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The c-Abl/p73 pathway induces neurodegeneration in a Parkinson's disease model

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ABSTRACT

Parkinson's disease is the second most common neurodegenerative disorder. Although it is clear that dopaminergic neurons degenerate, the underlying molecular mechanisms are still unknown, and thus, successful treatment is still elusive. One pro-apoptotic pathway associated with several neurodegenerative diseases is the tyrosine kinase c-Abl and its target p73. Here, we evaluated the contribution of c-Abl and p73 in the degeneration of dopaminergic neurons induced by the neurotoxin 6-hydroxydopamine as a model for Parkinson's disease. First, we found that in SH-SY5Y cells treated with 6-hydroxydopamine, c-Abl and p73 phosphorylation levels were up-regulated. Also, we found that the pro-apoptotic p73 isoform TAp73 was up-regulated. Then, to evaluate whether c-Abl tyrosine kinase activity is necessary for 6-hydroxydopamine-induced apoptosis, we co-treated SH-SY5Y cells with 6-hydroxydopamine and Imatinib, a c-Abl specific inhibitor, observing that Imatinib prevented p73 phosphorylation, TAp73 up-regulation, and protected SH-SY5Y cells treated with 6-hydroxydopamine from apoptosis. Interestingly, this observation was confirmed in the c-Abl conditional null mice, where 6-hydroxydopamine stereotaxic injections induced a lesser reduction of dopaminergic neurons than in the wild-type mice significantly. Finally, we found that the intraperitoneal administration of Imatinib prevented the death of dopaminergic neurons induced by injecting 6-hydroxydopamine stereotaxically in the mice striatum. Thus, our findings support the idea that the c-Abl/p73 pathway is involved in 6-hydroxydopamine degeneration and suggest that inhibition of its kinase activity might be used as a therapeutical drug in Parkinson's disease.

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder, with a prevalence of 1% in the population under 65 years old and 5% in the population over 85 years old (Berg et al., 2013; Troncoso-Escudero et al., 2018). PD is characterized by a degeneration of the dopaminergic neurons residing at the *substantia nigra* and, consequently, motor deficits (Hughes et al., 1992; Troncoso-Escudero et al., 2020). PD is a complex disorder involving abnormal cellular processes, including mitochondrial impairments, lysosomal dysfunction, and proteasomal stress, where oxidative stress might be an important factor in PD etiology. Indeed, PD patients have reduced reactive oxygen species (ROS) scavenging capability and overproduction of ROS and reactive nitrogen species (Zeevalk et al., 2008). Also, it has been reported that PD patients have increased oxidized proteins, lipids, and nucleic acids in the substantia nigra and a reduction in the antioxidant capacity measured in plasma (Alam et al., 1997; Sian et al., 1994; Sunday et al., 2014). Moreover, PD patients present an abnormal functioning of the complex I of the electron transport chain that overproduces ROS (Papa and De Rasmo, 2013). However, although oxidative stress has been extensively described in PD, the molecular mechanism between oxidative stress and the loss of dopaminergic neurons has not

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been fully clarified.

c-Abl is a non-receptor tyrosine kinase homologous to the Src kinase family (Hantshcel et al., 2003) with important cellular functions in the CNS, including the regulation of the growth cone movement and synaptogenesis (Zukerberg et al., 2000; Bradley and Koleske, 2009; Perez de Arce et al., 2010, González-Martín et al., 2021; Gutierrez et al., 2023). Also, c-Abl regulates a synaptic plasticity-related transcriptional program involved in memory and learning (González-Martín et al., 2021). Interestingly c-Abl has been found activated in the brains of PD patients and animal models (Ko et al., 2010; Imam et al., 2011, Brahmachari et al., 2016). Activated c-Abl phosphorylates Parkin, leading to inhibition of its activity (Ko et al., 2010, Iman et al., 2011; Kim et al., 2021), and α-synuclein (α-syn) regulating its aggregation (Mahul-Mellier et al., 2014, Brahmachari et al., 2016). Moreover, dopaminergic neuronal loss induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or α-syn preformed fibrils (PFF) is reduced by genetic loss or pharmacological inhibition of c-Abl in mice (Karuppagounder et al., 2014; Lee et al., 2018; Kwon et al., 2021). In addition, Nilotinib, a c-Abl inhibitor, reduce microglia-mediated neuroinflammation protecting against dopaminergic neuronal death in PD models (Wu et al., 2021). Although c-Abl inhibition has shown promising results in preclinical animal PD models (Brahmachari et al., 2017), in a clinical trial Nilotinib showed no significant symptomatic benefit in PD patients and no changes in dopamine metabolite levels in the cerebrospinal fluid (CSF), probably associated with low blood-brain barrier (BBB) penetrance (Simuni et al., 2021). Thus, it is necessary to understand how c-Abl activation contributes to the pathogenesis of sporadic PD, and if its inhibition is a promising therapeutic strategy.

However, c-Abl role in oxidative stress-dependent neurodegeneration in PD has been less studied. Interestingly, *in vitro* assays have shown that c-Abl kinase activity is incremented under oxidative stress and DNA damage (Kharbanda et al., 1995; Sun et al., 2000). During pro-apoptotic events, such as genotoxic and oxidative stress, c-Abl interacts and phosphorylates p73, a transcription factor member of the p53 family with functions associated with apoptosis (Nakagawa et al., 2002; Tsai and Yuan, 2003; Dötsch et al., 2010). Interestingly, hippocampal neurons treated with Aβ fibrils or H_2O_2 have shown increased c-Abl kinase activity and apoptosis by a p73-dependent mechanism and pharmacological inhibition of c-Abl by the FDA-approved drug Imatinib prevents this harm (Alvarez et al., 2004; Cancino et al., 2008). It has also been described that in Niemann-pick disease type C (NPC), another neurodegenerative disorder associated with oxidative stress, c-Abl/p73 signaling was activated (Klein et al., 2011). p73 induces apoptosis by up-regulating its target genes *Bim, Bax, Puma,* and *Noxa*, and reports have shown that oxidative stress induces the up-regulation of these pro-apoptotic genes in cortical neurons (Steckley et al., 2007). Indeed, it has been shown that Puma$^{-/-}$ neurons are resistant to induction of apoptosis by oxidants. Furthermore, an increase in the protein levels of Puma on PC12 cells treated with 1-methyl-4-phenylpyridinium (MPP^+) has been reported (Kook et al., 2011). Moreover, it has been described that c-Abl-p38α signaling plays an important role in MPTP-induced neuronal death (Wu et al., 2016), and the p38 activation is required for the c-Abl-dependent p73 activation (Mantovani et al., 2004). Thus, it is suggested that the c-Abl/p73 pathway is involved in oxidative stress-associated neurodegeneration in PD.

