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ORIGINAL ARTICLE

# BAG2 prevents Tau hyperphosphorylation and increases p62/SQSTM1 in cell models of neurodegeneration

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## Abstract

**Background** Protein aggregates are pathological hallmarks of many neurodegenerative diseases, however the physiopathological role of these aggregates is not fully understood. Protein quality control has a pivotal role for protein homeostasis and depends on specific chaperones. The co-chaperone BAG2 can target phosphorylated Tau for degradation by an ubiquitin-independent pathway, although its possible role in autophagy was not yet elucidated. In view of this, the aim of the present study was to investigate the association among protein aggregation, autophagy and BAG2 levels in cultured cells from hippocampus and locus coeruleus as well as in SH-SY5Y cell line upon different protein aggregation scenarios induced by rotenone, which is a flavonoid used as pesticide and triggers neurodegeneration.

**Methods and results** The present study showed that rotenone exposure at 0.3 nM for 48 h impaired autophagy prior to Tau phosphorylation at Ser199/202 in hippocampus but not in locus coeruleus cells, suggesting that distinct neuron cells respond differently to rotenone toxicity. Rotenone induced Tau phosphorylation at Ser199/202, together with a decrease in the endogenous BAG2 protein levels in SH-SY5Y and hippocampus cell culture, which indicates that rotenone and Tau hyperphosphorylation can affect this co-chaperone. Finally, it has been shown that BAG2 overexpression, increased p62/SQSTM1 levels in cells from hippocampus and locus coeruleus, stimulated LC3II recycling as well as prevented the raise of phosphorylated Tau at Ser199/202 in hippocampus.

**Conclusions** Results demonstrate a possible role for BAG2 in degradation pathways of specific substrates and its importance for the study of cellular aspects of neurodegenerative diseases.

**Keywords** Autophagy · Parkinson's disease · Alzheimer's disease · Rotenone · Alpha-synuclein · Hyperphosphorylated Tau

## Introduction

Several physiological processes and diseases are hallmarked by protein inclusions in intracellular and extracellular compartments [1]. Accumulation of reactive oxygen species (ROS) during aging induces protein oxidation that can trigger its aggregation and formation of plaques, which is commonly found throughout the brain during neurodegenerative disorders such as Alzheimer's (AD) and Parkinson's (PD) diseases.

PD is the second most common neurodegenerative disorder in the world and the most common motor age-related neurodegenerative disease. PD is pathologically characterized by degeneration of dopaminergic neurons in the substantia nigra and is associated to Lewy bodies which are protein deposits consisted mainly of α-synuclein (α-Syn) [2]. Alpha-Syn is more commonly found in presynaptic terminals, where it plays a role in synapses and synaptic vesicles recycling [3]. Mutations in the α-Syn gene (*SNCA*) linked to familial PD encompass duplications or triplications of the wild type gene [4], or point mutations that predispose to α-Syn misfolding. In this last category, a study including Germanic and Mediterranean families identified two missense mutations (A30P and A53T) in *SNCA* gene [4] that are associated with higher levels of α-Syn oligomers in human cell lines [5], suggesting that overexpression or anomalous conformation of this protein due to the presence of point mutations can influence the aggregation properties

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of α-Syn, leading to its oligomerization and the formation of amyloidogenic fibrils.

AD is a neurodegenerative disorder characterized by memory loss and decrease of cognitive and physical capabilities and is pathologically characterized by degeneration of neurons in the hippocampus. During AD progression it is common to find extracellular protein aggregation composed mainly by amyloid β peptides (Aβ) or intracellular aggregation of hyperphosphorylated Tau, known as neurofibrillary tangles [6]. Both molecules have important physiological functions in the healthy brain. Amiloid β peptide has a role in synaptic activity and neuronal survival [7], whilst Tau protein, a microtubule-associated protein (MAP), is fundamental to neuronal intracellular transport since it stabilizes microtubules and collaborates to motor proteins for proper axonal trafficking [8].

It is thought that the accumulation of these proteins and its consequent aggregation is due to deficits in cellular protein quality control systems [9]. Degradation of misfolded proteins can occur via the ubiquitin–proteasome system (UPS) or via autophagy, that are important pathways for protein quality control and cellular homeostasis.

In macroautophagy (hereafter mentioned as autophagy), large cargos, such as damaged organelles and protein aggregates, are degraded. There are important proteins involved in the autophagy process. Beclin-1 acts in the nucleation of the process, being essential for membrane recruitment for phagophore formation [10, 11]. The adaptor protein LC3 has three isoforms that participate of autophagy process, depending on cleavage and its lipidation status. As initially the early LC3 is cleaved by ATG4 to form LC3I, which then is conjugated to phosphatidylethalomanine, catalized by ATG3, to be transformed in LC3II [10, 11]. During the last steps of autophagy, autophagosomes fuse with lysosomes, and LC3II present in the autophagosomes inner membrane is degraded together with cargo. Therefore, the conversion of LC3I into LC3II is widely used as a readout of autophagic flux [12], together with the analysis of other proteins of autophagy pathway. It has been shown that autophagy can act as a regulated process mediated by different receptors, such as p62/SQSMT, that can promote autophagic clearance by its ability to bind LC3 and ubiquitilated cargos [13].

Regulation of protein quality control is also mediated by molecular chaperones. These proteins facilitate protein folding, transport across membranes, remodeling, disaggregation, refolding and/or degradation [14]. Heat shock proteins (HSPs) are the largest group of molecular chaperones, and many HSPs have been shown to prevent protein aggregation.

BAG proteins share a conserved C-terminal region (BAG domain) that interacts directly with the ATPase domain of Hsp70 chaperone [15], although their N-terminal region shows low homology [16]. BAG family is often implicated in degradation pathways [17].

BAG2 is a member of BAG protein that interacts with the ubiquitin ligase CHIP and Hsc70/Hsp70 to perform a diversity of cellular functions [18]. Indeed, BAG2 has the ability to deliver phosphorylated Tau for degradation to the proteasome in an ubiquitin-independent manner in AD models, playing a fundamental role in the maintenance of neuronal structure [19], and its overexpression was capable to shift Aβ1-42 neurotrophic to neurotoxic role in SH-SY5Y cells [20]. BAG2 and BAG5 also showed a protective effect through its regulation by the monoamine oxidase (MAO)-B inhibitor rasagiline in a mice model for PD [21].

It was postulated that BAG2 plays a role in mitophagy, since it also recognizes phosphorylated sites in Pink1 thereby leading to its degradation [22]. Pink1 (PTEN-induced kinase 1) is a protein associated with mitophagy and several mutations in *PINK1* gene are associated with an autosomal recessive early onset of PD [23]. BAG2 is also associated with autophagy activation, autophagosome-lysosome fusion improvement and endoplasmic reticulum stress through its association with p62/SQSTM1, as well as regulates the dissociation of the complex Beclin1-Bcl2 to trigger autophagy in macrophages [24]. However, to date this there is no studies on the relation between BAG2 and p62 in neurodegenerative diseases.

It was previously showed that low concentrations (0.5 and 1.0 nM) of the mitochondrial complex I inhibitor rotenone, commonly used in studies related to neurodegeneration [25–27], were able to induce Tau hyperphosphorylation and Aβ plaque formation in primary hippocampus and locus coeruleus cell cultures, and that even lower concentrations of the pesticide (0.3 nM) was able to mimic a scenario prior to protein aggregation [28]. Here, we propose to investigate the effect of low doses of rotenone exposure upon autophagy and BAG2 in hippocampal and locus coeruleus primary cell culture and SH-SY5Y cell line. Finally, we evaluated the effects of BAG2 overexpression on autophagy proteins levels and phosphorylation of Tau.

## Materials and methods

All the procedures were performed in strict accordance with Institutional and International Guidelines for animal care and use [29], as well as respecting the Brazilian federal law 11,794/08 for animal welfare and approved by the institutional ethics committee (CEUA 271/2016) of the Department of Genetics and Evolutionary Biology, Institute for Biosciences, University of Sao Paulo.

### SH-SY5Y cell culture and rotenone exposure

SH-SY5Y cells were cultured in DMEM (Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS, Sigma-Aldrich) and 1% penicillin/streptomycin (P/S). At 70–80% confluence, cells were passaged using trypsin/EDTA (Lonza) following general cell culture procedures. Split ratios ranging from 1/20 to 1/40 were used to ensure similar densities among the groups. It was previously reported that rotenone treatment was able to induce Tau phosphorylation by GSK3β activation with ROS, and LDH release in SH-SY5Y cells [30]. Medium was supplemented with 1 μM (stock at 1 mM in DMSO) rotenone for 4.5 h or DMSO at 0.002% as control. Cells were plated at $10^5$ cells/well in a 24-well plate (Nunc) for protein extraction and Western blot analysis or on confocal plates (MatTek) for immunocytochemistry.

