HHS Public Access

Author manuscript

Biochim Biophys Acta Mol Basis Dis. Author manuscript; available in PMC 2025 January 01.

Published in final edited form as:

Biochim Biophys Acta Mol Basis Dis. 2024 January ; 1870(1): 166898. doi:10.1016/j.bbadis.2023.166898.

Caspase-3 cleaved tau impairs mitochondrial function through the opening of the mitochondrial permeability transition pore

María José Pérez[1], **Rodrigo Ibarra-García-Padilla**[1], **Maoping Tang**[2], **George A. Porter Jr**[3], **Gail V.W. Johnson**[2], **Rodrigo A. Quintanilla**[1]

[1]Laboratory of Neurodegenerative Diseases, Centro de Investigaciones Biomédicas, Universidad Autónoma de Chile, Santiago, Chile.

[2]Department of Anesthesiology, University of Rochester Medical Center, New York, USA

[3]Department of Pediatrics, University of Rochester Medical Center, New York, USA

Abstract

Mitochondrial dysfunction is a significant factor in the development of Alzheimer's disease (AD). Previous studies have demonstrated that the expression of tau cleaved at Asp421 by caspase-3 leads to mitochondrial abnormalities and bioenergetic impairment. However, the underlying mechanism behind these alterations and their impact on neuronal function remains unknown. To investigate the mechanism behind mitochondrial dysfunction caused by this tau form, we used transient transfection and pharmacological approaches in immortalized cortical neurons and mouse primary hippocampal neurons. We assessed mitochondrial morphology and bioenergetics function after expression of full-length tau and caspase-3-cleaved tau. We also evaluated the mitochondrial permeability transition pore (mPTP) opening and its conformation as a possible mechanism to explain mitochondrial impairment induced by caspase-3 cleaved tau. Our studies showed that pharmacological inhibition of mPTP by cyclosporine A (CsA) prevented all mitochondrial length and bioenergetics abnormalities in neuronal cells expressing caspase-3 cleaved tau. Neuronal cells expressing caspase-3-cleaved tau showed sustained mPTP opening which is mostly dependent on cyclophilin D (CypD) protein expression. Moreover, the impairment of mitochondrial length and bioenergetics induced by caspase-3-cleaved tau were prevented in hippocampal neurons obtained from CypD knock-out mice. Interestingly, previous studies using these mice showed

Correspondence to: Dr. Rodrigo A. Quintanilla, Laboratory of Neurodegenerative Diseases, Universidad Autónoma de Chile, El Llano Subercaseaux 2801, 5to Piso, San Miguel 8910060, Santiago, Chile. rodrigo.quintanilla@uautonoma.cl.

Author's contributions

MJP designed the study, performed experiments, and wrote and edited the manuscript; RIGP, performed experiments; MT, performed experiments; GVWJ, edited the final draft of the manuscript; GAPJr, edited the final draft of manuscript; RAQ designed the study, performed experiments, and edited the manuscript. All authors read and approved the final manuscript draft.

Ethics approval and consent to participate

This work has the ethics approval of the Comité de Bioetica de la Universidad Autónoma, and Fondo de Ciencia y Tecnología (FONDECYT), ANID, Santiago, Chile. Resolution BE-05–20 (April 2020).

Competing interest

All authors hereby declare that they do not have any competing interests with respect to this work and do not intend to pursue any such interests in the future.

a prevention of mPTP opening and a reduction of mitochondrial failure and neurodegeneration induced by AD. Therefore, our findings showed that caspase-3-cleaved tau negatively impacts mitochondrial bioenergetics through mPTP activation, highlighting the importance of this channel and its regulatory protein, CypD, in the neuronal damage induced by tau pathology in AD.

Keywords

Tau; Mitochondria; Alzheime's disease; Mitochondrial permeability pore; Cyclophilin D

Introduction

Alzheimer's disease (AD) is an irreversible neurodegenerative disorder and the most common form of dementia in elderly (Querfurth and LaFerla 2010). AD is characterized by the accumulation of misfolded proteins, such as Aß peptide and tau protein, in the brain, leading to progressive neurodegeneration of the patients (Querfurth and LaFerla 2010). Tau protein is a microtubule-associated protein that promotes the assembly and stabilization of microtubules in physiological conditions, but in AD, it assembles into protein aggregates and undergoes pathological modifications that lead to neuronal dysfunction and death (Tapia-Rojas, Cabezas-Opazo et al. 2019). One of the pathological forms of tau present in AD is the caspase-3-cleaved tau, which is responsible for the initial stages of AD and the formation of neurofibrillary tangles (Gamblin, Chen et al. 2003; Rissman, Poon et al. 2004; de Calignon, Fox et al. 2010). Previous studies from our group have shown that truncated tau affects mitochondrial bioenergetics, transport, and morphology, leading to alterations in mitochondrial dynamics (by a reduction of optic atrophy 1 (Opa1) protein expression) (Perez, Jara et al. 2018a; Perez, Vergara-Pulgar et al. 2018c), an increase in oxidative stress, deregulations in calcium-buffering capacity, and an enhancement of Aß-induced mitochondrial failure (Quintanilla, Matthews-Roberson et al. 2009; Quintanilla, von Bernhardi et al. 2014; Perez, Vergara-Pulgar et al. 2018c; Tapia-Monsalves et al., 2023). These alterations suggests that caspase-3 cleaved tau affects mitochondrial function and sensitizes this organelle to toxic stimuli present in AD. However, the underlying mechanism involved in truncated tau toxicity remains unknown.

The mitochondrial permeability transition pore (mPTP) is a non-specific channel formed by cyclophilin D (CypD), the voltage-dependent anion channel (VDAC), adenine nucleotide translocase (ANT), ATP synthase, and the oligomycin sensitivity-conferring protein (OSCP) subunit of ATP synthase, which has been suggested to participate in mPTP activity (Jonas, Porter et al. 2015; Perez and Quintanilla 2017). Calcium impairment and increased oxidative stress can induce mPTP opening, resulting in an increase in mitochondrial permeability, release of mitochondrial content, and mitochondria-driven cell death, which is observed in most neurological disorders (Kroemer and Blomgren 2007; Du and Yan 2010; Bonora, Bononi et al. 2013; Bernardi and Di Lisa 2015; Jonas, Porter et al. 2015). Several studies have suggested an association between mPTP opening and mitochondrial dysfunction in the context of AD (Perez and Quintanilla 2017). Increased levels of VDAC and CypD have been observed in postmortem brains of AD patients and animal models of AD, which is significant due to the suggested role of elevated CypD levels as a relevant factor in

mPTP activation and mitochondrial failure (Du, Guo et al. 2011; Guo, Du et al. 2013; Gauba, Guo et al. 2017). Notably, crossing transgenic mAPP mice, a genetic model of AD that overexpresses mutated APP, with CypD (−/−) knockout mice resulted in complete restoration of mitochondrial function, as well as reduced neuronal damage and cognitive impairment compared to mAPP mice expressing endogenous levels of CypD (Du and Yan 2010; Du, Guo et al. 2011; Guo, Du et al. 2013). In addition, previous research from our group has demonstrated that tau deletion has positive effects on hippocampal neurons by improving mitochondrial function and inhibiting mPTP formation by reducing CypD protein (Jara, Cerpa et al. 2021). Moreover, tau deletion improved recognition memory and attentive capacity in juvenile mice and during normal aging, thereby enhancing brain function by improving mitochondrial health (Jara, Aranguiz et al. 2018; Jara, Cerpa et al. 2021). These findings underscore the importance of understanding the role of mPTP in mitochondrial dysfunction induced by tau pathology in AD.

In the current study, we used transient transfection and pharmacological approaches in immortalized cortical neurons and mouse primary hippocampal neurons to investigate the mechanism underlying mitochondrial dysfunction caused by caspase-3-cleaved tau overexpression. Our results indicate that caspase-3-cleaved tau has a negative impact on mitochondrial bioenergetics through mPTP activation, highlighting the significance of this channel and its regulatory protein, CypD, in the neuronal damage induced by tau pathology in AD.

Methods

Cell culture.

Conditionally immortalized neuronal cell lines (CN1.4 cells) (Quintanilla, Matthews-Roberson et al. 2009; Quintanilla, von Bernhardi et al. 2014; Perez, Vergara-Pulgar et al. 2018c) were cultured in DMEM media (Mediatech Inc., Corning, NY, USA) supplemented with 5% inactivated fetal bovine serum (Mediatech Inc., Corning, NY, USA) and 1% penicillin/streptomycin (Mediatech Inc., Corning, NY, USA) at 33ºC and 5% CO_2.

Hippocampal neuronal cultures were prepared from tau (−/−) knockout mice (B6.129 × 1-Mapttm1Hnd /J, 7,251) (Perez, Vergara-Pulgar et al. 2018c) obtained from Jackson Laboratories (USA) or CypD (−/−) knock-out mice (Hom, Quintanilla et al. 2011) on embryonic day 18. The cultures were maintained in Neurobasal growth medium (ThermoFisher) supplemented with B27 (ThermoFisher, MA, USA), L-glutamine, penicillin, and streptomycin (Mediatech Inc., Corning, NY, USA). Cytosine arabinoside (Sigma, MA, USA) was added to the cultured neurons on day 3 for 72 h to obtain cultures highly enriched in neurons (~ 5% glia), and the cultured neurons were transfected on day 8.

Transient transfection.

Tau constructs tagged with GFP, GFP-full-length-Tau, and GFP-caspase-3 cleaved-Tau (GFP-Tau (s)) as well as Mito-mCherry were generated according to previously described protocols (Quintanilla, Dolan et al. 2012; Perez, Vergara-Pulgar et al. 2018c). Lipofectamine 2000 (ThermoFisher, MA, USA) diluted in OptiMEM (ThermoFisher, MA, USA) was used

to transiently transfect CN1.4 cells and hippocampal neurons with plasmids containing GFP (1 μg) or GFP-Tau(s) (1 μg) constructs or to co-transfect them with Mito-mCherry (1 μg). Media were changed 24 h post-transfection, and analyses were conducted 48 h post-transfection for CN1.4 cells. For hippocampal neurons transfection, the protocol was applied after 8 days of culture and studies were made 48 h post-transfection. Live cell imaging was used to verify the transfection efficiency, which was found to be 30% and 5% for CN 1.4 and primary neurons, respectively. Western blotting was used to verify equal levels of transfection for all plasmids in CN 1.4 cells (Fig. 3C) (Perez, Vergara-Pulgar et al. 2018c).