Here, we investigated the role of the c-Abl/p73 pathway in a 6-hydroxydopamine (6-OHDA) PD model. Our studies reveal that c-Abl is activated in SH-SY5Y cells treated with 6-OHDA, inducing p73 phosphorylation and further apoptosis. Also, we found that the pro-apoptotic p73 isoform TAp73 was up-regulated. Then, to evaluate whether c-Abl tyrosine kinase activity was important for 6-OHDA-induced apoptosis, we treated SH-SY5Y cells with 6-OHDA and Imatinib. Interestingly, we observed that Imatinib prevented p73 phosphorylation, TAp73 upregulation, and apoptosis. Furthermore, we confirmed c-Abl involvement in this PD model using the c-Abl conditional null mice, where 6-OHDA stereotaxic injections induced significantly less reduction of dopaminergic neurons at the *substantia nigra* than the wild-type mice. Finally, we found that intraperitoneal administration of Imatinib prevented dopaminergic neuron death induced by 6-OHDA stereotaxic injection in the mice striatum, confirming the therapeutic potential of Imatinib for PD. Thus, our findings support the idea that c-Abl/p73 are involved in 6-OHDA neuronal degeneration and suggest that inhibition of its kinase activity could be a therapeutically option for PD patients.

2. Methods

2.1. Animal ethics

Procedures were conducted in accordance with Pontificia Universidad Católica de Chile CAC (protocol #150825007, approved June 2016), the Chilean Law 20.380 for Animal Protection, the Terrestrial Animal Health Code of the World Organization for Animal Health (OIE, 24a Edition, 2015), the European Directive 2010/63/UE and the Guide for the Care and Use of Experimental Animals (NRC, 8a Edition, 2011).

2.2. Animals

Homozygous c-Abl-floxed mice were kindly donated by Dr. AJ Koleske (Yale School of Medicine, US) and bred in our animal facility. c-Abl conditional null mice (c-Abl cKO) were bred from ABL1$^{loxP/loxP}$ and Nestin-Cre$^+$, obtained from Jackson Labs. These mice have loxP sites upstream and downstream of exon 5 of the *Abl1* gene. This strain originated and was maintained on a mixed B6.129S4, C57BL/6 background and did not display any gross physical or behavioral abnormalities. Genotyping was performed using a PCR-based screening to evaluate c-Abl ablation (Gutierrez et al., 2019). Male and female mice were housed on a 12/12-h light/ dark cycle at 24 °C with *ad libitum* access to food and water.

2.3. Animal perfusion and tissue fixation

WT or c-Abl cKO (González-Martín et al., 2021) mice were anesthetized with intraperitoneal injections (IP) of Ketamine (90–120 mg/Kg) and Xylazine (10 mg/Kg). Mice anesthesia status was continuously evaluated through the pinch response method. Once in deep anesthesia, a lateral incision of about 5–6 cm through the integument and abdominal wall just beneath the rib cage was made. Then, a small incision in the diaphragm that continued along the entire length of the rib cage was made to expose the pleural cavity. Then, the sternum was lifted to proceed with the heart perfusion. A small incision was done to the end of the right atrium using small scissors. Then, PBS buffer through a perfusion pump was injected into the left ventricle. Next, 50 ml of 4% paraformaldehyde in PBS buffer were circulated in the mice. Brains were dissected and kept in 4% paraformaldehyde (PFA) for 24 h at 4 °C. After this, brains were maintained in a 30% Sucrose solution for one week. After that, coronal sections were obtained using a Leica CM1520 criostat.

2.4. Cell cultures

Catecholaminergic PC12 cell line, derived from a rat pheochromocytoma, was grown in 100 mm diameter plaques in an atmosphere with 5% CO_2 and 37 °C with DMEM complete medium supplemented with glucose, 5% fetal bovine serum and 5% Horse serum, 100 U/ml of penicillin and 100 μg/ ml of streptomycin. SH-SY5Y cell line, derived from human neuroblastoma, was cultured in 250 ml Flask with F12/ DMEM 50:50 supplemented with 10% Fetal Bovine serum, 100 U/ml of penicillin and 100 μg/ ml of streptomycin. 6-OHDA (Sigma-Aldrich CAT# 28094–15–7) treatments were done in six-well plates starting from a 6-OHDA stock solution (5 mg/ml) with 0.2% w/v of Ascorbic

Acid (Sigma-Aldrich CAT# A7506) and protected from light exposure. 6-OHDA stock solution was used within a week otherwise discarded.

2.5. Western blot

Proteins were obtained of cell cultures lysed with RIPA buffer plus phosphatases and proteases inhibitors (50 mM Tris-HCl; pH 7.4; 150 mM NaCl; 1 mM EDTA; 1% NP-40; 0.25% Na-deoxycholate; 1 mM PMSF; 1 mM NaF; 1 mM Na_3VO_4, Pepstatin, PMSF, Leupeptin). Protein quantification was assessed by the BCA kit protocol (Life Technologies CAT# 23225). Western blots were carried out by loading 40–80 μg of proteins in an 8 – 12% polyacrylamide gel (SDS-PAGE). Proteins were then transferred to PDVF membranes and blocked 60 min with 5% BSA in TBS-T (0.9% NaCl, Tween 20 0.1%, 0.1 M Tris Base, pH 7.4) or 5% non-fat milk in TBS-T. Next, primary antibodies were incubated in blocking solution overnight at 4 °C. The following antibodies were used: Mouse anti-c-Abl (Santa Cruz); Rabbit anti-phospho Tyr 412-c-Abl 1:1500 (Sigma); Rabbit anti-phospho Tyr 245-c-Abl 1:1000 (Sigma); Rabbit phospho-p73 1:500 (Santa Cruz); Goat p73 1:1000 (Santa Cruz); Mouse GAPDH 1:5000 (Santa Cruz); Mouse anti-Actin 1:1000 (Santa Cruz). Then, membranes were washed three times for 8 min with TBS-T and incubated with the following secondary antibodies conjugated with HRP for 60 min at room temperature: anti-mouse IgG-HRP 1:1500; anti-rabbit-IgG-HRP 1:1500; anti-goat-IgG-HRP 1:5000. Finally, membranes were washed three times for 8 min with TBS-T and then incubated with chemiluminescent solutions (Bio-Rad) and then photodocumented using an imaging system (Bio-Rad).