### Primary neuron and glial cell culture from hippocampus and locus coeruleus and rotenone exposure

Methodology employed for primary cell culture was a modification of the previously described protocol [31]. Briefly, 10 neonate (1 day-old) Lewis rats had their brains dissected out to access the hippocampus and locus coeruleus, which were dissociated in cold sterile hank's Balanced Salt Solution (HBSS), pH 7.2. Cell solution was centrifuged at 300 × g for 10 min. The supernatant was discarded, and cells were suspended in Neurobasal A medium (Gibco) supplemented with 0.25 mM Glutamax (Gibco), 2% B27 (Gibco), 0.25 mM L-Glutamine (Sigma) and 40 mg/L Gentamicin (Gibco). Cells were plated on 24-well nunclon (Nunc) or confocal dishes (MatTek), coated with poli-D-lysine, at the density of 1800 cells/mm2. Cultures were kept in a humidified incubator with 5% CO2 at 37 °C for nine days with the media changed every 3 days.

It was previously reported that exposure of primary cell cultures to low concentration of rotenone (0.5 nM) during 48 h did not induce cell death and triggered protein aggregation of different proteins associated with neurodegenerative disorders [32]. Previous study also showed that 0.3 nM of rotenone for 48 h induced to a cell stress that preceded Tau aggregation [28]. Medium was supplemented with rotenone (Sigma) 0.3, 0.5 or 1 nM or with dimethyl sulfoxide (DMSO, Sigma), and applied to cell cultures during for 48 h. The concentration of DMSO was maintained at 0.002% to all groups. Chloroquin was used as a control for autophagy inhibition in a final concentration of 3 μM diluted in PBS, and applied to cell cultures during 48 h, same time as rotenone exposure. Cells plated on confocal dishes were used for immunocytochemistry assays for MAP2 and autophagy flux. Cells grown on 24-well plates were used for western blot.

### BAG2 transfection

SH-SY5Y cells were transfected with pEGFP-C1/BAG2 [19] or pEGFP-C1 control plasmid using Lipofectamine 3000 (Life Technologies) following manufacturer instructions. Primary cells were transfected with the same plasmids after nine days in a humidified incubator. Cells were transfected 2 h before rotenone treatment. Transfection was confirmed at 24 h by fluorescence microscopy. Supplementary Fig. 1 illustrates the protocol for BAG-2 transfection and rotenone exposure of primary cells.

### Immunocytochemistry

Primary and SH-SY5Y cells were fixed with methanol:acetone (1:1) for 10 min at − 20 °C, rinsed with PBS and permeabilized with 0.2% triton X-100 in PBS for 30 min. Non specific binding was blocked with 2% normal goat serum and 4% BSA in PBS for 30 min and then incubated overnight with primary antibody against MAP2 (1:1000, sc 74422; Santa Cruz, raised in mouse) in 1% NGS, 2% BSA and 0.2% triton X-100. Dishes were washed with cold PBS followed by incubation with FITC conjugated secondary antibody for 2 h. Cells were mounted with mounting medium containing DAPI (4,6-diamidino-2-phenylindole, Vector laboratories).

### Autophagy analysis

Autophagy dynamics was monitored expressing the LC3-eGFPmCherry construct [33]. The expression of eGFP is restricted to autophagosomes (non-acidic compartments) and the mCherry labeling can be found in the autophagosomes and autophagolysosomes (acidic compartment) vesicles. Autophagy scenario was obtained as the ratio between the number of vesicles presenting both fluorescence emitted by eGFP and mCherry, and the vesicles presenting only mCherry signal. After 9 days of culture, cells were transfected with 1000 ng of LC3-eGFP-mCherry using lipofectamine 3000 (Invitrogen), according to manufacturer's protocol, followed by 48 h of 0.3 or 0.5 nM of rotenone exposure for primary cell culture using phenol-free Neurobasal A medium. Six cells per dish from 4 or 3 different cultures were evaluated at excitation wavelengths of 488 nm for eGFP and 535 nm for mCherry, and emission 520 nm and 620 nm, respectively. Cells were imaged on Carl Zeiss LSM780 inverted Multiphoton microscope after 4% paraformaldehyde fixation for 20 min. Quantitative analysis of immunofluorescence data was performed using Image J (NIH) and the plug-in Find

Maxima. Autophagy was also monitored by Western blot assays evaluating LC3, Beclin-1, and p62 protein levels, as described in the next section.

### Protein extraction and western blot

After treatments, culture medium was removed, cells were washed three-times with ice-cold PBS, and total protein was extracted with RIPA buffer (1% Triton X-100, 0.5% Sodium deoxycholate, 0.1% SDS, 150 mM NaCl, 50 mM Tris, 1 µg/ml protease inhibitor cocktail, pH7.4) followed by incubation at 4 °C for 30 min. Homogenates were centrifuged at 20,000 × g, 4 °C, 30 min. Protein concentration of the supernatant was determined using Bradford's method [34]. Protein samples were boiled in Laemmli Buffer, loaded for separation through SDS-PAGE, transferred to nitrocellulose membrane and blocked (5% BioRad milk in TBS-T) during 1 h at room temperature, then probed with primary antibodies.

Membranes were incubated overnight at 4 °C with antibodies against hyperphosphorylated Tau (1:1000, Sigma, Ser 199/202, T6819), total Tau (1:1000, Sigma, Tau46, T9450), b-actin (1:1000, Santa Cruz, sc-47778), Beclin-1 (1:1000, Santa Cruz, sc-11427), p62 (1:1000, Cell Signaling, #5114), LC3 (1:1000, Sigma, 7543 or Sigma L8918), BAG2 (1:1000, Novus Biologicals, NBP1-59087), Flag (1:500, Sigma, 7425). Membranes were washed with TBS-T (3×, 5 min) followed by incubation with HRP conjugated secondary antibodies (antirabbit 1:10,000 or anti-mouse 1:6000, both from Amersham) during 2 h at room temperature. Blots were developed using ECL reagent according to manufacturers instructions (PerkinElmer). Ponceau-S (Sigma) incubation for 15 min was also used for loading control.

### Statistical analysis

Statistical analysis for Western Blot was performed using GraphPad Prism (GraphPad Software Inc., version 7.00, CA). One-way or two-way ANOVA with Bonferroni post-test was used to compare multiple experimental groups (> 2) or two categorical independent variables, respectively. Student's t test was used for comparison between two groups. Normal distribution was assessed by Kolmogorov–Smirnov and Shapiro–Wilk normality tests. Otherwise, a non-parametric analysis was used accordingly. A p value ≤ 0.05 was considered to indicate statistically significant differences. Values of p between 0.05 and 0.08 were considered as tendency. Each experiment was performed a minimum of three times.

## Results

### Rotenone exposure decreases the endogenous BAG2 protein levels in SH-SY5Y cells

In order to test if BAG2 was able to modulate autophagy pathway in neurodegenerative disorders, we first tested the effect of rotenone exposure for 4.5 h, in SH-SY5Y cells that were slightly positive for the neuronal marker MAP2 (Fig. 1A). Concentration of rotenone was adjusted to 1 µM for SH-SY5Y cells in order to achieve similar results of Tau phosphorylation as in primary cells [32].

The same samples were used to detect the endogenous levels of BAG2. Western blot analysis revealed a significant decrease in BAG2 expression relative to the control cells (Fig. 1B) suggesting that the BAG2 protein is sensitive to the rotenone treatment. In accordance, rotenone increased hyperphosphorylation of Tau protein (pTau) at Ser199/202 site, and a decrease in total Tau protein levels (Fig. 1C), similarly to what is present in AD patients.

Since some associations of p62 were already made with BAG3, another member of BAG family, we decided to test if the BAG2 overexpression was capable of modulating the p62 levels in this model. The cells were transfected with BAG2 plasmid (or GFP as control) and, after 24 h, cells were exposed to rotenone or DMSO. Results demonstrared that overexpression of BAG2 prevented the decrease of p62 levels after rotenone exposure in this cell model (Fig. 2) during acute pTau accumulation.

### Rotenone exposure decreased autophagy flux in hippocampal but not in locus coeruleus cell culture

Since endogenous BAG2 levels decreased in SH-SY5Y upon rotenone exposure, and pTau at Ser199/202 increase is associated with degradation pathway collapse, we decided to verify its role in an autophagy dysfunction scenario in primary cells models.

Previous results showed that low rotenone concentrations were able to induce dysfunction in proteins that are associated with neurodegenerative disorders, such as Tau, α-Synuclein and Aβ peptide [28, 32], mimicking different time points of protein insolubility.

Immunocytochemistry revealed that hippocampal cultures presented 49% of neurons labeled by MAP2 antibody (Fig. 3A), and locus coeruleus cultured cells were positive for the tyrosine hydroxylase enzyme (TH) (Fig. 4A).