For Opa1 expression studies, Opa1 (Myc-DDK-tagged) (Cat MR225663) plasmid was commercially obtained from Origene (Rockville, MD, USA). CN1.4 cells were double transfected with GFP (1μg) and GFP-tau(s) (1 μg) and Opa1-c-myc (1 μg) as described above. The Opa1 transfection was verified using immunofluorescence (Fig. 2B). To determine changes following a stimulus, cells were treated with thapsigargin (1μM) for 30 minutes, as indicated. For mPTP studies, cells were pre-treated with 0.5μM Cyclosporin A (CsA) (Tocris Bioscience, Bristol, UK) or 500 nM FK506 (Tocris Bioscience, Bristol, UK) for 2 hours, as indicated (Quintanilla et al., 2013; Tapia-Monsalves et al., 2023).

Measurement of mitochondrial length.

Mitochondrial length was calculated as previously described (Perez, Vergara-Pulgar et al. 2018c). Briefly, the length of individual mitochondria was measured in cells double transfected with GFP/Mito-mCherry and GFP-Tau(s)/Mito-mCherry. For CN1.4 cells double transfected with GFP-Tau(s) forms and Opa1-c-myc, cells were incubated with MitoTracker Red CMXRos (Molecular Probes, Thermo Fisher Scientific, MA, USA) for 35 min in KRH-glucose buffer. Then, cells were fixed in 4% paraformaldehyde-KRH-glucose for 15 min at 37°C and blocked with 10% bovine serum in KRH-glucose for 1 h. After that, the cells were incubated with a primary monoclonal anti-Opa1 antibody (Santa Cruz, Dallas, TX, USA; 1:500 dilution) overnight. The cells were then washed with KRH-glucose buffer, probed with the goat secondary monoclonal antibody Alexa Fluor 647 (Molecular Probes, Thermo Fisher Scientific, MA, USA; 1:1000) for 3 h, and finally stained with DAPI (Sigma, MA, USA; 1:5000 dilution) for 15 min. Fluorescence images were obtained using high-resolution fluorescence microscopy (Leica, Germany) with a 63x oil objective. We analyzed between 800 and 1000 isolated mitochondria from 30–40 cells, measuring 25 images per experiment. Fluorescence images were analyzed using Image J software.

Mitochondrial membrane potential determinations.

Mitochondrial membrane potential was determined using the mitochondrial dyes Mitotracker Red-H2ROS (Molecular Probes, Thermo Fisher Scientific, MA, USA), as previously described (Perez, Ponce et al. 2018b). Mitochondrial staining was verified using Mitotracker Green (Molecular Probes, Thermo Fisher Scientific, MA, USA) (Perez, Ponce et al. 2018b). Mitochondrial potential levels were calculated as the average fluorescence signal (F) per area in each image, subtracting the background fluorescence intensity (F0). To test the control for Mitotracker Red-H2ROS dye, mitochondrial depolarization was induced using 10 μM FCCP (Tocris Bioscience, Bristol, UK) for 15 min (Perez, Ponce et al. 2018b).

Cells were incubated with 0.5 μM Mitotracker Red-H2ROS and 0.5 μM Mitotracker Green in KRH-glucose buffer at 37°C for 35 min and then treated with 10 μM thapsigargin (Tocris Bioscience, Bristol, UK) for 30 min. The intensity of the signal was analyzed in 30 cells per experiment in at least 4 different experiments. Fluorescence images were obtained using a high-resolution fluorescence microscope (Leica, Germany) with a 63x oil objective. The images were analyzed using Image J software.

Western blot analysis.

The transfected CN1.4 cells were lysed in Triton lysis buffer containing a protease and phosphatase inhibitor cocktail (Roche, Vienna, Austria). Thirty micrograms of total protein extracts were resolved by electrophoresis on an SDS-polyacrylamide gel (BioRad, CA, USA) and transferred to a PVDF membrane (BioRad, CA, USA). The membranes were then incubated with the following primary antibodies: mouse monoclonal anti-GFP (Roche, Vienna, Austria; 1:1000 dilution), mouse monoclonal anti-CypD (Santa Cruz, CA, USA; 1:1000 dilution), mouse monoclonal anti-VDAC (Santa Cruz, CA, USA; 1:1000 dilution), mouse monoclonal anti-OSCP (Santa Cruz, CA, USA; 1:1000 dilution), mouse monoclonal anti-Opa1 (Santa Cruz, CA, USA; 1:1000 dilution), anti-Tau (Dako), or total OXPHOS antibody cocktail (Abcam, Cambridge, UK; 1:1000 dilution). The equal loading and transfer of protein to the membranes were determined by re-probing with an anti-actin antibody (Santa Cruz, CA, USA; 1:2000 dilution). Protein signals were detected using an HRP-linked goat anti-mouse or anti-rabbit secondary antibody (Thermo Fisher, MA, USA; 1:2000) as indicated. Finally, the immunoreactive protein signal was detected using enhanced chemiluminescence reagent (ECL, Thermo Fisher, MA, USA).

Co-Immunoprecipitation of OSCP.

CN 1.4 cells were lysed in buffer (50 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 0.5% NP-40, 5% glycerol, and protease inhibitor cocktail (Roche, Vienna, Austria), pH 7.4) following the protocol previously described by (Yan, Du et al. 2016). Following 7 freeze-thaw cycles, CN 1.4 cells transfected with GFP or GFP-full length or GFP-caspase-3-cleaved tau were lysed and pelleted at 12,500g at 4°C. The supernatant was used to immunoprecipitate OSCP (Santa Cruz antibody, 1 μg/100 μg protein) overnight at 4°C. Incubation with pre-cleaned protein A/G agarose (Thermo Fisher, MA, USA) for 2 h at room temperature was performed. Western blot against CypD (Abcam, Cambridge, UK; 1:1000 dilution) was performed.

In situ evaluation of the mPTP opening.

CN 1.4 cells were treated with 50 mM cobalt chloride for 15 minutes before incubation with 5 μM Calcein Blue (Molecular Probes, Thermo Fisher, MA, USA) and 0.5 μM Mitotracker Red (Molecular Probes, Thermo Fisher, MA, USA) in KRH-glucose buffer at 37°C for 30 minutes (Perez, Ponce et al. 2018c). The quenching of free calcein by cobalt chloride allows for observation of mitochondrial integrity as an mPTP opening indicator (Hom, Quintanilla et al. 2011; Quintanilla, Jin et al. 2013). Quantification of fluorescence intensity was carried out analyzing the calcein intensity in 25 images in 4 separate experiments, positive mitochondrial calcein signal was determined when it colocalized with Mitotracker Red dye. Image J software was used to perform these analyses.

ATP level determination.

Total ATP levels were determined in CN 1.4 cells transfected with GFP or GFP-Tau(s) forms, double transfected with GFP or GFP-Tau(s) forms plus Opa1-c-myc and double transfected with GFP or GFP-Tau(s) forms/Opa1-c-myc treated with CsA (0.5 μM, 2h) using bioluminescence assay. Transfected cells were lysed using radioimmunoprecipitation assay (RIPA) buffer (AMRESCO, OH, USA) (Quintanilla et al., 2020; Tapia-Monsalves-Tapia et al., 2023). The extracts were subsequently analyzed using a luciferin/luciferase bioluminescence assay kit (ATP Determination Kit #A22066 — Molecular Probes, Invitrogen) (Quintanilla et al., 2020; Tapia-Monsalves et al., 2023).

Statistical Analysis.

Statistical differences between two group of data were analyzed by Student t test. For multiple comparisons, one-way ANOVA was used, followed by Tukey test a posteriori. Differences were considered significant if $p < 0.05$. All experiments were repeated at least three times.

Results

Caspase-3 cleaved tau expression induces mitochondrial fragmentation and bioenergetics dysfunction in neuronal cells

We previously demonstrated that expression of caspase-3-cleaved tau leads to mitochondrial fragmentation and sensitizes mitochondria to neurotoxic stimuli associated with AD, such as sub-lethal doses of beta-amyloid fibrils (Perez, Vergara-Pulgar et al. 2018c). To validate our previous findings, we evaluated mitochondrial length in two models lacking endogenous tau expression, namely immortalized cortical neurons (CN 1.4) and primary hippocampal neurons from tau knockout mice (−/−), both of which were transfected with GFP, GFP-full-length (GFP-T4), and GFP-caspase-3-cleaved tau (GFP-T4C3) (Figure 1A). Our results show that expression of GFP-T4C3 leads to a significant decrease in mitochondrial length in hippocampal tau (−/−) neurons (Figure 1B, C) and an increase in the frequency of shorter mitochondria and a decrease in the population of longer mitochondria in CN 1.4 neuronal cortical lines (Figure 1 D, E). Moreover, we measured mitochondrial membrane potential under normal conditions in both primary neurons (Figure 1F) and CN 1.4 cells (Figure 1G) after GFP, GFP-T4, and GFP-T4C3 expression. We observed no significant effect on basal mitochondrial membrane potential levels. However, following a calcium stressor stimulus using 0.5 ¼M Thapsigargin (Thap) (Perez, Ponce et al. 2018b), truncated tau expression induced a significant decrease in mitochondrial potential levels in both tau (−/−) hippocampal neurons (Figure 1F) and CN1.4 cells (Figure 1G). These results support previous findings that truncated tau at Asp421 by caspase-3 significantly impacts mitochondrial health by decreasing mitochondrial length and mitochondrial membrane potential levels (Quintanilla, Matthews-Roberson et al. 2009; Perez, Vergara-Pulgar et al. 2018c).

Opa1 overexpression fails to prevent mitochondrial bioenergetic impairment induced by caspase-3-cleaved tau expression in immortalized cortical neurons

Opa1 (OPA1, mitochondrial dynamin-like-GTPase) is a crucial mitochondrial protein involved in mediating mitochondrial fusion of the inner mitochondrial membrane and regulating mitochondrial dynamics (Ranieri, Del Bo et al. 2012). In our previous study, we reported that the expression of GFP-T4C3 resulted in mitochondrial fragmentation and a significant decrease in Opa1 protein levels when compared to GFP-T4 expressing cells (Perez, Vergara-Pulgar et al. 2018c). Therefore, we aimed to investigate whether Opa1 expression could rescue the mitochondrial length and bioenergetics defects caused by truncated tau expression in immortalized cortical neurons. To achieve this, immortalized cortical neurons were co-transfected with both Opa1-c-myc and GFP or GFP-tau (s) forms (Figure 2A). Cells were loaded with Mitotracker red, fixed and immunostained against Opa1 (see methods). Our results indicate that overexpression of Opa1 did not affect mitochondrial length in cells expressing both GFP/Opa1-c-myc and GFP-T4/Opa1-c-myc (Figure 2B). Interestingly, the mitochondrial length reduction caused by GFP-T4C3 was reversed by Opa1 overexpression (Figure 2C). To further investigate the impact of Opa1 overexpression on bioenergetics performance, we measured mitochondrial depolarization before and after calcium overload with Thapsigargin (Figure 2D). We found that the loss of mitochondrial membrane potential induced by GFP-T4C3 was not recovered by Opa1 overexpression (Figure 2D). These findings suggest that the bioenergetics defects caused by caspase-3 cleaved tau are not mediated by changes in mitochondrial fusion induced by Opa1. This implies the possible involvement of a different mechanism in causing these mitochondrial abnormalities induced by truncated tau.