2.6. MTT and apoptotic nuclei

SH-SY5Y cells were grown in DMEM/F12 media supplemented with 10% FBS in 96-well plates. 5000 cells were cultured per well and grown for 24 h. Cells were treated with 6-OHDA in different concentrations for 24 h. Once treatments were finished, cell media was removed, and RPMI media with MTT was incubated for 6 h (final volume of 100 μl per well), protecting it from light. The MTT reaction was then stopped with a lysis buffer incubated for 24 h at 37 °C. MTT products were finally measured at 570 nm using a multiplate reader (Thermo). Apoptotic nuclei were analyzed by Hoechst 33342 staining (Life Technologies CAT # H1399). Hoechst was diluted 1:10000 in PBS buffer and was incubated for 10 min. After the incubation, coverslips were washed 3 times per 8 min and then mounted.

2.7. Immunofluorescence

Immunofluorescence in coverslips was initiated by plating 25,000 cells per well in a 24-well plate treated with Poly-Lysine. Cells were grown for 24 h, and before the treatment, media was removed and incubated with a fresh one. 6-OHDA treatments were made as previously described (cell cultures section). Then the cells were fixed in 4% PFA overnight. Next, coverslips were washed 3 times x 10 min with PBS Ca/Mg (Calcium Chloride 0.90 mM, Magnesium Chloride 0.49 mM) and cells permeabilizated with PBS + 0.2% Triton X-100 for 30 min. Coverslips were later washed twice x 8 min with PBS Ca/Mg, incubated with 0.2 M Glycine for 60 min to reduce autofluorescence of PFA, and subsequently, washed twice for 8 min with PBS Ca/Mg. Then, coverslips were blocked for 60 min in blocking solution (5% BSA in PBS Ca/Mg + 0.2% v/v Triton X-100). Once blocked primary antibodies were incubated in the blocking solution overnight (Anti-phospho-c-Abl sigma rabbit monoclonal 1:250). Primary antibodies were washed 3 times for 8 min with PBS Ca/Mg, and then secondary antibodies were incubated in the blocking solution (Anti-Rabbit IgG Alexa 555 conjugated, Life Technologies CAT # A-31572 1:250). Finally, coverslips were washed 3 times for 8 min and then mounted using Fluoromont. Images were acquired using a Leica DMi8 microscope and LAS X software (Leica).

2.8. Immunohistochemistry

25 μm thick brain slices were washed twice with PBS for 5 min each. Then endogenous catalase activity was inactivated with a 30 min wash with H_2O_2 0.3% v/v. Afterward, the slices were blocked for 60 min with a blocking solution containing 10% donkey serum, 5% BSA, 0.4% Triton x-100%, and 0.02% sodium azide. Then, the primary antibody Rabbit Anti-Tyrosine Hidroxylase (Merck) 1:5000 in blocking solution, was incubated at 4 °C overnight. Once incubation was finished, antibodies were washed up 3 times for 8 min with PBS. Then secondary antibody, Goat Anti-Rabbit IgG Biotinylated (CAT# BA-1000 VectorLabs) 1:2000 in blocking solution was incubated for one hour. Slices were then washed three times with PBS for 8 min and incubated for 60 min with the Biotin-Avidin peroxidase system previously prepared (1:100 A and 1:100 B) in PBS buffer (LifeTechnologies CAT# 32020). Then, slices were washed twice for 8 min with PBS. Slices were finally stained using the ImmPACT DAB substrate kit (Vector laboratories) for 5–10 min. Finally, slices were washed with PBS and mounted with Entellan (Millipore CAT# 107960). Images were acquired using a Leica DMi8 microscope and LAS X software (Leica).

2.9. Stereotaxic Injections

WT or c-Abl cKO mice (25–30 g) were anesthetized with intraperitoneal injections (IP) of Ketamine (90–120 mg/Kg) and Xylazine (10 mg/Kg). Mice were placed on an electronically controlled heating pad. Mice were then positioned in the stereotaxic equipment and fixed by the ears and mouth. Then the animal was disinfected using 70% ethanol in the affected areas. A sagittal incision was done with a scalpel, and the periosteum was removed with 100% H_2O_2. Next, Bregma coordinates were set in the stereotaxic frame. In order to obtain the injection point, specifically in the striatum (Coordinates: +0.07 cm rostro-caudal, −0.17 cm Lateral, −0.31 cm dorso-ventral) the syringe was lifted and moved to the correct coordinates previously described in Vidal et al., 2021. Using a sterilized drill, a hole was made, and the needle was placed. 2 μl of 6-OHDA (4 mg/ml) in 0.02% ascorbic acid solution were then injected slowly at a flow rate of 0.1 μl/min. Finally, 5 min after reaching dorso-ventral coordinate and having injected the desired volume, the needle was retracted slowly, and the mice closed up.

Animals were injected daily with Imatinib (25 mg/Kg) or vehicle (30% polyethylene glycol; 30% 1,2-propandiol) for 7 days post-stereotaxic injection. Observation of animal status was done daily. If pain signals were detected, animals were administered analgesics. If bad conditions were visualized on injected animals, they were adequately euthanized.

2.10. Statistical analysis

Data were analyzed using the Prism 6 Software for Mac OSX (GraphPad Software Inc., San Diego, CA). Mean and SEM values and the number of experiments are indicated in each figure legend and the specific test and post-test used.

3. Results

3.1. The c-Abl/p73 pathway participates in 6-OHDA-induced neurodegeneration

It has been shown that the non-receptor tyrosine kinase c-Abl is activated under different cellular insults such as genotoxic and oxidative stress, where it plays a pro-apoptotic role (Zhu and Wang, 2004; Cancino et al., 2011; Schlatterer et al., 2011). Therefore, we asked whether c-Abl, which is aberrantly activated in several neurodegenerative diseases, is also activated in an oxidative stress-dependent PD model. We first evaluated in the SH-SY5Y cell line, which is derived from human neuroblastoma, the phosphorylated levels of c-Abl using an

anti-phospho-specific antibody and immunofluorescence. We found that 6-OHDA induced a time-dependent increase in c-Abl phosphorylation (Fig. 1A). To confirm this observation, we evaluated the phosphorylation of c-Abl by Western blot. We observed that 6-OHDA induced a time-dependent increase in the phosphorylation of c-Abl at the tyrosines 412 and 245, both associated with c-Abl activity (Fig. 1B-D). Then, to discard a possible cell line-specific artifact, we also evaluated in the PC12 cell line derived from a rat pheochromocytoma. Fig. 1E-G also showed an upregulation in the phosphorylation of c-Abl at the tyrosine 412 and 245 when PC12 cells were treated with 6-OHDA, confirming that in both cell lines, there is a time-dependent increase in the phosphorylated form of c-Abl induced by 6-OHDA.

