neurochem. 80 (2002) 799–806.
[12] S. Sung, Y. Yao, K. Uryu, H. Yang, V.M. Lee, J.Q. Trojanowski, D. Pratico, Early vitamin E supplementation in young but not aged mice reduces Abeta levels and amyloid deposition in a transgenic model of Alzheimer's disease, FASEB J. – Off. Publ. Fed. Am. Soc. Exp. Biol. 18 (2004) 323–325.
[13] L. Liu, E.M. Rodriguez-Belmonte, N. Mazloum, B. Xie, M.Y. Lee, Identification of a novel protein, PDIP38, that interacts with the p50 subunit of DNA polymerase delta and proliferating cell nuclear antigen, J. Biol. Chem. 278 (2003) 10041–10047.
[14] B. Xie, H. Li, Q. Wang, S. Xie, A. Rahmeh, W. Dai, M.Y. Lee, Further characterization of human DNA polymerase delta interacting protein 38, J. Biol. Chem. 280 (2005) 22375–22384.
[15] A.N. Lyle, N.N. Deshpande, Y. Taniyama, B. Seidel-Rogol, L. Pounkova, P. Du, C. Papaharalambus, B. Lassegue, K.K. Griendling, Poldip2, a novel regulator of Nox4 and cytoskeletal integrity in vascular smooth muscle cells, Circ. Res. 105 (2009) 249–259.
[16] H. Park, T.I. Kam, Y. Kim, H. Choi, Y. Gwon, C. Kim, J.Y. Koh, Y.K. Jung, Neuropathogenic role of adenylate kinase-1 in Abeta-mediated tau phosphorylation via AMPK and GSK3beta, Hum. Mol. Genet. 21 (2012) 2725–2737.
[17] T.I. Kam, S. Song, Y. Gwon, H. Park, J.J. Yan, I. Im, J.W. Choi, T.Y. Choi, J. Kim, D.K. Song, T. Takai, Y.C. Kim, K.S. Kim, S.Y. Choi, S. Choi, W.L. Klein, J. Yuan, Y.K. Jung, FcgammaRIIb mediates amyloid-beta neurotoxicity and memory impairment in Alzheimer's disease, J. Clin. Invest. 123 (2013) 2791–2802.
[18] G.R. Jackson, M. Wiedau-Pazos, T.K. Sang, N. Wagle, C.A. Brown, S. Massachi, D.H. Geschwind, Human wild-type tau interacts with wingless pathway components and produces neurofibrillary pathology in Drosophila, Neuron 34 (2002) 509–519.
[19] C.W. Wittmann, M.F. Wszolek, J.M. Shulman, P.M. Salvaterra, J. Lewis, M. Hutton, M.B. Feany, Tauopathy in Drosophila: neurodegeneration without neurofibrillary tangles, Science 293 (2001) 711–714.
[20] S.M. Shim, W.J. Lee, Y. Kim, J.W. Chang, S. Song, Y.K. Jung, Role of S5b/PSMD5 in proteasome inhibition caused by TNF-alpha/NFkappaB in higher eukaryotes, Cell. Reports 2 (2012) 603–615.
[21] C. Schwab, P.L. McGeer, Inflammatory aspects of Alzheimer disease and other neurodegenerative disorders, J. Alzheimer's Dis. – JAD 13 (2008) 359–369.
[22] C.K. Glass, K. Saijo, B. Winner, M.C. Marchetto, F.H. Gage, Mechanisms underlying inflammation in neurodegeneration, Cell 140 (2010) 918–934.
[23] R. Scherz-Shouval, Z. Elazar, Regulation of autophagy by ROS: physiology and pathology, Trends Biochem. Sci. 36 (2011) 30–38.
[24] C.T. Aiken, R.M. Kaake, X. Wang, L. Huang, Oxidative stress-mediated regulation of proteasome complexes, Mol. Cell. Proteomics – MCP 10 (2011). R110.006924.
[25] Y. Wang, M. Martinez-Vicente, U. Kruger, S. Kaushik, E. Wong, E.M. Mandelkow, A.M. Cuervo, E. Mandelkow, Tau fragmentation, aggregation and clearance: the dual role of lysosomal processing, Hum. Mol. Genet. 18 (2009) 4153–4170.
[26] S. Pankiv, T.H. Clausen, T. Lamark, A. Brech, J.A. Bruun, H. Outzen, A. Overvatn, G. Bjorkoy, T. Johansen, p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy, J. Biol. Chem. 282 (2007) 24131–24145.
[27] T. Lamark, V. Kirkin, I. Dikic, T. Johansen, NBR1 and p62 as cargo receptors for selective autophagy of ubiquitinated targets, Cell. Cycle 8 (2009) 1986–1990 (Georgetown, Tex.).
[28] D.I. Brown, B. Lassegue, M. Lee, R. Zafari, J.S. Long, H.I. Saavedra, K.K. Griendling, Poldip2 knockout results in perinatal lethality, reduced cellular growth and increased autophagy of mouse embryonic fibroblasts, PloS One 9 (2014) e96657.
[29] D. Dias-Santagata, T.A. Fulga, A. Duttaroy, M.B. Feany, Oxidative stress mediates tau-induced neurodegeneration in Drosophila, J. Clin. Invest. 117 (2007) 236–245.
[30] H. Yamaguchi, K. Ishiguro, T. Uchida, A. Takashima, C.A. Lemere, K. Imahori, Preferential labeling of Alzheimer neurofibrillary tangles with antisera for tau protein kinase (TPK) I/glycogen synthase kinase-3 beta and cyclin-dependent kinase 5, a component of TPK II, Acta Neuropathol. 92 (1996) 232–241.
[31] J.T. Winston, D.M. Koepp, C. Zhu, S.J. Elledge, J.W. Harper, A family of mammalian F-box proteins, Curr. Biol. 9 (1999) 1180–1182.
[32] T. Hamano, S.H. Yen, T. Gendron, L.W. Ko, M. Kuriyama, Pitavastatin decreases tau levels via the inactivation of Rho/ROCK, Neurobiol. Aging 33 (2012) 2306–2320.
[33] P.O. Bauer, H.K. Wong, F. Oyama, A. Goswami, M. Okuno, Y. Kino, H. Miyazaki, N. Nukina, Inhibition of Rho kinases enhances the degradation of mutant huntingtin, J. Biol. Chem. 284 (2009) 13153–13164.
[34] J.N. Min, R.A. Whaley, N.E. Sharpless, P. Lockyer, A.L. Portbury, C. Patterson, CHIP deficiency decreases longevity, with accelerated aging phenotypes accompanied by altered protein quality control, Mol. Cell. Biol. 28 (2008) 4018–4025.
[35] D.C. Rubinsztein, G. Marino, G. Kroemer, Autophagy and aging, Cell 146 (2011) 682–695.
[36] R.A. Nixon, The role of autophagy in neurodegenerative disease, Nat. Med. 19 (2013) 983–997.
[37] R. Scherz-Shouval, Z. Elazar, ROS, mitochondria and the regulation of autophagy, Trends Cell Biol. 17 (2007) 422–427.