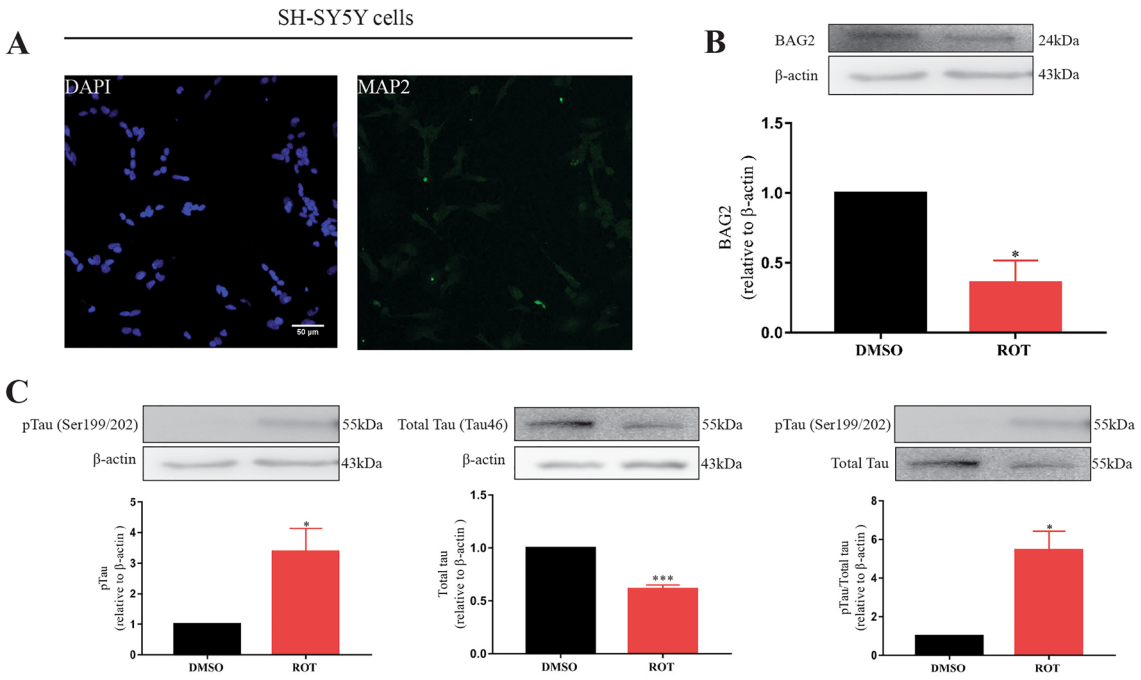



**Fig. 1** Rotenone exposure decreases BAG2 endogenous levels in SH-SY5Y cells. Illustrative digital images of cultured SH-SY5Y cells immunolabelled with antibody against MAP2 with DAPI as nuclear staining (**A**). Representative western blot image and quantification of cell extracts from SH-SY5Y line cells treated with 1 µM of rotenone or DMSO as control for 4.5 h, using the indicated antibodies (**B**, **C**). Protein levels were normalized to β-actin. Values are shown as percent control (DMSO) ± SD obtained from three independent experiments. *p < 0.05; ***p < 0.001; as compared to DMSO according to Student's *t* test

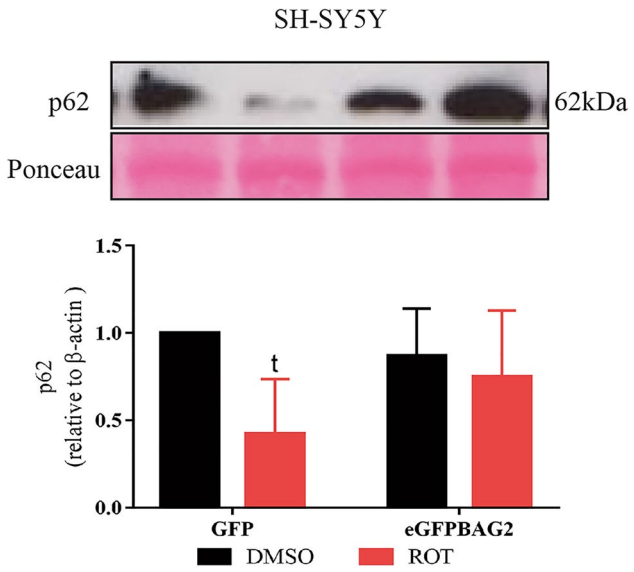
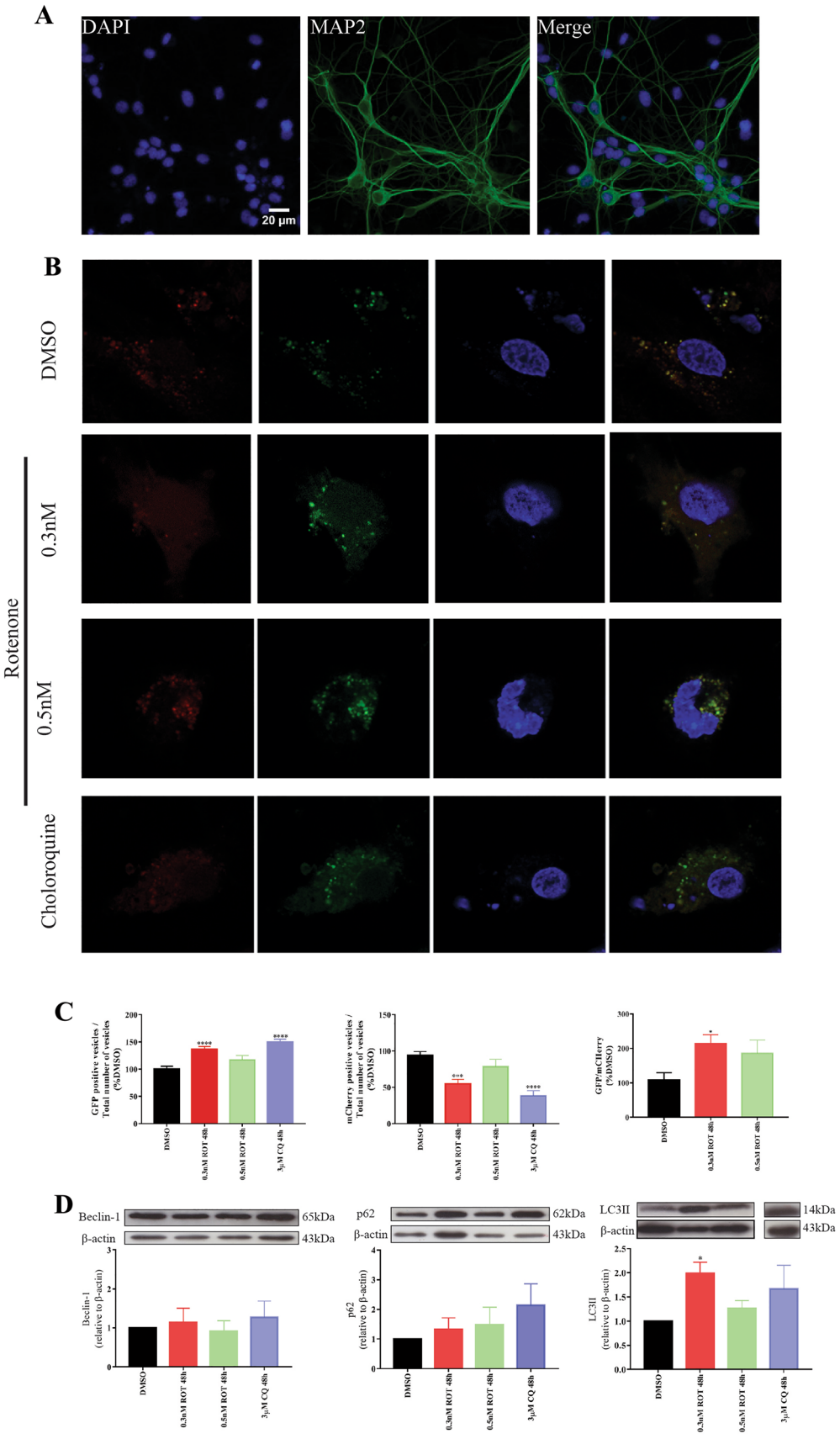



**Fig. 2** p62 levels in SH-SY5Y cells. Representative western blot image of cell extracts from SH-SY5Y transfected with GFP-BAG2 or GFP alone and exposed to with DMSO or 1 µM rotenone for 4.5 h with antibody against p62. Protein levels were normalized to Ponceau. Values are shown as percent control (DMSO) ± SD obtained from three independent experiments. t for tendency (t = 0.08) according to two-way ANOVA with Bonferroni's post hoc test

Cells were then transfected with LC3GFPmCherry plasmid to verify the autophagy dynamics within the different protein scenarios. Rotenone exposure increased non-acidic vesicles (GFP and mCherry positive) and decreased the acidic vesicles (mCherry only positive) when compared with the control in the hippocampus (Fig. 3B and C). According to previous results, rotenone (0.3 nM) reduced autophagy in these cells [28], suggesting that problems into this pathway preceded the increase of Tau insolubility. Meanwhile, in the presence of a higher rotenone concentration (0.5 nM) autophagy pathway did not change. Beclin-1 expression levels showed no alteration, suggesting that the autophagosome formation was preserved with the rotenone treatment (Fig. 3C). Consistent with the LC3GFPmCherry analysis, exposure to 0.3 nM of rotenone significantly increased LC3II protein levels, similarly to the chloroquine treatment with no change in 0.5 nM concentration (Fig. 3C). P62 levels showed a tendency to increase, after rotenone exposure, although not significant (Fig. 3D). Interestingly, the locus coeruleus analysis did not reveal any change in the autophagy dynamics or in the protein autophagy levels measurement (Fig. 4B-D). These data imply that the autophagy pathway responds differently to the rotenone exposure and protein insolubility depending on the cell type analyzed.