Furthermore, it has been described that aberrant activation of c-Abl induces cell death, up-regulating signaling pathways, including the p53 family of transcription factors. Indeed, the p53 family member p73 has been associated with apoptosis in different neurodegenerative diseases (Alvarez et al., 2004; Ramadan et al., 2005; Cancino et al., 2008; Killick et al., 2011; Cancino et al., 2013; Niklison-Chirou et al., 2016; Agostini et al., 2018). Then, we asked whether p73 expression and phosphorylation were altered in our *in vitro* PD model. First, we evaluated the total levels of p73 in SH-SY5Y cells treated with 6-OHDA. Immunoblotting against total p73 suggests that 6-OHDA induces an increase in its protein levels (Fig. 2A, B). Interestingly, using a specific antibody against p73 phosphorylated at Tyr99, a tyrosine-phosphorylated by c-Abl, we found

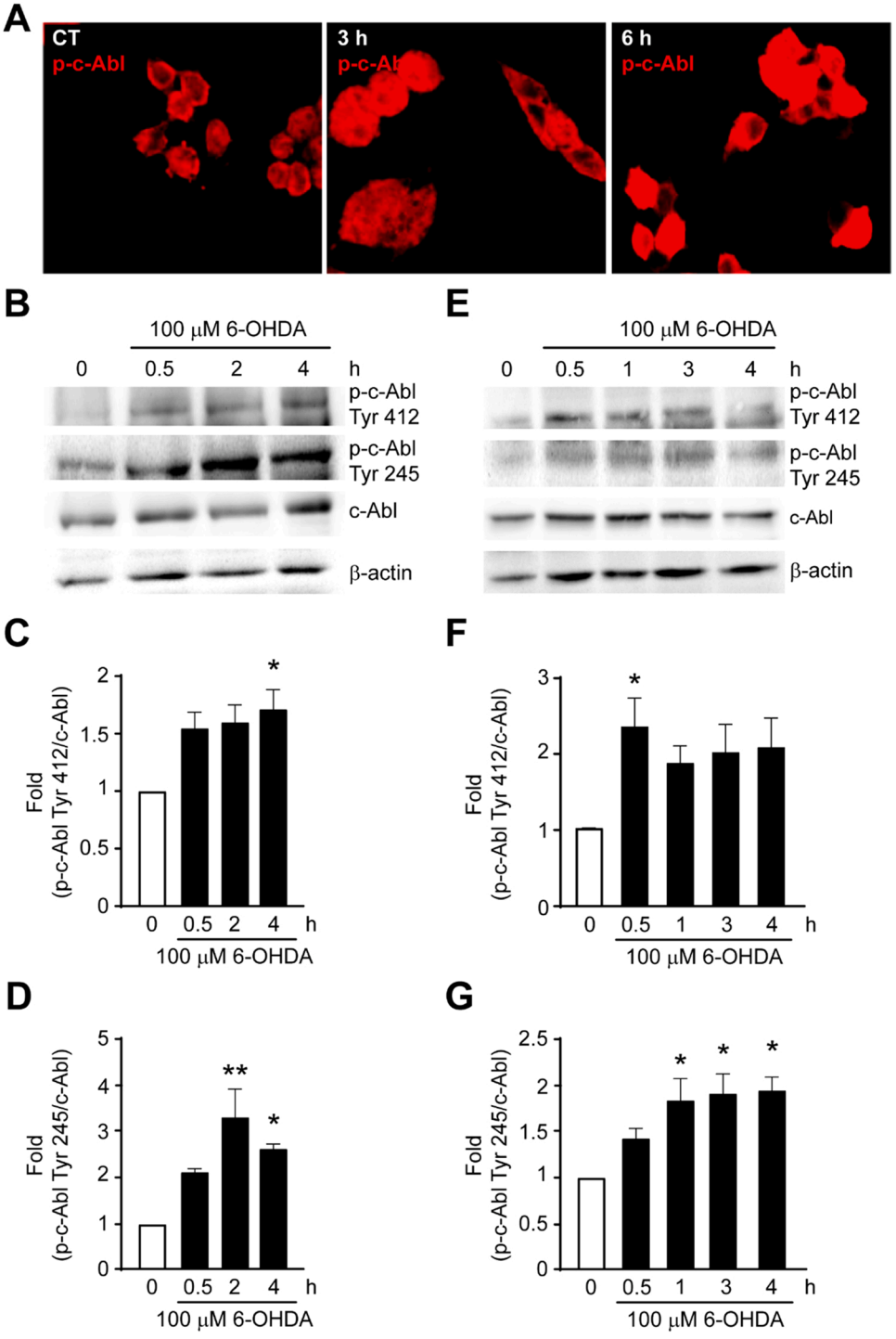

Fig. 1. 6-OHDA treatments up-regulate phosphorylated c-Abl protein levels. (A) Immunofluorescence of phospho-c-Abl-Tyr412 in SH-SY5Y cells treated with 100 μM 6-OHDA. (B-G) Western blot of total lysates of SH-SY5Y (B) and PC12 (E) cell cultures treated with 100 μM 6-OHDA and their densitometry quantifications (C and D, and F and G, respectively). β-actin was used as a loading control. Western blot quantifications for phosphorylated tyr 412 and tyr 245 were normalized by total c-Abl. $n = 4$ (C and D) and 5 (F and G). Statistical analysis was done using one-way ANOVA and Tukey's post hoc test * $p < 0.05$; ** $p < 0.001$. Mean ± SEM.