Truncated tau expression induces the opening of the mitochondrial permeability transition pore (mPTP).

Our previous reports have shown that tau deletion has positive effects on hippocampal bioenergetics by inhibiting mitochondrial permeability transition pore (mPTP) formation through a reduction in cyclophilin D (Cyp-D) protein (Jara, Cerpa et al., 2021). Furthermore, tau deletion increased ATP production and improved recognition memory and attentive capacity in juvenile and old mice (Jara, Aranguiz et al. 2018,; Jara, Cerpa et al. 2021). Here, we evaluated whether mitochondrial bioenergetic impairment induced by caspase-3-cleaved tau is related to mPTP formation by examining the expression of major mPTP components in CN 1.4 cells, including CypD, VDAC, and OSCP (Perez, Ponce et al., 2018b). Our results show that GFP-T4C3 expression leads to a significant increase in CypD levels compared to GFP or GFP-T4 expressing cells (Figure 3A). Since mPTP may directly affect energy metabolism through the direct involvement of OSCP/F-ATP synthase in mPTP formation (Bonora, Bononi et al., 2013), we also examined the protein expression of various mitochondrial oxidative phosphorylation complexes. We found no differences in mitochondrial complex expression between tau forms (Figure 3B). Also, expression of GFP protein in CN 1.4 cells indicates no difference in tau levels between conditions (Figure 3C).

Given that an increase in CypD could indicate an increase in mPTP conformation (Du and Yan 2010; Du, Guo et al., 2011; Hom, Quintanilla et al., 2011; Guo, Du et al., 2013), we performed co-immunoprecipitation studies of OSCP and CypD (Figure 3D) in

neuronal cells transfected with GFP and GFP-tau (s) forms. CN 1.4 cells transfected with caspase-3 cleaved tau showed a significant increase in CypD/OSCP interaction (Figure 3D). Indeed, when the cells were treated with CsA, the pharmacological inhibitor of the mPTP because of the high affinity with CypD, this interaction in no longer present (Figure 3D). Complementary, we analyzed the in-situ formation and subsequent opening of mPTP in CN 1.4 cells expressing GFP and GFP-Tau(s) forms by measuring calcein blue dye retained inside the mitochondria after a calcium overload (Perez, Ponce et al., 2018b) (Figure 3E, F). Cells transfected with GFP and GFP-T4 showed an increase in calsein blue localization in the mitochondrial population in control and after Thapsigargin treatment conditions (Figure 3E, F). However, GFP-T4C3-transfected cells showed an evident reduction in mitochondrial/ calcein blue localization (Figure 3E, F). To evaluate whether the release of calcein blue was related to mPTP opening, we treated cells with CsA, and we found that CsA treatment increasing the calcein blue mitochondrial localization in caspase-3 cleaved tau cells (Figure 3E, F), suggesting that expression of GFP-T4C3 form induces the opening of mPTP.

Cyclosporine-A treatment prevented mitochondrial damage induced by caspase-3 cleaved tau.

A reduction in CypD expression has been shown to improve mitochondrial function in the hippocampus of transgenic mice overexpressing the mutant human form of APP (Du, Guo et al., 2011). Additionally, pharmacological inhibition of CypD using CsA has been demonstrated to prevent mPTP opening and neurodegeneration caused by Aß and other stressors (Giorgio, Bisetto et al., 2009; Giorgio, Soriano et al., 2010; Perez, Ponce et al., 2018b). Based on these findings, we investigated whether CypD inhibition could also alleviate the mitochondrial defects induced by truncated tau. To evaluate the contribution of mPTP opening to changes in mitochondrial morphology in cells transfected with truncated tau, we double-transfected CN 1.4 cells (Mito-mCherry/GFP or GFP-Tau(s) forms) and treated them with 0.5 μM CsA for 2 hours. Mitochondrial length changes were then measured, and CsA treatment was found to restore mitochondrial length in GFP-T4C3 CN 1.4 cells (Figure 4A, B, C). This suggests that the mPTP opening could be involved in dysregulation of mitochondrial dynamics previously observed (Figure 1). To determine if mPTP blockage protects against mitochondrial depolarization in cells transfected with truncated tau, we treated transfected cells (GFP and GFP-Tau(s)) with CsA (Figure 4 D, E). We found that CsA treatment significantly rescued the mitochondrial membrane potential loss induced by truncated tau in cells treated with Thapsigargin (Figure 4D, E). Complementary, we evaluate ATP levels in CN 1.4 cells transfected with GFP or GFP-Tau(s) forms (black bars), cells double transfected with GFP or GFP-Tau(s) forms plus Opa1-c-myc (light grey bars), and cells transfected with GFP or GFP-Tau(s) forms treated with CsA (0.5 μm, 2h, dark grey bars) (Fig. 4F). Interestingly, treatment with CsA prevented ATP levels reduction induced by truncated tau expression in CN 1.4 cells (Figure 4F). Also, CN 1.4 cells double transfected with GFP or GFP-Tau(s) forms plus Opa1-c-myc did not present any improvement in ATP production decrease in truncated tau expressing cells which corroborates our observations regarding Opa1 involvement in mitochondrial bioenergetics defects induced by caspase-3 cleaved tau (Figure 2).

Complementary, to confirmed specific CsA inhibition against mPTP and CypD, we used FK506, an inhibitor of calcineurin (Perez, Ponce et al. 2018b), a calcium-dependent enzyme that may be affected by CsA treatment (Quintanilla, Jin et al. 2013). Incubation with FK-506 (500 nM, 2h) did not prevent mitochondrial depolarization induced by GFP-T4C3, confirming that this recovery pathway is specifically dependent on mPTP opening (Figure S1A, B). Finally, we transfected primary hippocampal neurons from tau knockout mice (−/−) with GFP, GFP-T4, and GFP-T4C3 to confirm our findings in a more applicable model (Figure 5). We found that CsA treatment prevented both mitochondrial length and mitochondrial membrane potential deficiencies after Thapsigargin treatment in GFP-T4C3 transfected neurons (Figure 5A, B, C). Together, these results shows that CsA alleviates mitochondrial dysfunction induced by caspase-3-cleaved tau in neuronal cells, indicating a crucial role of mPTP opening in tau pathology.

Genetic ablation of CypD prevents mitochondrial damaged induced by caspase-3 cleaved tau.

To corroborate the potential role of mPTP in the mitochondrial dysfunction induced by GFP-T4C3, we utilized hippocampal neurons from CypD (−/−) mice (Baines, Kaiser et al. 2005; Hom, Quintanilla et al. 2011), which are known to exhibit enhanced resistance to mitochondrial stress and mPTP blocked (Du, Guo et al. 2011; Guo, Du et al. 2013; Gauba, Chen et al. 2019) (Figure 6A). We found that transfection of caspase-3 cleaved tau into hippocampal CypD (−/−) neurons did not induce alterations in mitochondrial length, indicating that the CypD-dependent mitochondrial fragmentation is caused by this toxic tau form (Figure 6B, C). Furthermore, we observed complete prevention of Thap-induced mitochondrial depolarization in cells transfected with GFP-T4C3 in hippocampal neurons deficient in CypD expression (Figure 6D). Consequently, our results demonstrate that genetic ablation of CypD can prevent the opening of mPTP and mitochondrial dysfunction caused by truncated tau by caspase-3.

Discussion

Mitochondrial dysfunction has been identified as a critical player in the early neurodegenerative events of AD, including synaptic failure, oxidative damage, and impairment of neuronal communication (Gibson and Shi 2010; Cabezas-Opazo, Vergara-Pulgar et al. 2015). Previous studies suggest that defects in mitochondria result in an imbalance in calcium regulation, which could lead to a decrease in ATP levels and the accumulation of synaptic vesicles in axons. While mitochondria play a vital role in supplying energy through ATP synthesis and regulating redox homeostasis for cellular maintenance, they also regulate the calcium buffering capacity of the cell, which is essential for neurotransmitter release (Di Monte, Tokar et al. 1999; Bobba, Atlante et al. 2004; Gendron and Petrucelli 2009). Therefore, it is imperative to investigate the mechanism involved in mitochondrial dysfunction and neuronal damage induced by soluble pathological forms of tau in AD to better understand the underlying pathogenesis of the disease.

Tau, a microtubule-associated protein, undergoes specific post-translational modifications that affect its function and solubility in physiological conditions. These modifications can

play various roles in the onset and progression of neurodegenerative diseases, including AD (Kosik, Joachim et al. 1986; Tapia-Rojas, Cabezas-Opazo et al. 2019). Although phosphorylation is considered one of the most important modifications in AD, proteolytic processing by caspases may also be involved in early neurodegeneration (Johnson 2006; Perez, Jara et al. 2018a). Our group has shown that caspase-3-mediated truncation of tau leads to specific mitochondrial dysfunction, including fragmented morphology, increased oxidative stress, decreased calcium-buffering capacity, and mitochondrial depolarization (Quintanilla, Matthews-Roberson et al., 2009). Additionally, primary neurons expressing truncated tau showed increased sensitivity to amyloid-beta (Aβ) and calcium overload stress (Quintanilla, Matthews-Roberson et al. 2009; Quintanilla, Dolan et al. 2012). Our previous study also demonstrated that Opa1 levels (main regulator of the fusion of the inner mitochondrial membrane) were decreased when truncated tau was expressed and that could lead to mitochondrial dysfunction (Perez, Vergara-Pulgar et al. 2018c). However, here we found that the overexpression Opa1 reversed only mitochondrial length deficits induced by truncated tau but was unable to rescue bioenergetics deficits such as mitochondrial depolarization and ATP loss (Figure 2). These observations suggests that mitochondrial dysfunction induced by caspase-3 cleaved tau is triggered by an alternative mechanism.