A
DAPI
MAP2
Merge
20 μm
B
DMSO
Rotenone
0.3nM
0.5nM
Choloroquine
C
GFP positive vesicles / Total number of vesicles (%DMSO)
mCherry positive vesicles / Total number of vesicles (%DMSO)
GFP/mCHerry (%DMSO)
DMSO
0.3nM ROT 48h
0.5nM ROT 48h
3μM CQ 48h
D
Beclin-1
65kDa
β-actin
43kDa
p62
62kDa
LC3II
14kDa
Beclin-1 (relative to β-actin)
p62 (relative to β-actin)
LC3II (relative to β-actin)

◂**Fig. 3** Rotenone exposure decreases autophagic dynamics in hippocampal cell culture. Illustrative digital images of cultured hippocampal cells immunolabelled with antibody against MAP2 (neuron marker) with DAPI as nuclear staining (**A**). Illustrative confocal images of LC3eGFPmCherry expression in hippocampal neurons following DMSO or rotenone exposure for 48 h (**B**). Quantification of GFP and mCherry positive vesicles per cell and the evaluation of the autophagy dinamics in LC3eGFPmCherry transfected neurons (**C**). Representative western blot image of cell extracts from hippocampal cells treated with DMSO or rotenone for different autophagy proteins, as indicated (**D**). Protein levels were normalized to β-actin. Values are shown as percent control (DMSO) ± SD obtained from four (confocal images) or five (western blot) independent experiments. *$p < 0.05$; ***$p < 0.001$; ****$p < 0.0001$; as compared to DMSO according to One-way ANOVA followed by Bonferroni post-hoc test

## Higher concentration of rotenone decreases endogenous BAG2 protein levels in hippocampal cell culture

The co-chaperone BAG2 is able to deliver pTau for degradation to the proteasome in an ubiquitin-independent pathway [19]. Moreover, a previous report demonstrated that BAG2 acts in a specific context for Tau degradation, suggesting that BAG2 expression is dependent on the cell differentiation status [35]. In line with these previous observations, and the data for the SH-SY5Y cells, we decided to verify if a higher concentration of rotenone in hippocampus cells would change BAG2 levels. To test this, the hippocampal cells were exposed to 1 nM of rotenone for 48 h with DMSO as control (Fig. 5). Rotenone treatment lead to an increase in pTau at Ser199/202 and in the ratio between pTau at Ser199/202 and total Tau (Fig. 5A–C), and LC3 (Fig. 5F), suggesting that the treatment impaired Tau and autophagy functions, simmilar to the effects caused by the negative control chloroquine. Exposure to rotenone (1 nM) into these cells did not result in a change in Beclin-1 expression levels, supporting the idea that rotenone treatment at low concentrations does not interfere in the first phases of the autophagy pathway (Fig. 5D). Endogenous levels of BAG2 decreased with rotenone treatment similarly to the chloroquine treatment (Fig. 5E), which was also observed for the SH-SY5Y cells (Fig. 1). These data imply that, besides the autophagic impairment, the pesticide also affects the protein levels of endogenous BAG2.

To further investigate whether BAG2 overexpression was able to decrease Tau phosphorylation at Ser199/202 we decided to transfect the hippocampal cell culture with eGFPBAG2 plasmid (or GFP as control) and treated cells with 1 nM of rotenone for 48 h. BAG2 overexpression prevented the increase of pTau Ser 199/202 levels (Fig. 5G), as observed in Fig. 5C.

GFP transfection (control) in hippocampus cell culture leaded to pTau Ser199/202 expression in the DMSO treatment (Fig. 5G), effect not observed in cells not transfected treated with DMSO (Fig. 5A). This result may indicate that the GFP transfection by itself is enough to lead to physiological changes in phosphorylation of Tau protein at Ser199/202 site.

## BAG2 expression activates autophagy pathway in hippocampal and locus coeruleus cell culture during protein aggregation

To evaluate if BAG2 was able to modulate the autophagy protein levels in the different protein insolubility scenarios, hippocampal cells were transfected with eGFPBAG2 plasmids (Fig. 6A and B), and exposed to 0.3 or 0.5 nM rotenone for 48 h (Fig. 6C). Overexpression of BAG2 significantly increased p62 levels in these cells and reduced LC3II levels (Fig. 6C) when proteostasis is impaired. As observed for the hippocampus, locus coeruleus cells also showed increased p62 levels mediated by BAG2 overexpression (Fig. 7), however this effect seems to be present even in the control scenario. Taken together, these data suggest that BAG2 is able to interact with the autophagy pathways in the regions analyzed, although the differential effect are present only when autophagy is disturbed.

## Discussion

Although rotenone is toxic and causes neurodegeneration when employed in agriculture, it is useful in experimental studies to trigger protein dysfunction making the evaluation of cell pathways related to neurodegeneration possible to understand and propose feasible mechanisms to fight against neurodegenerative diseases [25, 26]. Low doses of rotenone (0.3 or 0.5 nM) are capable of mimicking cell impairments that occur before and during the protein aggregation process in primary cell culture model of hippocampus and locus coeruleus that present Aβ and pTau protein aggregates, without triggering cell death [28, 32]. Moreover, Tau hyperphosphorylation is a relevant hallmark for AD and is considered the primary event in Tau dysfunction. Several kinases can trigger Tau phosphorylation, such as glycogen synthase kinase-3b (GSK3b), c-Jun N-terminal kinase (JNK) and mitogen-activated protein kinase (MAPK), all associated with AD progression [36]. Undifferentiated SH-SY5Y cell exposed to rotenone is a model able to show increase pTau (Ser199/202) protein levels as previously proposed probably by an increase in Tyr216 residue phosphorylation, that lead to GSK3β activation [30].

Several publications have shown impairment in protein degradation by disruption of UPS and autophagy in AD [37, 38]. A previous study had shown that low doses of rotenone decrease proteasome function, what could result in a compensatory upregulation of other pathways,