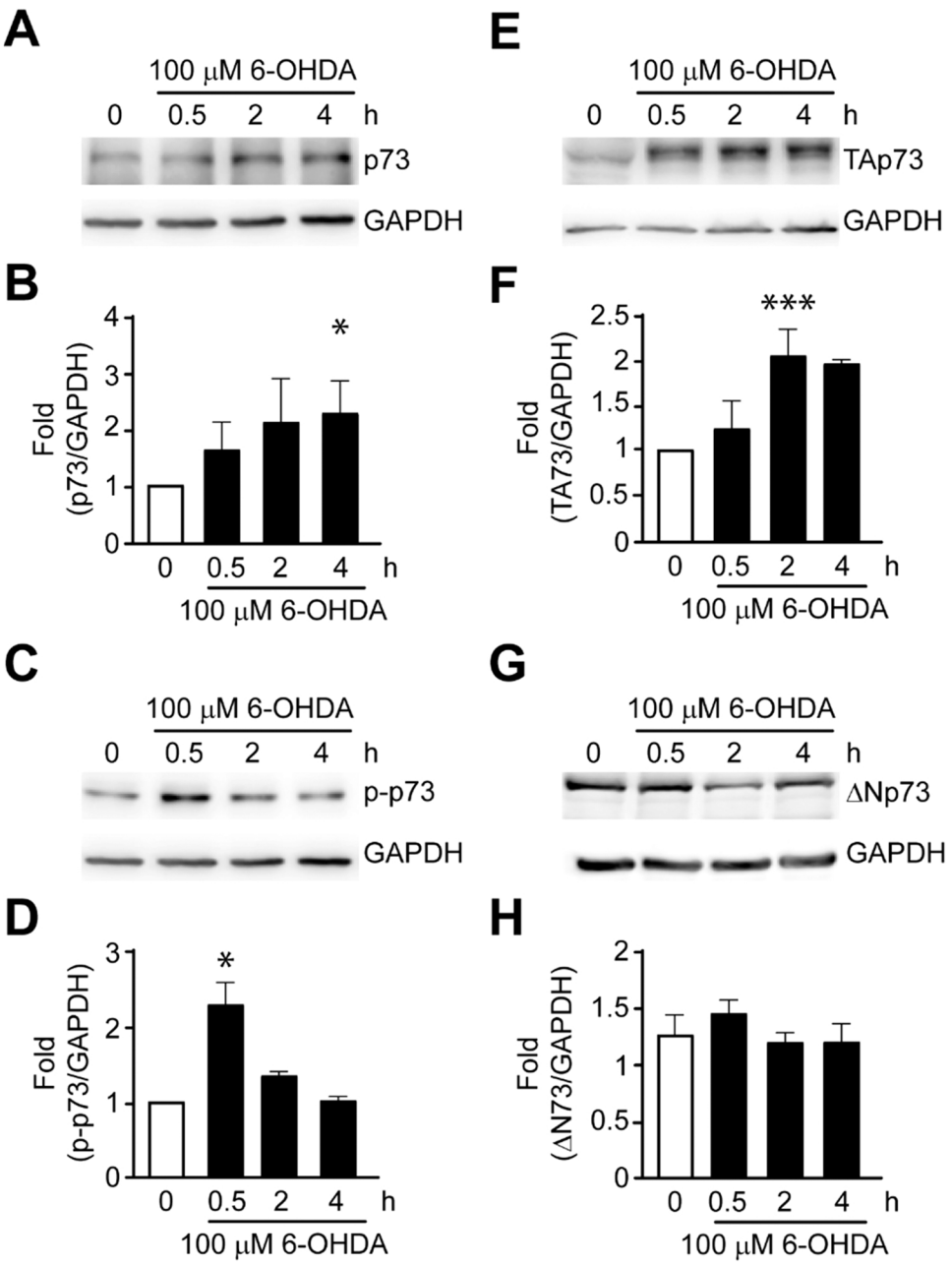

Fig. 2. Phosphorylated and pro-apoptotic TAp73 specific isoform form of p73 are increased in cells treated with 6-OHDA. Western blot was performed from total lysates of SH-SY5Y cell culture treated with 100 μM 6-OHDA at 0.5, 2 or 4 h. The membranes were incubated with total p73 (A), phospho-p73 (C), TAp73 (E) or ΔNp73 (G) antibodies. Then, membranes were reprobed with GAPDH antibody as loading control. Densitometric quantifications were performed for total p73 (B), phospho-p73 (D), TAp73 (F) or ΔNp73 (H), normalized by GAPDH, its loading control. One-way ANOVA Tukey's post hoc test were performed. * $p < 0.05$; *** $p < 0.001$; ns = not significant. $n = 3$. Mean ± SEM.

that at 30 min after treatment with 6-OHDA SH-SY5Y cells shown higher levels of p73 phosphorylated at Tyr99 than control cells (Fig. 2C, D). Moreover, p73 can be translated into different isoforms depending on the promotor and alternative splicing. TAp73 is the full-length p73 containing a trans-activating domain with transcriptional activity and pro-apoptotic function (Nakagawa et al., 2002). On the other hand, ΔNp73 is a short isoform without the TA domain that works as a negative dominant and has an anti-apoptotic nature. Therefore, we evaluated if 6-OHDA differentially affects p73 isoforms levels. For this purpose, we used SH-SY5Y cells treated with 6-OHDA and evaluated TAp73 and ΔNp73 levels using isoform-specific antibodies. We found that treatments with 6-OHDA induced an increase in TAp73 isoform at 2 h after treatment (Fig. 2E, F). Furthermore, there were no changes when ΔNp73 protein levels were evaluated in the same conditions (Fig. 2G, H). These results suggest that the c-Abl/p73 signaling pathway is activated in cells treated with 6-OHDA.

3.2. The pharmacological inhibition of c-Abl prevents 6-OHDA-induced neurodegeneration in vitro

Our results suggest that 6-OHDA induced neurodegeneration due to the up-regulation of c-Abl tyrosine kinase activity. These results are consistent with previous reports that showed that c-Abl is up-regulated in different stressing contexts, e.g., beta-amyloid, oxidative stress, and genotoxic stress (Alvarez et al., 2004; Cancino et al., 2008; Cancino et al., 2011; Maiani et al. 2011), where it has been shown that c-Abl orchestrates pro-apoptotic fates. Then, to test whether c-Abl kinase activity is important to 6-OHDA-induced neuronal degeneration, we performed *in vitro* assays of SH-SY5Y cells co-treated with 6-OHDA and Imatinib. As expected, the percentage of apoptotic nuclei in SH-SY5Y cells treated with 6-OHDA increased in a time and concentration-dependent manner. Treatments of 100 μM 6-OHDA reach approximately 7% and 18% of apoptotic nuclei at 12 or 24 h treatment, respectively, and adding 10 μM Imatinib significantly prevented the

appearance of apoptotic nuclei (Fig. 3A). Similar results were obtained when cells were treated with 250 μM 6-OHDA (Fig. 3B). Furthermore, MTT assay of SH-SY5Y treated for 12 h with 6-OHDA (100 or 250 μM) showed a reduction in cell viability of approximately 70% with 100 μM and 50% with 250 μM of 6-OHDA relative to the control, which was prevented when co-treated with 10 μM Imatinib (Fig. 3C). These results suggest that c-Abl tyrosine kinase activity is necessary to 6-OHDA-induced neurodegeneration.

Then, to confirm that c-Abl is a key regulator of p73 stability and activity in our model, we wondered whether the increment of phosphorylated p73 levels induced by 6-OHDA was c-Abl dependent. To evaluate this, we used SH-SY5Y cell cultures treated with 100 μM 6-OHDA plus 10 μM Imatinib or vehicle. In 6-OHDA treatments, there was a consistent increase in phosphorylated-p73. Interestingly, this increase was prevented when incubated with Imatinib (Fig. 4A). And as previously described, Imatinib successfully inhibited c-Abl phosphorylation induced by 6-OHDA (Fig. 4B). Together, these data suggest that c-Abl tyrosine kinase activity is necessary to induce cell death by 6-OHDA in a p73-dependent mechanism.