CypD, a peptidyl-prolyl cis-trans isomerase, is a key regulator of mPTP formation and opening, which consequently causes mitochondrial and cellular damage (Du and Yan 2010; Du, Guo et al. 2011). Decreases in CypD expression improved mitochondrial function in the hippocampus of transgenic mice overexpressing the mutant human form of APP (Du, Guo et al. 2011), and pharmacological inhibition of CypD using CsA, has been shown to prevent mPTP opening and neurodegeneration caused by Aß (Du and Yan 2010). Interestingly, we found that overexpression of caspase-3-cleaved tau induce an increase in the expression of CypD (Figure 3A). However, recent evidence have suggested that in AD brains and old APP transgenic mice VDAC expression is increased and could be interacting with hyperphosphorylated tau (PHF-1) and Aβ peptide aggregates contributing to mitochondrial dysfunction showed in AD (Manczak and Reddy, 2012). Although genetic ablation of VDACs revealed no differences in the effects on mPTP of a variety of inducers and inhibitors (Krauskopf et al., 2006; Baines et al., 2007), recent studies are consistent with a modulatory role of VDACs on mPTP (Tanno et al., 2017: Koushi et al., 2020). Therefore, it is possible that VDAC could be contributing to mPTP opening showed in caspase-3 cleaved tau expressing cells.

The latest models that explain mPTP activity suggest that this megacomplex channel is formed principally by the interaction of CypD and the ATP synthase subunit, OSCP (Giorgio et al., 2013; Jonas, Porter et al. 2015), and this interaction could be crucial for ageing and AD pathogenesis (Gauba, Guo et al. 2017; Gauba, Chen et al. 2019). In our study, we found that only when caspase-3 cleaved tau was expressed in CN 1.4 cells, an interaction between OSCP and CypD occurred (Figure 3D). Treatment with CsA, a pharmacological inhibitor of the mPTP due to its high affinity with CypD, eliminated this interaction (Figure 3D). Furthermore, we analyzed the opening of the mPTP in vivo using the mitochondrial calcein blue retention assay after quenching with cobalt (Hom, Quintanilla et al. 2011; Perez, Ponce et al. 2018b). We observed a decrease in the retention of the calcein dye in mitochondria under basal conditions when truncated tau was expressed, and that the

release of mitochondrial dye was prevented by CsA treatment (Figure 3E). Surprisingly, we also found that pharmacological and/or genetic inhibition of CypD regulates mPTP opening in our model, which in consequence modulates mitochondrial dynamics and prevent the mitochondrial potential loss induced by calcium overload. These results, strongly indicate that caspase-3-cleaved tau is promoting mPTP opening and consequently trigger mitochondrial impairment.

Several studies have reported that impairment of calcium regulation and increased oxidative stress can induce the opening of mPTP, leading to decreased ATP production, mitochondrial content release, mitochondrial damage, and ultimately cell death (Kroemer and Blomgren 2007; Du and Yan 2010; Bonora, Bononi et al. 2013; Bernardi and Di Lisa 2015; Bernardi, Di Lisa et al. 2015; Jonas, Porter et al. 2015). In this study, we found that caspase-3 cleaved tau may also be a modulator of mPTP opening which is sensitive to ROS stress and cytosolic calcium increase (Perez and Quintanilla, 2017). In this context, we recently showed that inhibition of mPTP using CsA (0.5 μm, 2h) prevented ROS increase induced by caspase-3 cleaved tau expression in CN 1.4 cells and mice hippocampal neurons (Tapia-Monsalves et al., 2023). Therefore, we can hypothesize that the pathological opening of the pore caused by this pathological tau form may result in a pathological state that could be either increasing ROS species or changing the metabolic state of mitochondria (Olesen and Quintanilla, 2023). Also, since alterations in mitochondrial metabolism can influence mitochondrial structure, the mitochondrial fragmentation could be a secondary response to the metabolic imbalance of the cells expressing truncated tau (Perez and Quintanilla 2017). Interestingly, several reports suggest that misfolded proteins may interact with components of the mPTP, potentially leading to pathological pore opening. For example, Aβ peptide, derived tau fragments, and phosphorylated tau have been shown to interact with the mPTP (Amadoro, Corsetti et al. 2012; Manczak and Reddy 2012; Gauba, Chen et al. 2019). Interactome analysis suggests that full-length and phosphorylated forms of tau interact with proteins involved in mPTP opening, including many ANT and OSCP (Tracy, Madero-Perez et al. 2022). Furthermore, less common forms of tau, such as N-terminal tau fragments, also interact with components of the mPTP, leading to its opening and mitochondrial failure (Atlante, Amadoro et al. 2008; Amadoro, Corsetti et al. 2012). Taken together these findings suggest that caspase-3-cleaved tau could also activate mPTP formation by increasing its interaction with components of the pore.

Mitochondrial dysfunction induced by caspase-3 cleaved tau could be involved in the synaptic function alterations presented in AD (reviewed in Olesen and Quintanilla, 2023). In fact, we recently showed that truncated tau expression affects synaptic plasticity including the reduction of dendritic spine density, synaptic vesicle, and affecting synaptophysin localization in mature hippocampal neurons (Tapia-Monsalves et al., 2023). More importantly, inhibition of mPTP opening using CsA (0.5 μm, 2h) prevented all these synaptic plasticity defects produced by truncated tau expression indicating an important role of mitochondrial failure induced by this toxic tau form in the synaptic and cognitive decline present in AD (Tapia-Monsalves et al., 2023).

In conclusion, our study sheds light on a novel mechanism by which soluble pathological tau modifications, such as caspase-3 cleaved tau, contribute to neuronal injury through

mitochondrial impairment. We found that this tau form influences CypD and OSCP interaction, leading to mPTP opening and subsequent mitochondrial dysfunction. Our data suggest that this pathological mechanism may play a critical role in the early cognitive impairment seen in AD, where mitochondrial function is essential for synaptic processes. Moreover, we found a direct association between tau pathology, mPTP opening, and AD, highlighting the potential therapeutic value of targeting CypD to reduce mitochondrial dysfunction and neuronal damage. This may lead to the development of new therapeutic strategies to eventually decrease or ameliorate the neurodegenerative changes showed in AD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Funding

This work was supported by ANID, Fondo de Ciencia y Tecnología (FONDECYT), Santiago, Chile, Grants #1200178, [RAQ], NIH grant AG073121 [GVWJ], and NIH grant HL144776 [GAP]. MJP was financed by Ph.D. fellowship from Universidad Autónoma de Chile, Santiago, Chile.

Availability of supporting data

Further information and requests for resources, reagents, protocols should be submitted to Dr. Rodrigo A. Quintanilla (rodrigo.quintanilla@uautonoma.cl), corresponding author of this manuscript.

Abbreviations

Aβ	Amyloid beta
AD	Alzheimer’s disease
ANT	Adenine nucleotide translocase
CypD	Cyclophilin D
Cyclosporin A	CsA
GFP-T4	GFP-full length tau
GFP-T4C3	GFP-caspase-3-cleaved tau
mPTP	Mitochondrial permeability transition pore
Opa1	Optic atrophy 1 protein
OSCP	Oligomycin sensitivity-conferring protein
ROS	Reactive Oxygen Species
Thapsigargin	Thap
VDAC	Voltage-dependent anion channel

References

Amadoro G, Corsetti V, Atlante A, Florenzano F, Capsoni S, Bussani R, Mercanti D. and Calissano P. (2012). “Interaction between NH(2)-tau fragment and Abeta in Alzheimer’s disease mitochondria contributes to the synaptic deterioration.” Neurobiol Aging 33(4): 833 e831–825.

Atlante A, Amadoro G, Bobba A, de Bari L, Corsetti V, Pappalardo G, Marra E, Calissano P. and Passarella S. (2008). “A peptide containing residues 26–44 of tau protein impairs mitochondrial oxidative phosphorylation acting at the level of the adenine nucleotide translocator.” Biochim Biophys Acta 1777(10): 1289–1300. [PubMed: 18725189]

Baines CP, Kaiser RA, Purcell NH, Blair NS, Osinska H, Hambleton MA, Brunskill EW, Sayen MR, Gottlieb RA, Dorn GW, Robbins J. and Molkentin JD (2005). “Loss of cyclophilin D reveals a critical role for mitochondrial permeability transition in cell death.” Nature 434(7033): 658–662. [PubMed: 15800627]

Baines C, Kaiser R, Sheiko T. et al. Voltage-dependent anion channels are dispensable for mitochondrial-dependent cell death. Nat Cell Biol 9, 550–555 (2007). 10.1038/ncb1575. [PubMed: 17417626]

Bernardi P. and Di Lisa F. (2015). “The mitochondrial permeability transition pore: molecular nature and role as a target in cardioprotection.” J Mol Cell Cardiol 78: 100–106. [PubMed: 25268651]

Bernardi P, Di Lisa F, Fogolari F. and Lippe G. (2015). “From ATP to PTP and Back: A Dual Function for the Mitochondrial ATP Synthase.” Circ Res 116(11): 1850–1862. [PubMed: 25999424]

Bobba A, Atlante A, Azzariti A, Sgaramella G, Calissano P. and Marra E. (2004). “Mitochondrial impairment induces excitotoxic death in cerebellar granule cells.” Int J Mol Med 13(6): 873–876. [PubMed: 15138628]

Bonora M, Bononi A, De Marchi E, Giorgi C, Lebiedzinska M, Marchi S, Patergnani S, Rimessi A, Suski JM, Wojtala A, Wieckowski MR, Kroemer G, Galluzzi L. and Pinton P. (2013). “Role of the c subunit of the FO ATP synthase in mitochondrial permeability transition.” Cell Cycle 12(4): 674–683. [PubMed: 23343770]

Cabezas-Opazo FA, Vergara-Pulgar K, Perez MJ, Jara C, Osorio-Fuentealba C. and Quintanilla RA (2015). “Mitochondrial Dysfunction Contributes to the Pathogenesis of Alzheimer’s Disease.” Oxid Med Cell Longev 2015: 509654.

de Calignon A, Fox LM, Pitstick R, Carlson GA, Bacskai BJ, Spires-Jones TL and Hyman BT (2010). “Caspase activation precedes and leads to tangles.” Nature 464(7292): 1201–1204. [PubMed: 20357768]

Di Monte DA, Tokar I. and Langston JW (1999). “Impaired glutamate clearance as a consequence of energy failure caused by MPP(+) in astrocytic cultures.” Toxicol Appl Pharmacol 158(3): 296–302. [PubMed: 10438663]

Du H, Guo L, Zhang W, Rydzewska M. and Yan S. (2011). “Cyclophilin D deficiency improves mitochondrial function and learning/memory in aging Alzheimer disease mouse model.” Neurobiol Aging 32(3): 398–406. [PubMed: 19362755]

Du H. and Yan SS (2010). “Mitochondrial permeability transition pore in Alzheimer’s disease: cyclophilin D and amyloid beta.” Biochim Biophys Acta 1802(1): 198–204. [PubMed: 19616093]