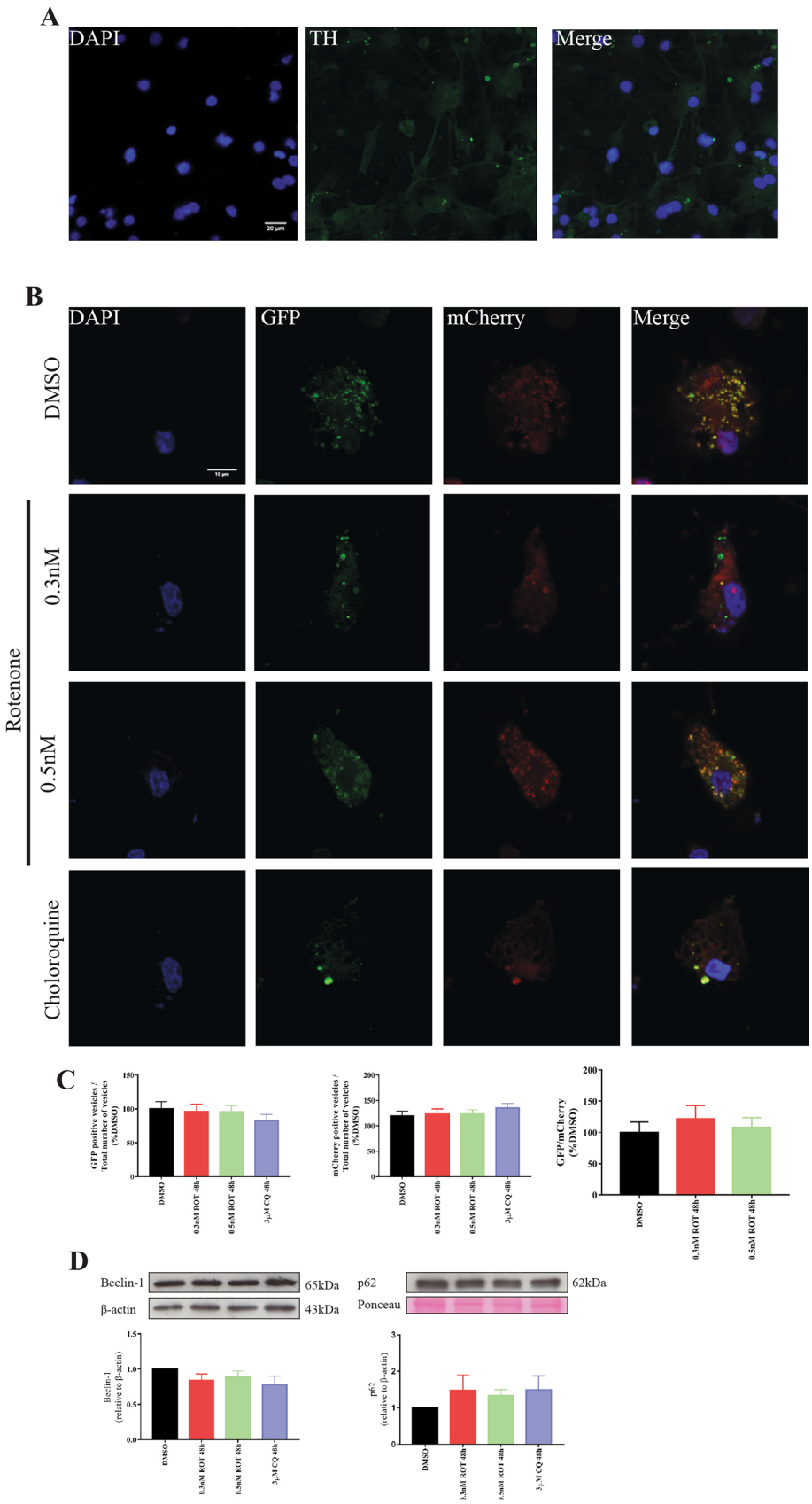



**Fig. 4** Rotenone exposure does not affect autophagic dinamics in locus coeruleus cell culture. Illustrative digital images of cultured locus coeruleus cells immunolabelled with antibody against tyrosine hydroxylase (TH) with DAPI as nuclear staining (**A**). Illustrative confocal images of LC3eGFP-mCherry expression in locus coeruleus neurons following DMSO or rotenone exposure (**B**). Quantification of GFP and mCherry positive vesicles per cell and the evaluation of the autophagy dinamics in LC3eGFPmCherry transfected neurons (**C**). Representative western blot image of cell extracts from locus coeruleus cells treated with DMSO or rotenone for different autophagy proteins, as indicated (**D**). Protein levels were normalized to β-actin. Values are shown as percent control (DMSO) ± SD obtained from three independent experiments. Protein levels were not statistically different in any of the conditions shown, according to One-way ANOVA with Bonferroni's post hoc test

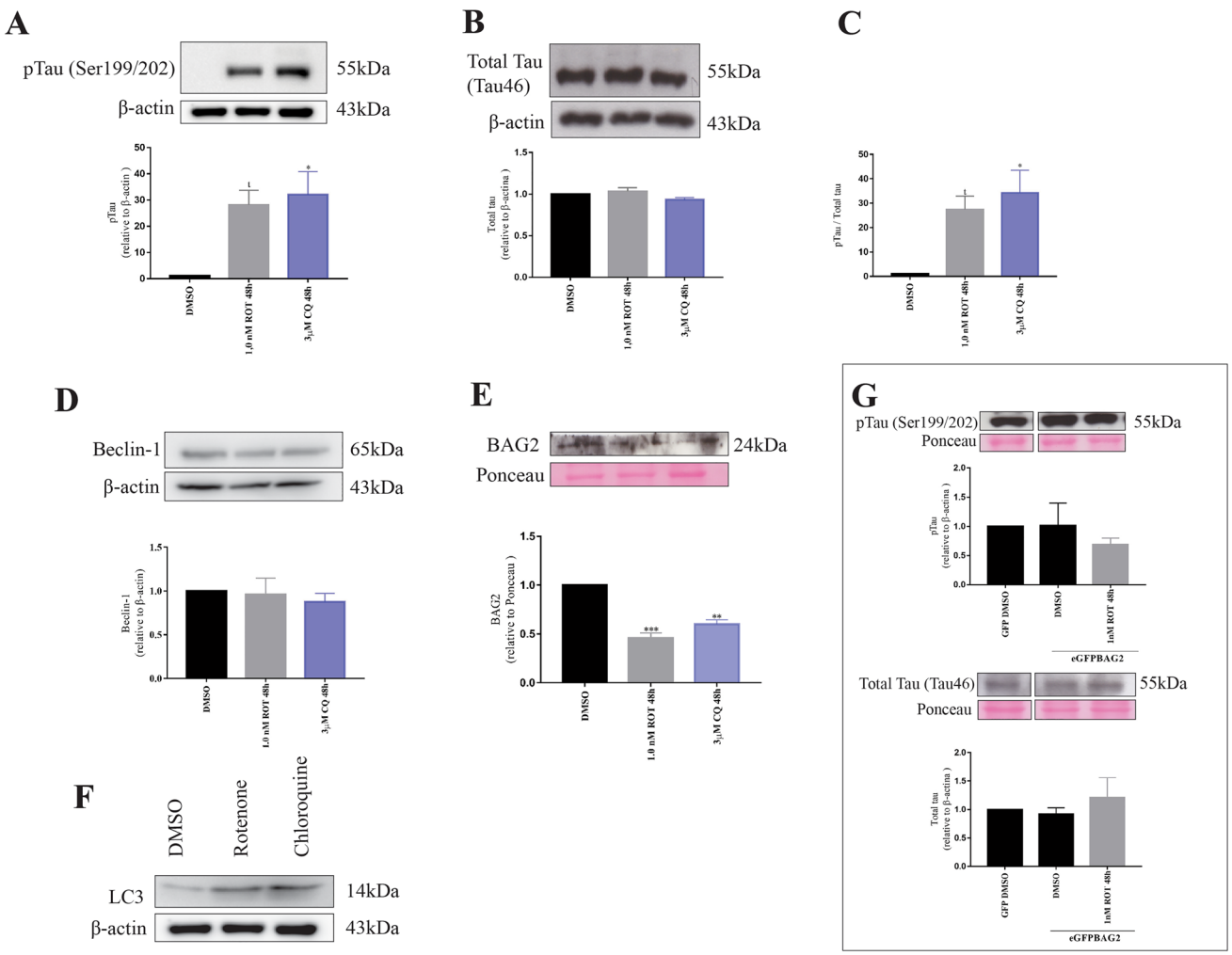



**Fig. 5** Rotenone exposure decreases endogenous BAG2 levels and increase pTau at Ser199/202 in hippocampal cell culture and BAG2 overexpression prevents the raise in pTau. Representative western blot image and quantification of cell extracts from hippocampal cells treated with 1 nM of rotenone (gray), or 3 μM of choloroquine (blue) or DMSO (black) as control for 48 h using antibodies against pTau Ser199/202 (**A**), Total Tau (Tau 46) (**B**), and pTau Total Tau ratio (**C**). Quantification of Beclin-1 (**D**) and endogenous BAG2 (**E**) protein levels. Representative western blot image for antibody against LC3 (**F**). Hippocampal cells were transfected with eGFPBAG2 plasmids (or GFP as control) and treated with 1 nM of rotenone for 48 h and western blot analyses were performed with the indicated antibodies (**G**). Data are shown as mean ± SD of three independent experiments. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ or t for tendency ($t = 0.07$) compared to DMSO, according to One-way ANOVA (or Kruskal–Wallis test when necessary) with Bonferroni's post hoc test. (Color figure online)

such as autophagy [28]. To verify this, hippocampal cells were exposed to low concentrations of rotenone for 48 h that increase ROS production as shown before [28]. Here we have shown by LC3GFPmCherry detection and Western blot analysis that an autophagic dysfunction is present in the early stages of protein aggregation (0.3 nM of rotenone) in hippocampus cells (Fig. 3), supporting our hypothesis that impairment of protein degradation precedes the formation of aggregates. Autophagy, as a complex degradation pathway, can be dysregulated at different and multiple steps. In our results, LC3II are accumulated with 0.3 nM of rotenone, similarly to what occurs with chloroquine treatment (Fig. 3D). Interestingly, Beclin-1 levels remain unchanged in the samples analyzed, what points to a scenario were only latter phases of the autophagy pathway is impaired (Fig. 3D). Despite these results corroborate previous studies [28] it is important to highlight that the role of protein aggregation in neurodegeneration is still not well understood in the literature, and some results are controversial depending on the cell type and model studied [39–41].