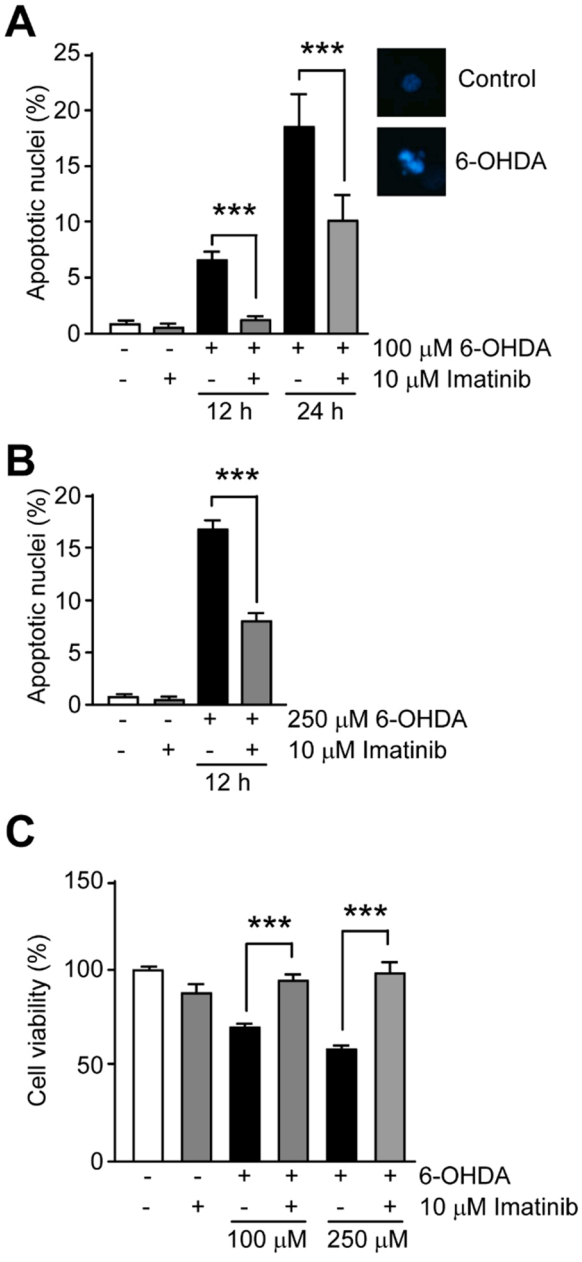

Fig. 3. Imatinib prevents cell death induced by 6-OHDA. (A-B) Apoptotic nuclei assay was assessed by Hoetsch staining in SH-SY5Y cell culture co-treated with 100 μM (A) or 250 μM (B) 6-OHDA and 10 μM Imatinib. Representative control and apoptotic (6-OHDA) nuclei are shown in A. (C) MTT assay of SH-SY5Y cell culture treated with 100 μM or 250 μM 6-OHDA and 10 μM Imatinib. One-way ANOVA and Tukey's post hoc test were performed *** $p < 0.001$; ns = not significant. n = 4. Mean ± SEM.

3.3. *The genetic or pharmacological inhibition of c-Abl prevents 6-OHDA-induced neurodegeneration in vivo*

It is well established that the nigrostriatal pathway neurodegenerates in Parkinson's disease, and it has been suggested that the c-Abl tyrosine kinase is involved in this process. To confirm the contribution of c-Abl in the neurodegeneration of the nigrostriatal tract in this PD model, we used neuronal-specific c-Abl null mice (c-Abl cKO) that were stereotaxically injected with 6-OHDA in the striatum (Berger et al. 1991). 7 days post-injection, mice were euthanized, and tyrosine hydroxylase (TH)-positive neurons were evaluated in the *substantia nigra*. As expected, immunohistochemistry at the *substantia nigra* showed a reduced detection of TH+ neurons on the ipsilateral side in wild-type mice (Fig. 5A, B; upper panel). Importantly, this reduction was significantly ameliorated in the conditional c-Abl null mice (Fig. 5A, B; bottom panel), suggesting that c-Abl participates in the neurodegeneration induced by 6-OHDA.

Finally, we evaluated whether *in vivo* inhibition of c-Abl kinase activity by systemic administration of Imatinib prevented dopaminergic cell loss induced by stereotaxic injections of 6-OHDA in the striatum. We immunostained coronal sections for TH containing the *substantia nigra*. As previously described, mice injected with 6-OHDA show decreased TH-positive neurons on the ipsilateral side compared with the control contralateral side in the *substantia nigra* (Fig. 6A, B; upper panel). Notably, we found that mice intraperitoneally injected with Imatinib presented more TH-positive neurons on the ipsilateral side than vehicle-treated animals (Fig. 6A, B; lower panel). Thus, Imatinib protects against the 6-OHDA-induced reduction of TH-positive neurons in the *substantia nigra*.

4. Discussion

In this study, we found that the injection of neurotoxin 6-OHDA, a model of Parkinson's disease, activates c-Abl and its target p73 and that this signaling mediates the degeneration of dopaminergic neurons. We showed that c-Abl is activated in neuronal cell lines treated with 6-OHDA. Also, 6-OHDA induced an increase in p73 phosphorylation and TAp73 levels. Interestingly, c-Abl inhibition prevented the cell death induced by 6-OHDA. Furthermore, loss of TH-positive neurons induced by 6-OHDA injection at the striatum was prevented by pharmacological or genetic inhibition of c-Abl in mice, confirming c-Abl involvement in PD.