Gamblin TC, Chen F, Zambrano A, Abraha A, Lagalwar S, Guillozet AL, Lu M, Fu Y, Garcia-Sierra F, LaPointe N, Miller R, Berry RW, Binder LI and Cryns VL (2003). “Caspase cleavage of tau: linking amyloid and neurofibrillary tangles in Alzheimer’s disease.” Proc Natl Acad Sci U S A 100(17): 10032–10037. [PubMed: 12888622]

Gauba E, Chen H, Guo L. and Du H. (2019). “Cyclophilin D deficiency attenuates mitochondrial F1Fo ATP synthase dysfunction via OSCP in Alzheimer’s disease.” Neurobiol Dis 121: 138–147. [PubMed: 30266287]

Gauba E, Guo L. and Du H. (2017). “Cyclophilin D Promotes Brain Mitochondrial F1FO ATP Synthase Dysfunction in Aging Mice.” J Alzheimers Dis 55(4): 1351–1362. [PubMed: 27834780]

Gendron TF and Petrucelli L. (2009). “The role of tau in neurodegeneration.” Mol Neurodegener 4: 13. [PubMed: 19284597]

Gibson GE and Shi Q. (2010). “A mitocentric view of Alzheimer’s disease suggests multi-faceted treatments.” J Alzheimers Dis 20 Suppl 2(0 2): S591–607. [PubMed: 20463407]

Giorgio V, Bisetto E, Soriano ME, Dabbeni-Sala F, Basso E, Petronilli V, Forte MA, Bernardi P. and Lippe G. (2009). “Cyclophilin D modulates mitochondrial F0F1-ATP synthase by interacting with the lateral stalk of the complex.” J Biol Chem 284(49): 33982–33988. [PubMed: 19801635]

Giorgio V, Soriano ME, Basso E, Bisetto E, Lippe G, Forte MA and Bernardi P. (2010). “Cyclophilin D in mitochondrial pathophysiology.” Biochim Biophys Acta 1797(6–7): 1113–1118. [PubMed: 20026006]

Giorgio V, von Stockum S, Antoniel M, Fabbro A, Fogolari F, Forte M, Glick GD, Petronilli V, Zoratti M, Szabó I, Lippe G, Bernardi P. Dimers of mitochondrial ATP synthase form the permeability transition pore. Proc Natl Acad Sci U S A. 2013 Apr 9;110(15):5887–92. doi: 10.1073/pnas.1217823110. [PubMed: 23530243]

Guo L, Du H, Yan S, Wu X, McKhann GM, Chen JX and Yan SS (2013). “Cyclophilin D deficiency rescues axonal mitochondrial transport in Alzheimer’s neurons.” PLoS One 8(1): e54914.

Hom JR, Quintanilla RA, Hoffman DL, de Mesy Bentley KL, Molkentin JD, Sheu SS and Porter GA Jr. (2011). “The permeability transition pore controls cardiac mitochondrial maturation and myocyte differentiation.” Dev Cell 21(3): 469–478. [PubMed: 21920313]

Jara C, Aranguiz A, Cerpa W, Tapia-Rojas C. and Quintanilla RA (2018). “Genetic ablation of tau improves mitochondrial function and cognitive abilities in the hippocampus.” Redox Biol 18: 279–294. [PubMed: 30077079]

Jara C, Cerpa W, Tapia-Rojas C. and Quintanilla RA (2021). “Tau Deletion Prevents Cognitive Impairment and Mitochondrial Dysfunction Age Associated by a Mechanism Dependent on Cyclophilin-D.” Front Neurosci 14: 586710.

Johnson GV (2006). “Tau phosphorylation and proteolysis: insights and perspectives.” J Alzheimers Dis 9(3 Suppl): 243–250.

Jonas EA, Porter GA Jr., Beutner G, Mnatsakanyan N. and Alavian KN (2015). “Cell death disguised: The mitochondrial permeability transition pore as the c-subunit of the F(1)F(O) ATP synthase.” Pharmacol Res 99: 382–392. [PubMed: 25956324]

Kosik KS, Joachim CL and Selkoe DJ (1986). “Microtubule-associated protein tau (tau) is a major antigenic component of paired helical filaments in Alzheimer disease.” Proc Natl Acad Sci U S A 83(11): 4044–4048. [PubMed: 2424016]

Krauskopf A, Eriksson O, Craigen WJ, Forte MA, Bernardi P. Properties of the permeability transition in VDAC1(−/−) mitochondria. Biochim Biophys Acta. 2006 May-Jun;1757(5–6):590–5. doi: 10.1016/j.bbabio.2006.02.007. [PubMed: 16626625]

Koushi M, Aoyama Y, Kamei Y. et al. Bisindolylpyrrole triggers transient mitochondrial permeability transitions to cause apoptosis in a VDAC1/2 and cyclophilin D-dependent manner via the ANT-associated pore. Sci Rep 10, 16751 (2020). 10.1038/s41598-020-73667-z. [PubMed: 33046783]

Kroemer G. and Blomgren K. (2007). “Mitochondrial cell death control in familial Parkinson disease.” PLoS Biol 5(7): e206. [PubMed: 17638420]

Manczak M. and Reddy PH (2012). “Abnormal interaction of VDAC1 with amyloid beta and phosphorylated tau causes mitochondrial dysfunction in Alzheimer’s disease.” Hum Mol Genet 21(23): 5131–5146. [PubMed: 22926141]

Olesen MA, Quintanilla RA. Pathological Impact of Tau Proteolytical Process on Neuronal and Mitochondrial Function: a Crucial Role in Alzheimer’s Disease. Mol Neurobiol. 2023 Oct;60(10):5691–5707. doi: 10.1007/s12035-023-03434-4. [PubMed: 37332018]

Perez MJ, Jara C. and Quintanilla RA (2018a). “Contribution of Tau Pathology to Mitochondrial Impairment in Neurodegeneration.” Front Neurosci 12: 441. [PubMed: 30026680]

Perez MJ, Ponce DP, Aranguiz A, Behrens MI and Quintanilla RA (2018b). “Mitochondrial permeability transition pore contributes to mitochondrial dysfunction in fibroblasts of patients with sporadic Alzheimer’s disease.” Redox Biol 19: 290–300. [PubMed: 30199818]

Perez MJ and Quintanilla RA (2017). “Development or disease: duality of the mitochondrial permeability transition pore.” Dev Biol 426(1): 1–7. [PubMed: 28457864]

Perez MJ, Vergara-Pulgar K, Jara C, Cabezas-Opazo F. and Quintanilla RA (2018c). “Caspase-Cleaved Tau Impairs Mitochondrial Dynamics in Alzheimer’s Disease.” Mol Neurobiol 55(2): 1004–1018. [PubMed: 28084594]

Querfurth HW and LaFerla FM (2010). “Alzheimer’s disease.” N Engl J Med 362(4): 329–344. [PubMed: 20107219]

Quintanilla RA, Dolan PJ, Jin YN and Johnson GV (2012). “Truncated tau and Abeta cooperatively impair mitochondria in primary neurons.” Neurobiol Aging 33(3): 619 e625–635.

Quintanilla RA, Jin YN, von Bernhardi R. and Johnson GV (2013). “Mitochondrial permeability transition pore induces mitochondria injury in Huntington disease.” Mol Neurodegener 8: 45. [PubMed: 24330821]

Quintanilla RA, Matthews-Roberson TA, Dolan PJ and Johnson GV (2009). “Caspase-cleaved tau expression induces mitochondrial dysfunction in immortalized cortical neurons: implications for the pathogenesis of Alzheimer disease.” J Biol Chem 284(28): 18754–18766. [PubMed: 19389700]

Quintanilla RA, von Bernhardi R, Godoy JA, Inestrosa NC and Johnson GV (2014). “Phosphorylated tau potentiates Abeta-induced mitochondrial damage in mature neurons.” Neurobiol Dis 71: 260–269. [PubMed: 25134729]

Quintanilla RA, Tapia-Monsalves C, Vergara EH, Pérez MJ, Aranguiz A. Truncated Tau Induces Mitochondrial Transport Failure Through the Impairment of TRAK2 Protein and Bioenergetics Decline in Neuronal Cells. Front Cell Neurosci. 2020 Jul 30;14:175. doi: 10.3389/fncel.2020.00175. [PubMed: 32848607]

Ranieri M, Del Bo R, Bordoni A, Ronchi D, Colombo I, Riboldi G, Cosi A, Servida M, Magri F, Moggio M, Bresolin N, Comi GP and Corti S. (2012). “Optic atrophy plus phenotype due to mutations in the OPA1 gene: two more Italian families.” J Neurol Sci 315(1–2): 146–149. [PubMed: 22197506]

Rissman RA, Poon WW, Blurton-Jones M, Oddo S, Torp R, Vitek MP, LaFerla FM, Rohn TT and Cotman CW (2004). “Caspase-cleavage of tau is an early event in Alzheimer disease tangle pathology.” J Clin Invest 114(1): 121–130. [PubMed: 15232619]

Tanno M, Kuno A, Ishikawa S, Miki T, Kouzu H, Yano T, Murase H, Tobisawa T, Ogasawara M, Horio Y, Miura T. Translocation of glycogen synthase kinase-3β (GSK-3β), a trigger of permeability transition, is kinase activity-dependent and mediated by interaction with voltage-dependent anion channel 2 (VDAC2). J Biol Chem. 2014 Oct 17;289(42):29285–96. doi: 10.1074/jbc.M114.563924. [PubMed: 25187518]

Tapia-Monsalves C, Olesen MA, Villavicencio-Tejo F, Quintanilla RA. Cyclosporine A (CsA) prevents synaptic impairment caused by truncated tau by caspase-3. Mol Cell Neurosci. 2023 Jun;125:103861. doi: 10.1016/j.mcn.2023.103861.