Indeed, it is possible that different areas of the brain respond differently to the same treatment. For example, rotenone exposure did not cause a significant autophagy impairment in locus coeruleus (Fig. 4), despite previous publish

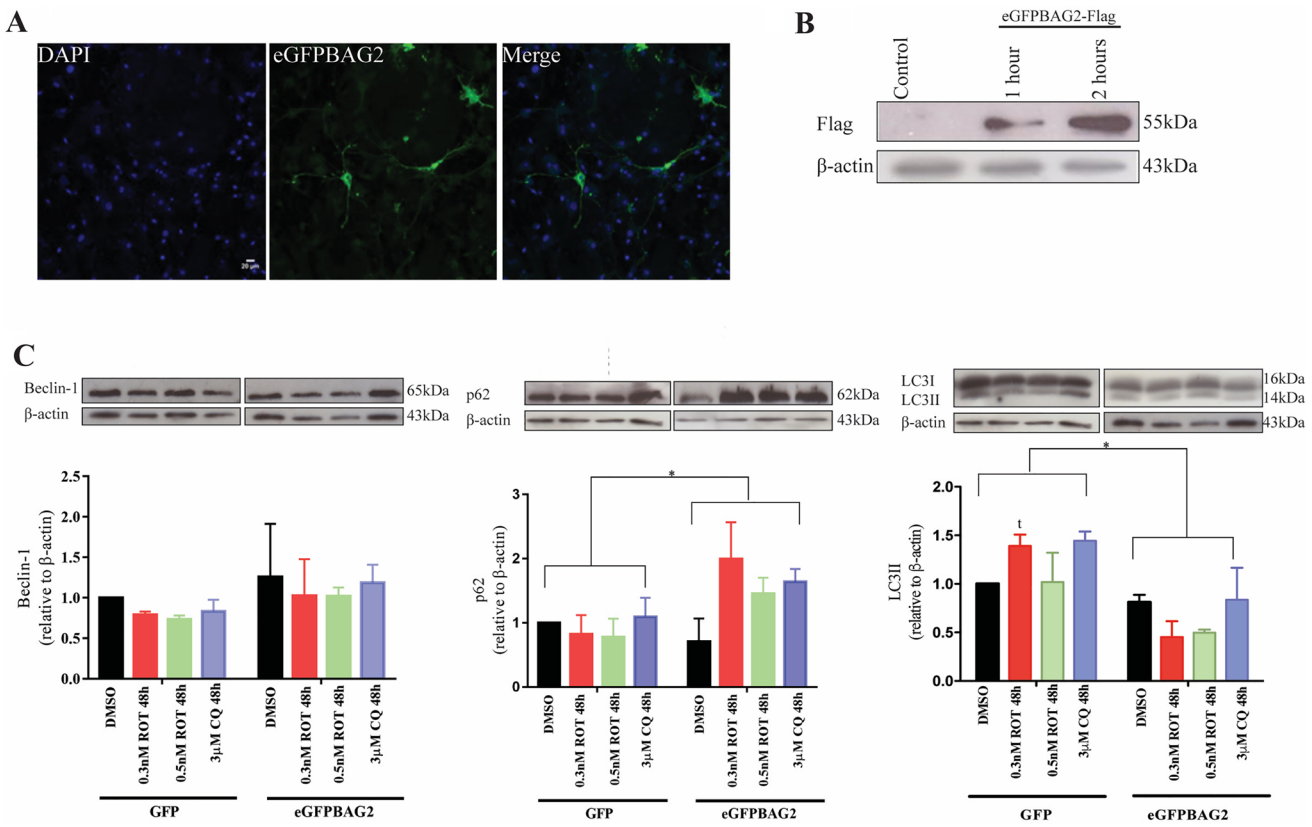



**Fig. 6** BAG2 overexpression increases p62 protein levels in hippocampal cell culture exposed to rotenone. Illustrative digital images of cultured hippocampal cells overexpressing GFP-BAG2-flag construct, with DAPI as nuclear staining (**A**). Representative western blot image of cell extracts from hippocampal cells transfected with GFP-BAG2-flag, or no transfected (as control) for 1 or 2 h before medium change using the indicated antibodies (**B**). Representative western blot images of cell extracts from hippocampal cells transfected with GFP-BAG2-flag or GFP alone, for 2 h followed by specific treatments: 0.3 (red) or 0.5 nM (green) of rotenone, 3 μM of choloroquine (blue) or DMSO (black) as control, for 48 h, using the indicated antibodies. Data are shown as mean ± SD of three independent experiments. *$p < 0.05$ or t for tendency ($t = 0.07$) compared to DMSO GFP, according to Two-way ANOVA with Bonferroni's post hoc test. (Color figure online)

work from our lab had shown that the treatment was efficient in trigger protein aggregation for pTau, Aβ, and α-Syn [32]. These results were not expected, since it is known that this region is rich in noradrenergic neurons and, therefore, more susceptible to the damage induced by ROS increase. Locus coeruleus is the center for norepinephrine synthesis and is the only cortical source of this neurotransmitter [42], where a defect in its regulation is associated with emotional memory deficit in old rats [43]. Moreover, it is known that dysfunction in norepinephrine occurs early in AD pathogenesis [44] together with a loss of neurons projections [45, 46], that can correspond to almost 80% in some patients [47], features that were not achieved in our model.

Different chaperones are being studied in autophagy context, especially in neurodegenerative disorders, since it is fundamental for substrate selection, and delivery to degradation, and also in generating intracellular responses for protein dysfunction. Recent studies demonstrated that members of the BAG family act as modulators for other chaperones. BAG2, for example, can induce pTau degradation by the proteasome in an ubiquitin-independent manner [19, 48] through inhibition of the E3 ubiquitin ligase CHIP, important for the canonical Tau degradation machinery [48]. Moreover, BAG2 can also be associated directly with PINK-1 wild-type and mutant leading towards its stabilization by decreasing its ubiquitylation [49], suggesting a possible role in mitochondria degradation.

De Paula and colleagues [35] demonstrated that a decrease in BAG2 expression levels increased pTau mRNA and protein levels in SH-SY5Y cells exposed to temperature reduction. The same study revealed that the response mediated by BAG2 is dependent on the status of cell differentiation [35]. In our cell model, SH-SY5Y undifferentiated cells and hippocampal cells treated with 1 nM of rotenone showed a decrease in BAG2 endogenous levels (Figs. 1B and 5E), what could be important, together with kinases activation, for the increase of pTau levels and dysfunction of Tau homeostasis.

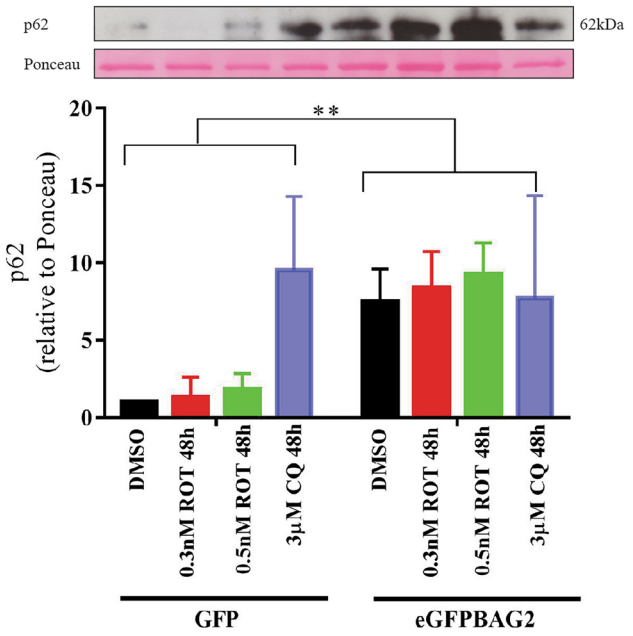



**Fig. 7** BAG2 overexpression increases p62 protein levels in locus coeruleus cell culture. Representative western blot images of cell extracts from locus coeruleus cells transfected with GFP-BAG2-flag or GFP alone, for 2 h followed by specific treatments: 0.3 (red) or 0.5 nM (green) of rotenone, 3 µM of choloroquine (blue) or DMSO (black) as control, for 48 h, using the indicated antibodies. Data are shown as mean ± SD of two independent experiments. $^{**}p < 0.01$ compared to DMSO GFP, according to Two-way ANOVA with Bonferroni's post hoc test. (Color figure online)

Some relations had been proposed between BAG proteins and autophagy, especially with BAG3 [17, 50, 51]. BAG3 can act in the complex BAG3-HSPB8-HSP70 together with p62 and regulate protein misfolding degradation, or can be associated with dynein, a motor protein important in intracellular transport, showing some role in protein transport to the aggresomes [50]. It is known that the co-chaperone BAG2 can associate with different cell components, such as mitochondria, endoplasmic reticulum, and microtubules [16], however, only recently that the BAG2 possible role in selective autophagy started to be elucidated [24].