Indeed, previous studies have already associated c-Abl with PD (Ko et al., 2010; Imam et al., 2011, Brahmachari et al., 2016, Schlatterer et al., 2011; Werner and Olanow, 2022). It has been shown that the hyperactivated c-Abl critically regulates α-synuclein-induced neuropathology. Deletion of the c-Abl gene reduced α-syn aggregation, neuropathology, and neurobehavioral deficits of mice expressing a human α-synucleinopathy-associated mutation (hA53Tα-syn) while overexpression of constitutively active c-Abl accelerated α-syn aggregation, neuropathology, and neurobehavioral deficits. It has also been described that α-syn is a substrate of c-Abl where its phosphorylation in tyrosine 39 decreases the clearance of α-syn, resulting in the aggregation of this protein that is characteristic of PD (Mahul-Mellier et al., 2014; Brahmachari et al., 2016). Also, it has been described that c-Abl phosphorylates Parkin in its tyrosine 143, inhibiting its E3 ubiquitin ligase activity and protective capacity, producing an accumulation of Parkin's substrates in MPTP models (Ko et al., 2010). Moreover, Nilotinib, a

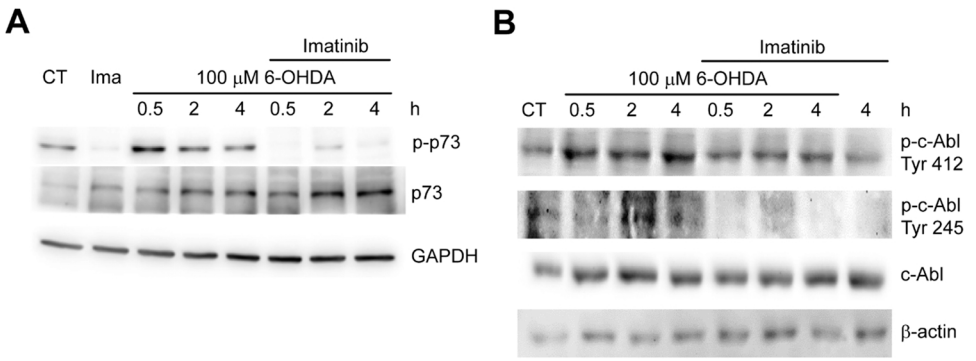

Fig. 4. Imatinib reduces phosphorylated-p73 protein levels induced by 6-OHDA. (A) Total lysates of SH-SY5Y cell cultures treated with 100 μM 6-OHDA or 10 μM Imatinib for 0.5, 2, or 4 h were incubated with antibodies against total or phosphorylated p73. Then, membranes were reprobed with GAPDH antibodies as a loading control. (B) Total lysates of SH-SY5Y cell cultures treated with 100 μM 6-OHDA or 10 μM Imatinib for 0.5, 2, or 4 h were incubated with antibodies against total or phosphorylated c-Abl. Then, membranes were reprobed with β-actin antibodies as a loading control.

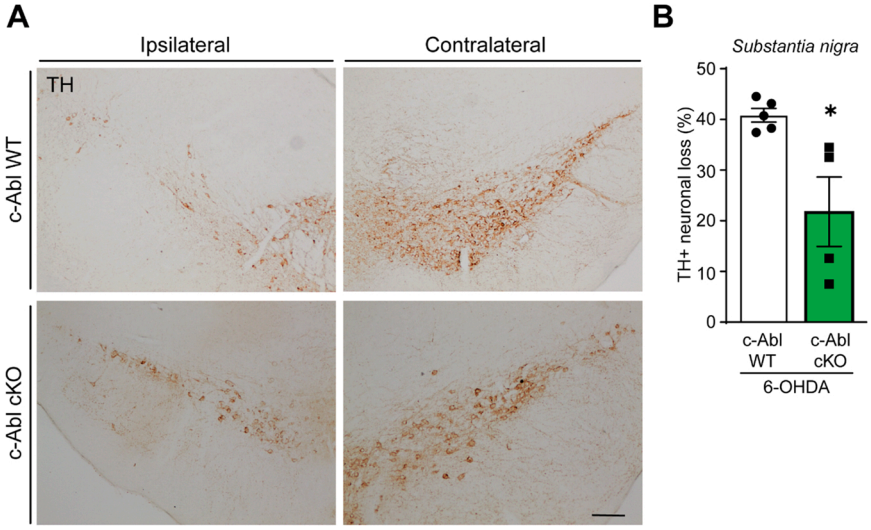

Fig. 5. Conditional ablation of c-Abl prevents reduction of tyrosine hydroxylase-positive cells induced by 6-OHDA stereotaxic injection. (A-B) Wild-type (c-Abl WT) and neuronal-specific c-Abl null mice (c-Abl cKO) were stereotaxically injected with 6-OHDA at the striatum, and 7 days after, immunohistochemistry for tyrosine hydroxylase was performed in coronal brain sections at the *substantia nigra* (A). Then TH+ neurons were quantified (B). Data are expressed as mean ± SEM. n = 5 (c-Abl WT) and n = 4 (c-Abl cKO). * $p < 0.05$. Scale bar: 100 μm.

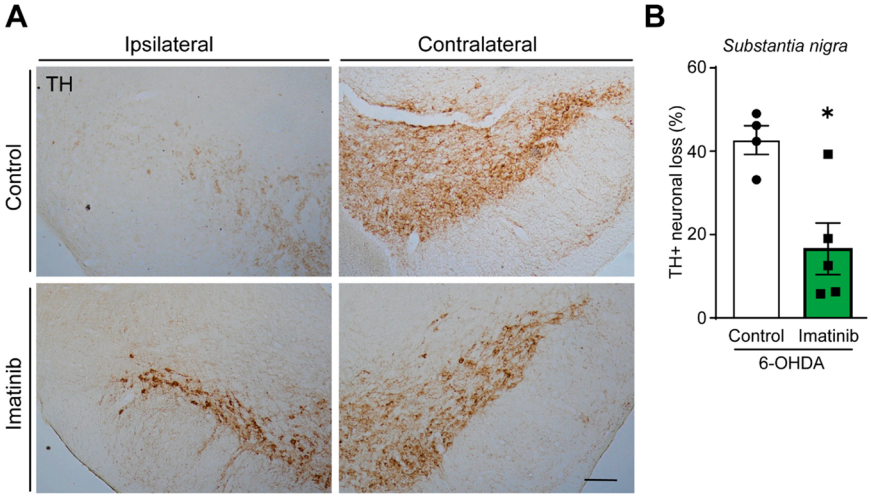

Fig. 6. Pharmacological c-Abl inhibition reduced 6-OHDA-induced dopaminergic cell loss. (A-B) WT C57B adult mice were injected with 6-OHDA (2 μl of 5 mg/ml stock solution) in the striatum. Mice were divided into two groups; untreated and treated with Imatinib (25 mg/kg) for 7 days, one injection per day. Then, immunostaining for tyrosine hydroxylase was performed in coronal slices containing the *substantia nigra* (A). Then TH+ neurons were quantified (B). Data are expressed as mean ± SEM. n = 4 (control) and n = 5 (Imatinib). * $p < 0.05$. Scale bar: 100 μm.

second-generation specific c-Abl inhibitor, increases α-syn clearance, cell death, and behavioral impairments, strongly suggesting a role of c-Abl in PD (Karuppagounder et al., 2014; Mahul-Mellier et al., 2014; Tanabe et al., 2014). More recently, a novel selective blood-brain barrier penetrant c-Abl inhibitor significantly reduced activation of c-Abl and the neurotoxicity caused by α-syn PFF in cortical neurons. Additionally, it ameliorated the loss of dopaminergic neurons, neuroinflammatory responses, and behavioral deficits induced by α-syn PFF injection in vivo (Kwon et al., 2021). However, it is necessary to study further whether Imatinib can ameliorate motor dysfunction using pharmacological or genetic PD preclinical models such as MPTP injection, α-syn PFF injected mice, or α-syn overexpression transgenic mice. Moreover, it is necessary to determine the possible impact of this pharmacological treatment on PD progression, considering motor dysfunction, dopaminergic neuronal loss, and α-syn accumulation. Therefore, our data and previous findings strongly suggest using c-Abl inhibitors with high brain penetrances such

as Imatinib and Neurotinib (Marín et al., 2022) to reduce the main hallmarks of PD.