Tapia-Rojas C, Cabezas-Opazo F, Deaton CA, Vergara EH, Johnson GVW and Quintanilla RA (2019). “It’s all about tau.” Prog Neurobiol 175: 54–76. [PubMed: 30605723]

Tracy TE, Madero-Perez J, Swaney DL, Chang TS, Moritz M, Konrad C, Ward ME, Stevenson E, Huttenhain R, Kauwe G, Mercedes M, Sweetland-Martin L, Chen X, Mok SA, Wong MY, Telpoukhovskaia M, Min SW, Wang C, Sohn PD, Martin J, Zhou Y, Luo W, Trojanowski JQ, Lee VMY, Gong S, Manfredi G, Coppola G, Krogan NJ, Geschwind DH and Gan L. (2022). “Tau interactome maps synaptic and mitochondrial processes associated with neurodegeneration.” Cell 185(4): 712–728 e714. [PubMed: 35063084]

Yan S, Du F, Wu L, Zhang Z, Zhong C, Yu Q, Wang Y, Lue LF, Walker DG, Douglas JT and Yan SS (2016). “F1F0 ATP Synthase-Cyclophilin D Interaction Contributes to Diabetes-Induced Synaptic Dysfunction and Cognitive Decline.” Diabetes 65(11): 3482–3494. [PubMed: 27554467]

- Caspase-3 cleaved tau induces mitochondrial fragmentation and bioenergetics failure in AD
- Overexpression of Opa1-mitochondrial fusion protein failed to prevent bioenergetics impairment produced by truncated tau
- Caspase-3 cleaved tau induces mitochondrial permeability transition pore (mPTP) opening affecting bioenergetics function
- Pharmacological inhibition of mPTP using cyclosporine A prevents mitochondrial impairment induced by truncated tau
- Genetic reduction of the mPTP regulator, cyclophilin D (CypD), prevents mitochondrial dysfunction induced by truncated tau

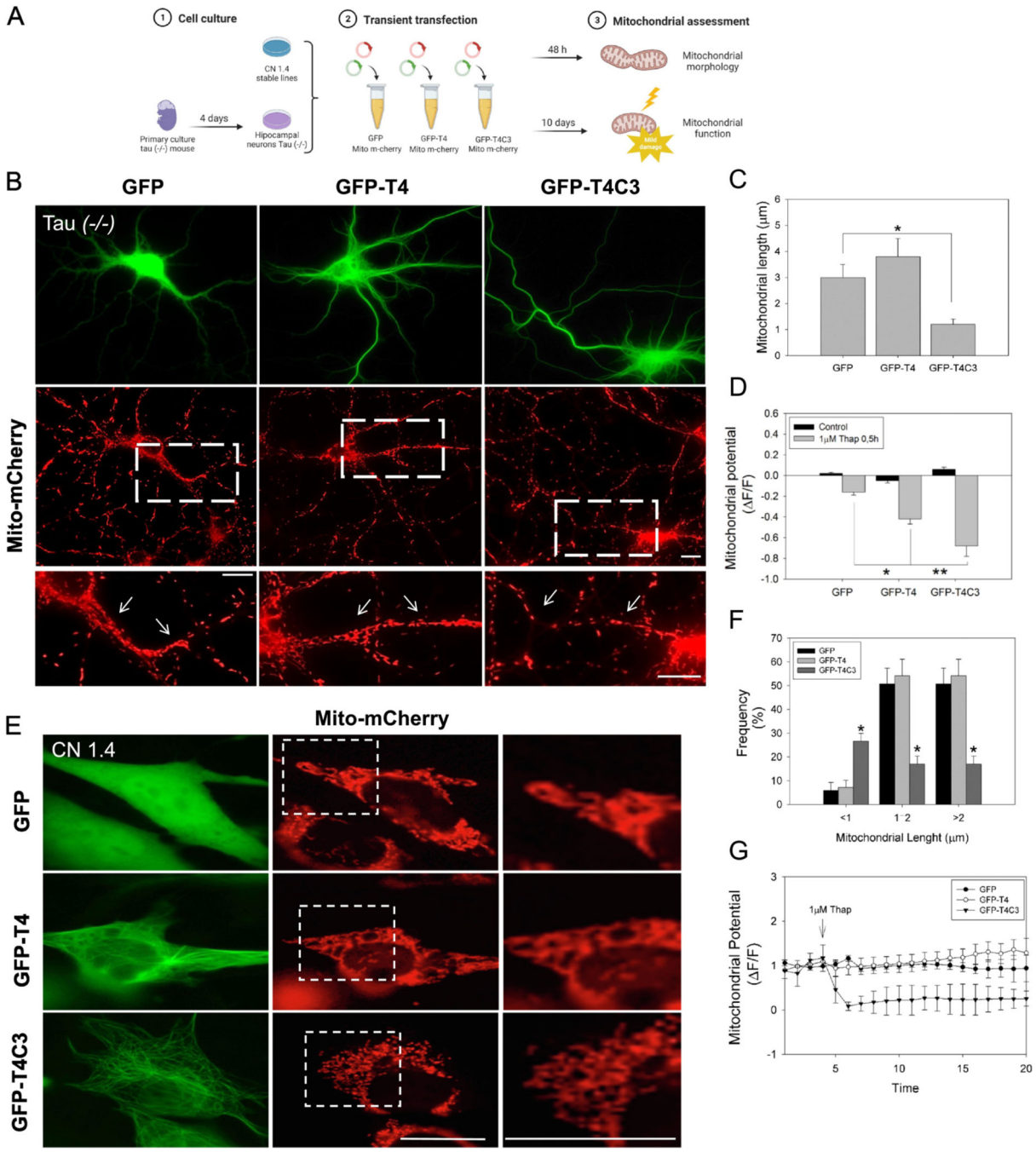

Figure 1. Caspase-3 cleaved tau induces mitochondrial fragmentation and bioenergetics dysfunction in neuronal cells.

(**A**) Schematic representation of the experimental procedure, showing cell culture, transient transfection, and mitochondrial assessment protocols. (**B**) Representative images of tau (−/−) neurons double-transfected with Mito-mCherry and either GFP-full length (GFP-T4) or GFP-caspase-3 cleaved tau (GFP-T4C3). Arrows in the magnified section indicate isolated mitochondria in neuronal prolongations. Quantification of mitochondrial length is shown in (**C**). *p<0.05 indicates differences between groups calculated by ANOVA

test. (**D**) Measurements of mitochondrial membrane potential performed with Mitotracker Red CMxRos in tau (−/−) neurons transfected with either GFP or GFP-tau (s) forms and treated with Thapsigargin (Thap, 0.5 μM, 30 min). **p<0.001, *p<0.05, indicate differences between groups calculated by ANOVA test. (**E**) Representative images of CN 1.4 cells double-transfected with Mito-mCherry and either GFP or GFP-Tau (s) forms to determine mitochondrial length. The magnified area allows visualization of the mitochondrial network. Quantification of mitochondrial length distribution expressed in percentage (%) of frequency is shown in (**F**). *p<0.05 indicates differences between groups calculated by ANOVA test. (**G**) Representative trends of mitochondrial membrane potential levels over 20 min in CN 1.4 cells transfected with either GFP or GFP-Tau (s) forms. After 5 min, the cells were treated with Thap (1 μM, indicated by arrow). Data are the mean ± SE. All experiments were performed with n = 4. Scale bar = 10 μm in (**B**, **E**).

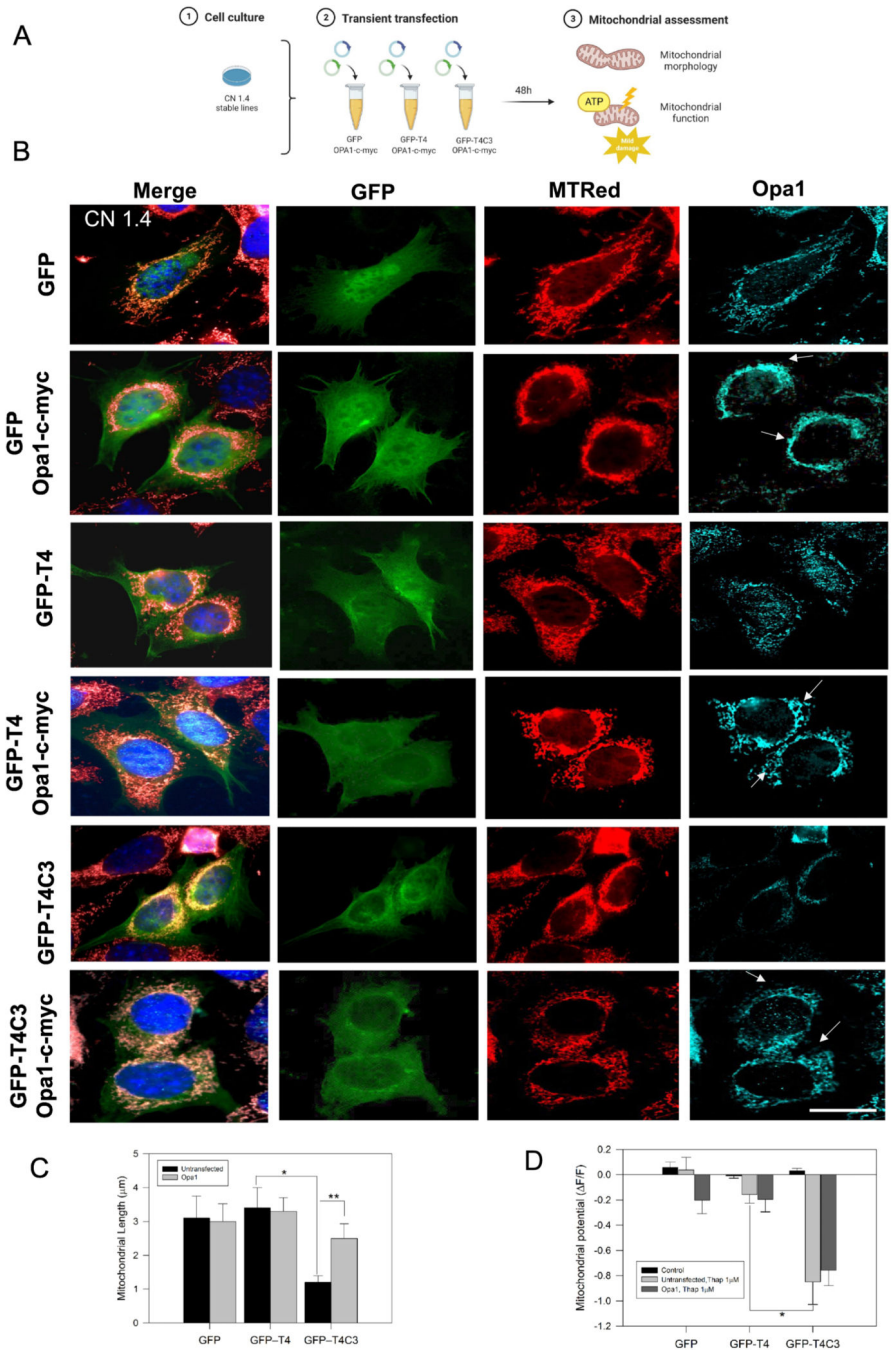

Figure 2. Opa1 overexpression prevents mitochondrial fragmentation without affecting mitochondrial bioenergetics dysfunction induced by caspase-3 cleaved tau in CN 1.4 immortalized neurons.