Since our rotenone model was able to induce Tau dysfunction at Ser199/202 in SH-SY5Y cells and hippocampal primary cells culture [32] associated with an autophagy impairment, we hypothesized that BAG2 overexpression would be able to lead some autophagy response upon rotenone treatment. To verify these, cells overexpressing BAG2 were analyzed by immunoblot assays for different autophagy proteins. Overexpression of BAG2 in undifferentiated cells prevented the decrease in p62 after rotenone exposure. Moreover, BAG2 was able to significantly increase p62 levels in hippocampus and locus coeruleus (Figs. 6C and 7) and also led to a decrease in LC3II/LC3I ratio in hippocampus cells (Fig. 6C), suggesting that BAG2 overexpression might have an important role in autophagy response during protein aggregation. It is noteworth that acute effects, as those observed in SH-SY5Y cells exposed to rotenone during 4.5 h, may trigger different responses as compared to more prolonged exposure to rotenone, as in primary cells in the present study. Indeed, p62 levels might be downregulated in the acute phase of protein aggregation and upregulated during the prolonged autophagy impairment, demonstrating that the results achieved here are not contradictory.

Recently, BAG2 had been proposed as a potential plasma biomarker candidate for early-diagnosis for PD that might be associated to autophagy dysfunction [52]. In this research, the authors showed a decrease in BAG2 and cathepsin D (a lysosomal aspartic endopeptidase) expression in the plasma of those patients. Suprisingly, BAG2 decrease was significantly correlated with olfactory dysfunction [52]. Our results showed that rotenone treatment, highly used in neurodegenerative models, was efficient in leading the BAG2 decrease supporting the recent findings in literature. Moreover, Liang et al. [24] explored the interplay between BAG2 and p62 in immunology and the present study can also bring new information to this field.

P62 can generate multiple responses regarding autophagy. In some scenarios, p62 can induce autophagy and problems in this protein are associated with misfolding protein aggregation [53]. However, when high levels of amino acids are present, p62 can inhibit autophagy by mTOR ubiquitylation [54]. Moreover, a p62 decrease was detected in AD patients, with autophagy impairment associated with Tau and Aβ accumulation [55], and a reduction in ubiquitylated pTau degradation [56]. These results suggest that an increase in p62 protein levels could be effective in AD [53].

Although the levels of endogenous BAG2 in the beginning of Tau hyperphosphorylation (after 0.3 and 0.5 nM rotenone) remains to be determined (mainly because de poor BAG2 signal in primary cells), it is clear that overexpression of BAG2 prevents Tau phosphorylation at Ser199/202, p62 decrease and LC3II accumulation which contributes to the literature concerning the role of BAG2 during protein insolubility and opens a new venue of investigation on a possible target to deepen the knowledge in neurodegeneration field.

In conclusion, data suggest that BAG2 is decreased when pTau Ser199/202 increases and overexpression of BAG2 was able to increase p62 levels in hippocampus and locus coeruleus as well as prevented the raise in pTau Ser199/202 in hippocampus after rotenone exposure. It might be speculated that the involvement of BAG2 with p62 could allow specific substrate marking towards degradation to autophagy.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s11033-022-07577-w.

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**Author contributions** RSL performed the experiments; RSL, DCC and MFRF analysed data and wrote the manuscript; MFRF supervised the study.

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**Data availability** Data will be available upon request.

**Code availability** Not applicable.

## Declarations

**Conflict of interest** Authors declare that there are no conflicts of interest to be reported.

**Ethical approval** All the procedures were performed in strict accordance with Institutional and International Guidelines for animal care and use [29], as well as respecting the Brazilian federal law 11794/08 for animal welfare and approved by the institutional ethics committee (CEUA 271/2016) of the Department of Genetics and Evolutionary Biology, Institute for Biosciences, University of Sao Paulo.

**Consent to participate** Not applicable.

**Consent for publication** Not applicable.

## References

1. Grune T, Jung T, Merker K, Davies KJA (2004) Decreased proteolysis caused by protein aggregates, inclusion bodies, plaques, lipofuscin, ceroid, and 'aggresomes' during oxidative stress, aging, and disease. Int J Biochem Cell Biol 36:2519–2530. https://doi.org/10.1016/j.biocel.2004.04.020
2. Yamamura Y, Kuzuhara S, Kondo K et al (1998) Clinical, pathologic and genetic studies on autosomal recessive early-onset parkinsonism with diurnal fluctuation. Parkinsonism Relat Disord 4:65–72. https://doi.org/10.1016/S1353-8020(98)00015-7
3. Tolmasov M, Djaldetti R, Lev N, Gilgun-Sherki Y (2016) Pathological and clinical aspects of alpha/beta synuclein in Parkinson's disease and related disorders. Expert Rev Neurother 16:505–513. https://doi.org/10.1586/14737175.2016.1164600
4. Polymeropoulos MH, Lavedan C, Leroy E et al (1997) Mutation in the α-synuclein gene identified in families with Parkinson's disease. Science (80–) 276:2045–2047. https://doi.org/10.1126/science.276.5321.2045
5. Marmolino D, Foerch P, Atienzar FA et al (2016) Alpha synuclein dimers and oligomers are increased in overexpressing conditions in vitro and in vivo. Mol Cell Neurosci 71:92–101. https://doi.org/10.1016/j.mcn.2015.12.012
6. Buée L, Bussière T, Buée-Scherrer V et al (2000) Tau protein isoforms, phosphorylation and role in neurodegenerative disorders11These authors contributed equally to this work. Brain Res Rev 33:95–130. https://doi.org/10.1016/S0165-0173(00)00019-9
7. Kamenetz F, Tomita T, Hsieh H et al (2003) APP processing and synaptic function. Neuron 37:925–937. https://doi.org/10.1016/S0896-6273(03)00124-7
8. De Vos KJ, Grierson AJ, Ackerley S, Miller CCJ (2008) Role of axonal transport in neurodegenerative diseases. Annu Rev Neurosci 31:151–173. https://doi.org/10.1146/annurev.neuro.31.061307.090711
9. Vilchez D, Saez I, Dillin A (2014) The role of protein clearance mechanisms in organismal ageing and age-related diseases. Nat Commun 5:5659. https://doi.org/10.1038/ncomms6659
10. Aman Y, Schmauck-Medina T, Hansen M et al (2021) Autophagy in healthy aging and disease. Nat Aging 1:634–650. https://doi.org/10.1038/s43587-021-00098-4
11. Hansen M, Rubinsztein DC, Walker DW (2018) Autophagy as a promoter of longevity: insights from model organisms. Nat Rev Mol Cell Biol 19:579–593. https://doi.org/10.1038/s41580-018-0033-y
12. Mizushima N, Yoshimori T, Levine B (2010) Methods in mammalian autophagy research. Cell 140:313–326. https://doi.org/10.1016/j.cell.2010.01.028
13. Pankiv S, Clausen TH, Lamark T et al (2007) p62/SQSTM1 binds directly to Atg8/LC3 to facilitate degradation of ubiquitinated protein aggregates by autophagy. J Biol Chem 282:24131–24145. https://doi.org/10.1074/jbc.M702824200
14. Kampinga HH, Craig EA (2010) The HSP70 chaperone machinery: J proteins as drivers of functional specificity. Nat Rev Mol Cell Biol 11:579–592. https://doi.org/10.1038/nrm2941
15. Xu Z, Page RC, Gomes MM et al (2008) Structural basis of nucleotide exchange and client binding by the Hsp70 cochaperone Bag2. Nat Struct Mol Biol 15:1309–1317. https://doi.org/10.1038/nsmb.1518
16. Qin L, Guo J, Zheng Q, Zhang H (2016) BAG2 structure, function and involvement in disease. Cell Mol Biol Lett 21:18. https://doi.org/10.1186/s11658-016-0020-2
17. Ji C, Tang M, Zeidler C et al (2019) BAG3 and SYNPO (synaptopodin) facilitate phospho-MAPT/Tau degradation via autophagy in neuronal processes. Autophagy 15:1199–1213. https://doi.org/10.1080/15548627.2019.1580096
18. Arndt V, Daniel C, Nastainczyk W et al (2005) BAG-2 acts as an Inhibitor of the chaperone-associated ubiquitin ligase CHIP. Mol Biol Cell 16:5891–5900. https://doi.org/10.1091/mbc.e05-07-0660
19. Carrettiero DC, Hernandez I, Neveu P et al (2009) The cochaperone BAG2 sweeps paired helical filament- insoluble Tau from the microtubule. J Neurosci 29:2151–2161. https://doi.org/10.1523/JNEUROSCI.4660-08.2009
20. Santiago FE, Almeida MC, Carrettiero DC (2015) BAG2 Is Repressed by NF-κB signaling, and its overexpression is sufficient to shift Aβ1-42 from neurotrophic to neurotoxic in undifferentiated SH-SY5Y neuroblastoma. J Mol Neurosci 57:83–89. https://doi.org/10.1007/s12031-015-0579-5
21. Guo JF, He S, Kang JF et al (2015) Involvement of Bcl-2-associated athanogene (BAG)-family proteins in the neuroprotection by rasagiline. Int J Clin Exp Med 8:18158–18164
22. Qu D, Hage A, Don-Carolis K et al (2015) BAG2 gene-mediated regulation of PINK1 protein is critical for mitochondrial translocation of PARKIN and neuronal survival. J Biol Chem 290:30441–30452. https://doi.org/10.1074/jbc.M115.677815
23. Siuda J, Jasinska-Myga B, Boczarska-Jedynak M et al (2014) Early-onset Parkinson's disease due to PINK1 p. Q456X mutation—Clinical and functional study. Parkinsonism Relat Disord 20:1274–1278. https://doi.org/10.1016/j.parkreldis.2014.08.019

24. Liang S, Wang F, Bao C et al (2020) BAG2 ameliorates endoplasmic reticulum stress-induced cell apoptosis in *Mycobacterium tuberculosis*-infected macrophages through selective autophagy. Autophagy 16:1453–1467. https://doi.org/10.1080/15548627.2019.1687214
25. Radad K, Gille G, Rausch W-D (2008) Dopaminergic neurons are preferentially sensitive to long-term rotenone toxicity in primary cell culture. Toxicol In Vitro 22:68–74. https://doi.org/10.1016/j.tiv.2007.08.015
26. Ullrich C, Humpel C (2009) Rotenone induces cell death of cholinergic neurons in an organotypic co-culture brain slice model. Neurochem Res 34:2147–2153. https://doi.org/10.1007/s11064-009-0014-9
27. Xie C, Zhuang X-X, Niu Z et al (2022) Amelioration of Alzheimer's disease pathology by mitophagy inducers identified via machine learning and a cross-species workflow. Nat Biomed Eng 6:76–93. https://doi.org/10.1038/s41551-021-00819-5
28. Chaves RS, Kazi AI, Silva CM et al (2016) Presence of insoluble Tau following rotenone exposure ameliorates basic pathways associated with neurodegeneration. IBRO Reports. https://doi.org/10.1016/j.ibror.2016.09.001
29. Demers G, Griffin G, De Vroey G et al (2006) Harmonization of animal care and use guidance. Science (80–) 312:700–701. https://doi.org/10.1126/science.1124036
30. Hongo H, Kihara T, Kume T et al (2012) Glycogen synthase kinase-3β activation mediates rotenone-induced cytotoxicity with the involvement of microtubule destabilization. Biochem Biophys Res Commun 426:94–99. https://doi.org/10.1016/j.bbrc.2012.08.042
31. Kivell BM, McDonald FJ, Miller JH (2001) Method for serum-free culture of late fetal and early postnatal rat brainstem neurons. Brain Res Protoc 6:91–99. https://doi.org/10.1016/S1385-299X(00)00037-4
32. Chaves RS, Melo TQ, Martins SA, Ferrari MFR (2010) Protein aggregation containing beta-amyloid, alpha-synuclein and hyperphosphorylated tau in cultured cells of hippocampus, substantia nigra and locus coeruleus after rotenone exposure. BMC Neurosci. https://doi.org/10.1186/1471-2202-11-144
33. Kimura S, Noda T, Yoshimori T (2007) Dissection of the autophagosome maturation process by a novel reporter protein, tandem fluorescent-tagged LC3. Autophagy 3:452–460. https://doi.org/10.4161/auto.4451
34. Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254. https://doi.org/10.1016/0003-2697(76)90527-3
35. de Paula CAD, Santiago FE, de Oliveira ASA et al (2016) The co-chaperone BAG2 mediates cold-induced accumulation of phosphorylated Tau in SH-SY5Y cells. Cell Mol Neurobiol 36:593–602. https://doi.org/10.1007/s10571-015-0239-x
36. Pei J-J, Braak E, Braak H et al (2001) Localization of active forms of C-jun kinase (JNK) and p38 kinase in Alzheimer's disease brains at different stages of neurofibrillary degeneration. J Alzheimer's Dis 3:41–48. https://doi.org/10.3233/JAD-2001-3107
37. Huang Q, Figueiredo-Pereira ME (2010) Ubiquitin/proteasome pathway impairment in neurodegeneration: therapeutic implications. Apoptosis 15:1292–1311. https://doi.org/10.1007/s10495-010-0466-z
38. Wolfe DM, Lee J, Kumar A et al (2013) Autophagy failure in Alzheimer's disease and the role of defective lysosomal acidification. Eur J Neurosci 37:1949–1961. https://doi.org/10.1111/ejn.12169
39. Cherra SJ, Kulich SM, Uechi G et al (2010) Regulation of the autophagy protein LC3 by phosphorylation. J Cell Biol 190:533–539. https://doi.org/10.1083/jcb.201002108
40. Hernandez D, Torres CA, Setlik W et al (2012) Regulation of presynaptic neurotransmission by macroautophagy. Neuron 74:277–284. https://doi.org/10.1016/j.neuron.2012.02.020
41. Ravikumar B, Vacher C, Berger Z et al (2004) Inhibition of mTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of Huntington disease. Nat Genet 36:585–595. https://doi.org/10.1038/ng1362
42. Daulatzai MA (2016) Dysfunctional sensory modalities, locus coeruleus, and basal forebrain: early determinants that promote neuropathogenesis of cognitive and memory decline and Alzheimer's disease. Neurotoxicol Res 30:295–337. https://doi.org/10.1007/s12640-016-9643-3
43. Luo Y, Zhou J, Li M et al (2015) Reversal of aging-related emotional memory deficits by norepinephrine via regulating the stability of surface AMPA receptors. Aging Cell 14:170–179. https://doi.org/10.1111/acel.12282
44. Brunnström H, Friberg N, Lindberg E, Englund E (2011) Differential degeneration of the locus coeruleus in dementia subtypes. Clin Neuropathol 30:104–110. https://doi.org/10.5414/NPP30104
45. Grudzien A, Shaw P, Weintraub S et al (2007) Locus coeruleus neurofibrillary degeneration in aging, mild cognitive impairment and early Alzheimer's disease. Neurobiol Aging 28:327–335. https://doi.org/10.1016/j.neurobiolaging.2006.02.007
46. Weinshenker D (2008) Functional consequences of locus coeruleus degeneration in Alzheimers disease. Curr Alzheimer Res 5:342–345. https://doi.org/10.2174/156720508784533286
47. Bondareff W, Mountjoy CQ, Roth M (1982) Loss of neurons of origin of the adrenergic projection to cerebral cortex (nucleus locus ceruleus) in senile dementia. Neurology 32:164–164. https://doi.org/10.1212/WNL.32.2.164
48. Wang Y, Mandelkow E (2012) Degradation of tau protein by autophagy and proteasomal pathways. Biochem Soc Trans 40:644–652. https://doi.org/10.1042/BST20120071
49. Che X, Tang B, Wang X et al (2013) The BAG2 protein stabilises PINK1 by decreasing its ubiquitination. Biochem Biophys Res Commun 441:488–492. https://doi.org/10.1016/j.bbrc.2013.10.086
50. Gamerdinger M, Kaya AM, Wolfrum U et al (2011) BAG3 mediates chaperone-based aggresome-targeting and selective autophagy of misfolded proteins. EMBO Rep 12:149–156. https://doi.org/10.1038/embor.2010.203
51. Sariyer IK, Merabova N, Patel PK et al (2012) Bag3-induced autophagy is associated with degradation of JCV oncoprotein, T-Ag. PLoS ONE 7:e45000. https://doi.org/10.1371/journal.pone.0045000
52. Kang J, Kim JW, Heo H et al (2021) Identification of BAG2 and cathepsin D as plasma biomarkers for Parkinson's disease. Clin Transl Sci 14:606–616. https://doi.org/10.1111/cts.12920
53. Liu H, Dai C, Fan Y et al (2017) From autophagy to mitophagy: the roles of P62 in neurodegenerative diseases. J Bioenerg Biomembr 49:413–422. https://doi.org/10.1007/s10863-017-9727-7
54. Sánchez-Martín P, Komatsu M (2018) p62/SQSTM1—steering the cell through health and disease. J Cell Sci. https://doi.org/10.1242/jcs.222836
55. Tanji K, Miki Y, Ozaki T et al (2014) Phosphorylation of serine 349 of p62 in Alzheimer's disease brain. Acta Neuropathol Commun 2:50. https://doi.org/10.1186/2051-5960-2-50
56. Jung H-J, Kim Y-J, Eggert S et al (2013) Age-dependent increases in tau phosphorylation in the brains of type 2 diabetic rats correlate with a reduced expression of p62. Exp Neurol 248:441–450. https://doi.org/10.1016/j.expneurol.2013.07.013