(**A**) Experimental procedure picture, showing cell culture, transient transfection, and mitochondrial assessment protocols. (**B**) Representative images of immunofluorescence of Opa1 in cells preloaded with MitoTracker Red (MTRed) in CN 1.4 cells transfected with GFP or GFP-Tau(s) forms and double transfected with GFP or GFP-Tau(s) forms plus Opa1-c-myc. White arrows shows Opa1 overexpression in double transfected cultures. Scale bar = 10 μm. Quantitative analysis of mitochondrial length in GFP or GFP-Tau(s) cells

(black bars) and double transfected with GFP or GFP-Tau(s) forms/Opa1-c-myc (grey bars) is shown in (**C**). **p < 0.001, *p < 0.05. Differences were calculated by ANOVA test. (**D**) Mitochondrial membrane potential measurements in GFP or GFP-Tau(s) cells (control, black bars), GFP or GFP-Tau (s) cells treated with thapsigargin (Thap 1 μM, 30 min, light grey bars), and double transfected cells with GFP or GFP-Tau(s) forms/Opa1-c-myc treated with Thap (Thap 1 μM, 30 min, dark grey bars) is shown. *p < 0.001. Differences were calculated by ANOVA test. Data are the mean ± SE. (E) All experiments were performed with n = 4.

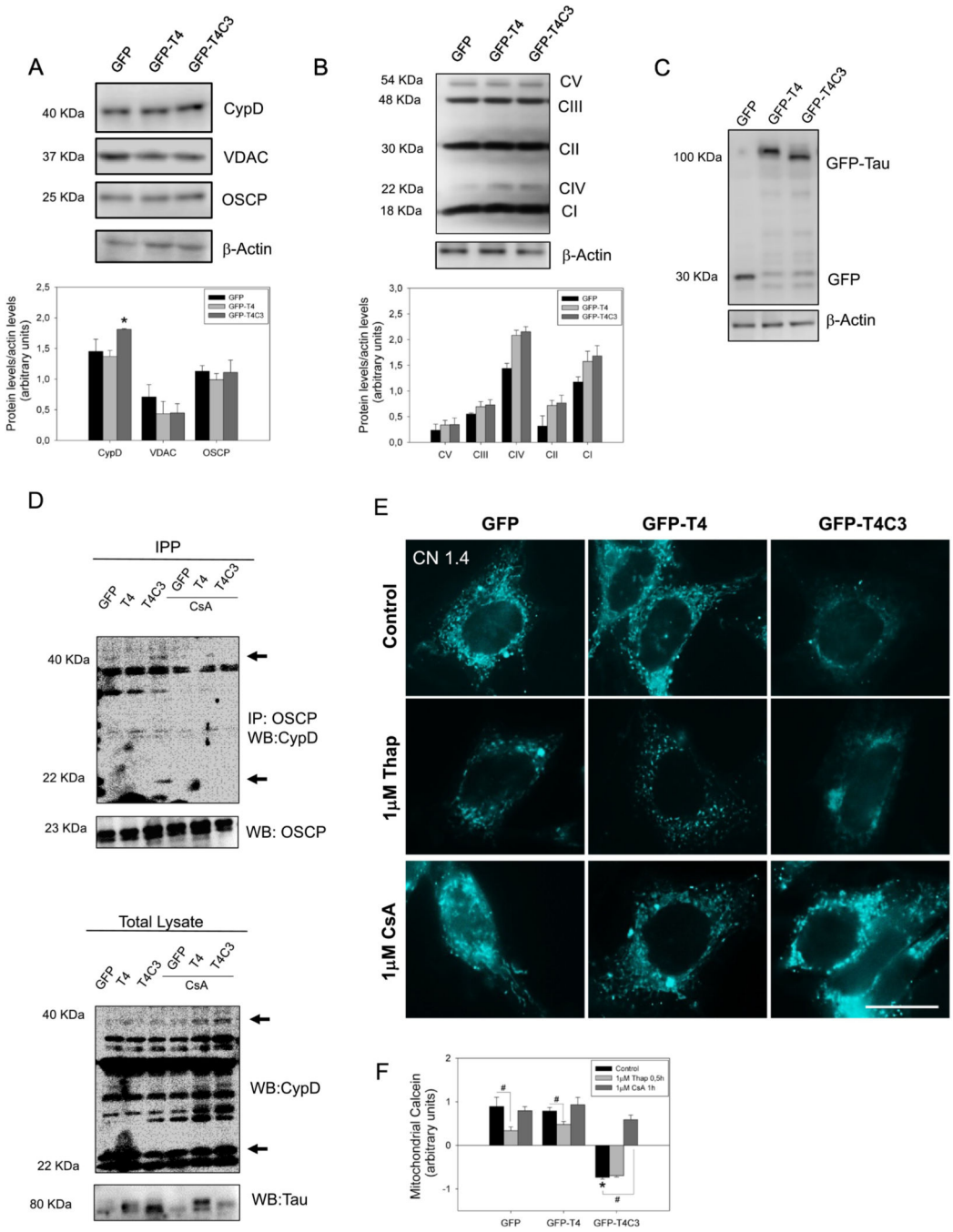

Figure 3. Caspase-3 cleaved tau expression induces the opening of the mitochondrial permeability transition pore (mPTP) in CN 1.4 immortalized neurons.

(**A**) Representative western blot indicating the expression levels of mPTP components in CN 1.4 cells transfected with GFP or GFP-tau (s) forms. Quantification of mPTP expression levels using ß-actin as a loading control. *p<0.05. (**B**) Representative western blot assay showing the expression levels of mitochondrial respiratory complexes in transfected CN 1.4 cells. Quantification of OXPHOS expression levels using ß-actin as a loading control. (**C**) Expression of GFP and GFP-Tau (s) forms in CN 1.4 immortalized neurons. (**D**)

Coimmunoprecipitation of OSCP from CN 1.4 cells transfected with GFP and GFP-tau (s) forms. Cells were treated with CsA (1 μM, 2h) as indicated. Representative western blot against CypD, OSCP, and tau in the coimmunoprecipitation assay or total lysate. (**E**) Representative images of CN 1.4 cells transfected with GFP or GFP-Tau (s) forms labeled with calcein blue. Calcein blue mitochondrial fluorescence intensity was evaluated after $CoCl_2$ treatment. Cells were treated with 1μM Thap for 30 min or 1μM CsA for 2 h as indicated. Scale bar =10 μm. (**F**) Quantitative analysis of mitochondrial fluorescent intensity. *$p<0.05$ indicates differences with GFP control, #$p<0.05$ indicates differences between groups. Differences were calculated by ANOVA test. Data are the mean ± SE. All experiments were performed with n = 3.

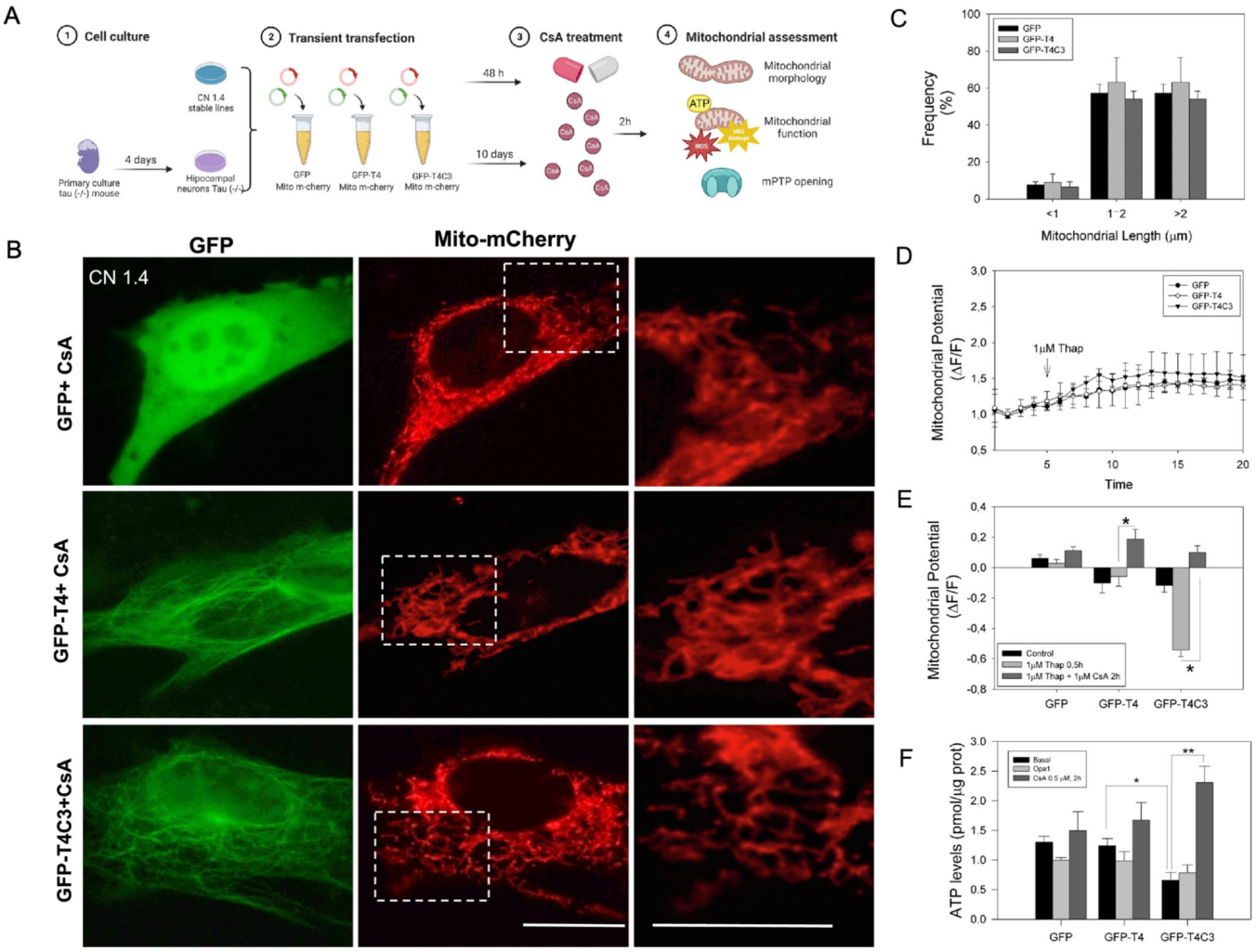

Figure 4. Pharmacological blocking of the mitochondrial permeability transition pore (mPTP) prevents mitochondrial dysfunction induced by caspase-3 cleaved tau in CN 1.4 immortalized neurons.

(**A**) Schematic of the experimental procedure, showing cell culture, transient transfection, cyclosporine A (CsA) treatment, and mitochondrial assessment protocols. (**B**) Representative images of CN 1.4 cells double transfected with Mito-mCherry and GFP or GFP-Tau (s) forms. A magnified area allows visualization of the mitochondrial network. Scale bar = 10 μm. Quantification of mitochondrial length frequency is shown in (**C**). (**D**) Measurements of mitochondrial membrane potential performed with Mitotracker Red CMxRos in live CN 1.4 cells transfected with GFP or GFP-tau (s) forms over time. Thapsigargin (Thap, 1 μM) treatment is indicated in the figure. (**E**) Mitochondrial membrane potential determination before and after Thap (1μm, 30 min) and CsA (0.5 μm, 2h) treatments. Data are the mean ± SE, *p<0.001 indicates differences with and without CsA calculated by Student's t-test. (**F**) ATP content was measured in CN 1.4 cells transfected with GFP or GFP-Tau(s) forms (black bars), double transfected with GFP or GFP-Tau(s) forms/Opa1-c-myc (light grey bars) and double transfected with GFP or GFP-Tau(s) forms/ Opa1-c-myc treated with CsA (0.5 μM, 2h, dark grey bars) using bioluminescence assay.

Data are mean ± SE, *p<0.05, **p< 0.001, differences were estimated by one-way ANOVA test. All experiments were performed with n=4.

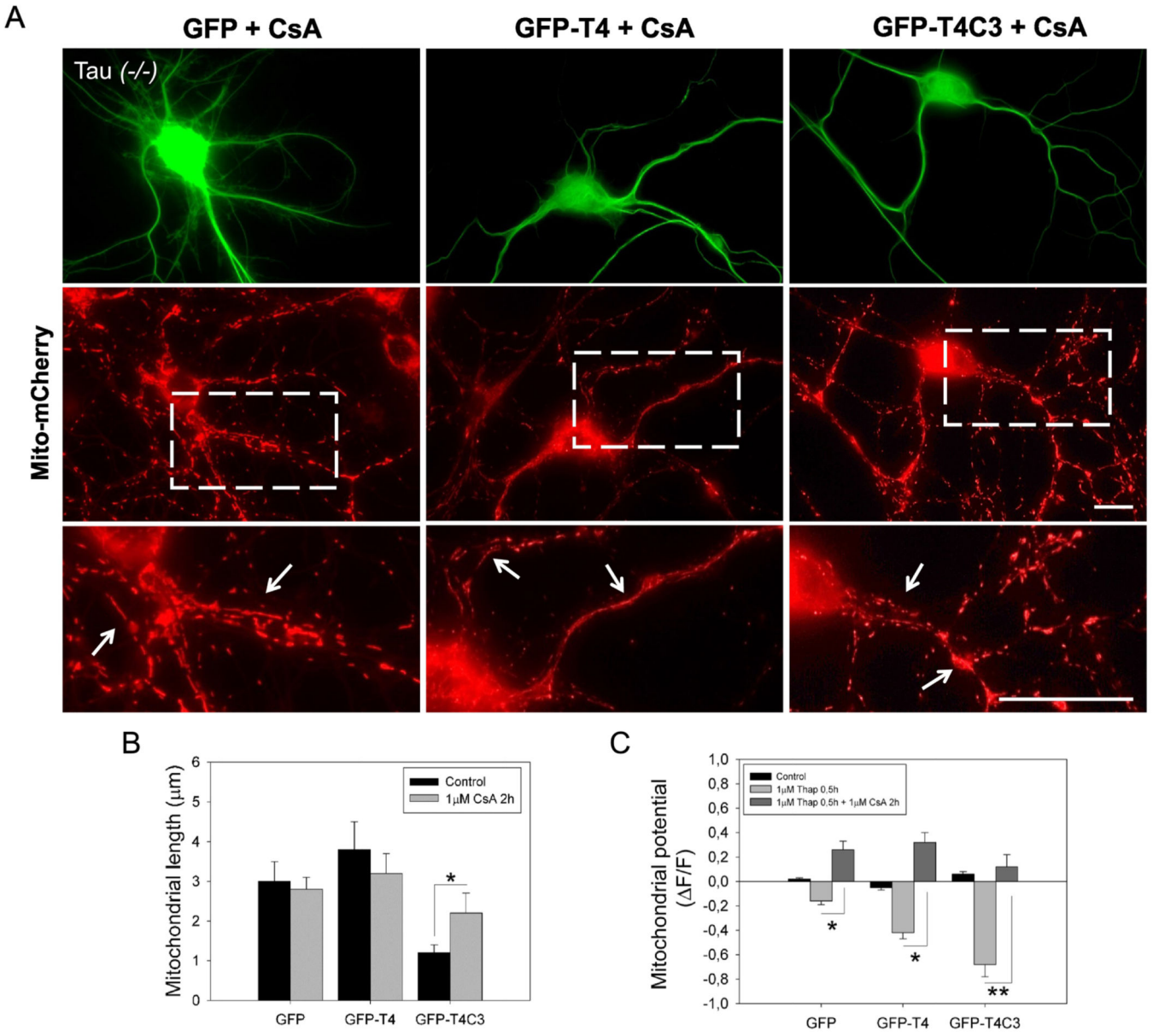

Figure 5. Pharmacological blocking of the mitochondrial permeability transition pore (mPTP) prevents mitochondrial dysfunction induced by caspase-3 cleaved tau expression in tau *(−/−)* hippocampal neurons.

(**A**) Representative images of tau (−/−) neurons double transfected with Mito-mCherry and GFP or GFP-Tau (s) forms, following CsA treatment (0.5 µM, 2h). Arrows in magnified section indicate isolated mitochondria in neuronal prolongations. Scale bar = 10 µm. Quantification of mitochondrial length is shown in (**B**). *p<0.05, indicates differences with and without CsA calculated by t Student test. (**C**) Mitochondrial membrane potential determinations before and after Thap and CsA treatments. **p<0.001, *p<0.05, indicates differences with and without CsA calculated by t Student test. Data are the mean ± SE. All experiments were performed with n = 4.

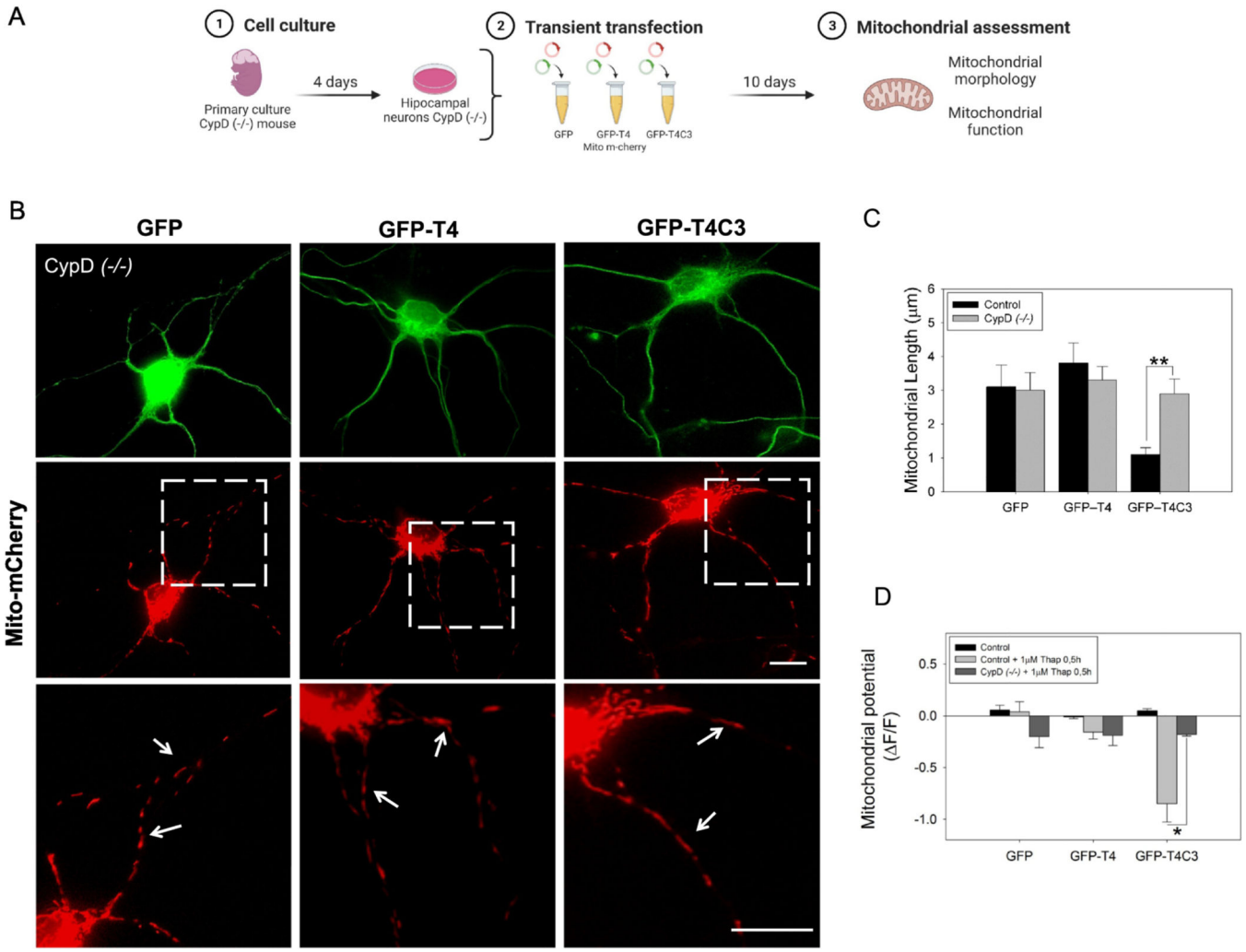

Figure 6. Genetic ablation of Cyclophilin D prevents mitochondrial impairment induced by caspase-cleaved tau expression

(**A**) Schematic of the experimental procedure, showing cell culture, transient transfection, and mitochondrial assessment protocols. (**B**) Representative images of CypD (−/−) neurons transfected with Mito-mCherry and GFP or GFP-Tau (s) to determine mitochondrial length. Arrows in the magnified section indicate isolated mitochondria in neuronal prolongations. Scale bar = 10 µm. (**C**) Quantification of mitochondrial length. **p < 0.01 indicates differences with control neurons calculated by t-Student test. (**D**) Measurement of mitochondrial membrane potential in GFP and GFP-tau (s) transfected neurons. Cells were treated with Thap (1 µM, 30 min) as indicated. *p < 0.05 indicates differences between control and CypD (−/−) neurons transfected after Thap treatment. Differences were calculated by t-Student test. Data are the mean ± SE. All experiments were performed with n = 4.