Furthermore, p73 is a key c-Abl target that mediates the induction of apoptosis. p73 transcription factor has similar roles to p53, including binding and transactivation of some p53-responsive genes involved in apoptosis and cell cycle regulation (Murray-Zmijewski et al. 2006). Our results showed that in SH-SY5Y cells treated with 6-OHDA, an increment of p73 protein total levels and its phosphorylated form in residue 99. Furthermore, when pharmacological inhibiting c-Abl with Imatinib, in 6-OHDA treatments, a decrease in p73 phosphorylated levels was reduced compared to the vehicle-treated cells, which supports the role of c-Abl/p73 signaling in Parkinson's disease. Here we demonstrated that TAp73 protein levels are up-regulated in 6-OHDA treatments. This is coherent with literature where it has been proposed that in pro-apoptotic environments, TAp73 is up-regulated, and ΔNp73 is downregulated or unchanged (Benosman et al., 2011). p73 has distinctive pro-apoptotic target genes (e.g., *puma* and *noxa*), so it might be valuable to study if there is a transcriptional upregulation with a consequent protein level increment and if c-Abl pharmacological inhibition correlates with a decrease in this upregulation. Interestingly, Melino et al. (2004), using an inducible form of p73, showed that upon induction of p73 over 12 h, mRNA quantification of the apoptotic gene *puma* was significantly increased, revealing a possible explanation of the results observed previously where it appeared a gap between p73 activation and neuronal death (Melino et al. 2004).

Several stressing conditions have described the c-Abl/p73 interaction, enabling p73 phosphorylation by c-Abl and triggering pro-apoptotic events. For example, in hippocampal neurons exposed to Aβ fibrils, it has been shown that an increased c-Abl/p73 interaction is associated with Aβ-induced neuronal apoptosis. Moreover, inhibiting c-Abl kinase activity with Imatinib reduces c-Abl/p73 complex formation preventing Aβ fibrils neuronal death (Yuan et al., 1999, Alvarez et al., 2004). Interestingly c-Abl/p38 signaling activation mediates the p73 activation (Mantovani et al., 2004) and plays an important role in MPTP-induced neuronal death (Wu et al., 2016). Although we have not assessed whether 6-OHDA treatments induce an increment in c-Abl-p73 interaction, p73 tyrosine 99 has only been reported to be a substrate of c-Abl. Hence, these results suggest a possible direct interaction under these conditions. Furthermore, we confirmed that c-Abl participates in 6-OHDA-induced neurodegeneration of dopaminergic cells *in vivo*. The 6-OHDA-induced dopaminergic neuron loss was reduced in c-Abl null mice, confirming the role of c-Abl mediating neuronal death in this Parkinson's disease model. Moreover, the Imatinib systemic administration prevented the loss of TH-positive neurons in the *substantia nigra*, suggesting that activation of c-Abl in either post-synaptic striatal neurons or presynaptic DA neurons in the *substantia nigra* is triggering neuronal death. This is consistent with previous reports showing that intraperitoneal MPTP injections yield dopaminergic cell loss of the nigrostriatal tract and that administration of Nilotinib, a c-Abl specific inhibitor, prevents this harm and improves mice scores in behavioral tests (Karuppagounder et al., 2014). Both c-Abl and TAp73 activation also has been linked to inflammatory responses (Schlatterer et al., 2011; Tomasini et al., 2013). Indeed c-Abl inhibition protects against dopaminergic neuronal death in the LPS-induced neuroinflammation PD model, while its overexpression results in severe neurodegeneration and neuroinflammation (Wu et al., 2021).

It has been proposed that c-Abl can respond by mechanisms upon oxidative stress. In hippocampal neurons treated with hydrogen peroxide, c-Abl kinase activity is up-regulated and translocated to the nucleus. On the other hand, mouse embryonic fibroblast treated with H_2O_2 has c-Abl enrichment in the mitochondrial fraction correlated with a mitochondrial transmembrane potential loss, which is prevented by Imatinib (Alvarez et al., 2004, Kumar et al., 2001). Neurotoxic effects produced by 6-OHDA have been suggested mainly by two mechanisms. First, after reaching effective cytoplasmic concentrations distributed through the DA transporter, 6-OHDA is oxidized by monoamine oxidase and auto-oxidation, generating H_2O_2 and depleting antioxidant enzymes (Cohen and Heikkila, 1984). It has also been reported that 6-OHDA disrupts mitochondrial electron transport chain complex I independent of ROS production. (Glinka and Youdim, 1995). Interestingly, it has been described that the c-Abl/p73 pathway is activated in neurons in oxidative stress-associated damage such as Aβ peptide toxicity and lysosomal cholesterol accumulation (Alvarez et al., 2004; Klein et al., 2011). Thus, it might suggest that 6-OHDA oxidative stress activates the c-Abl/p73 pathway and pro-apoptotic response. Interestingly we recently found that the c-Abl activation participates in necroptosis activation in Gaucher disease (GD), a lysosomal storage disease linked to PD (Hruska et al., 2008, Klein and Mazzulli, 2018), where active c-Abl is increased in Gaucher disease models and participates in RIPK3 and MLKL signaling activation (Yañez et al., 2021).

In conclusion, our findings suggest that c-Abl is implicated in the loss of dopaminergic cells induced by 6-OHDA and that inhibiting this kinase prevents that harm. We have also shown a possible mechanism of 6-OHDA-induced apoptotic events through activation of the c-Abl/p73 pathway, where 6-OHDA-induced activation of c-Abl, probably by its oxidative capacity, phosphorylates p73, producing an increment in p73 stability and consequently, triggering pro-apoptotic events. This study contributes to understanding Parkinson's disease etiology and may be considered when developing new therapeutic targets.

CRediT authorship contribution statement

Tamara Marín: Investigation, Methodology, Formal analysis, Writing – review & editing. **Cristian Valls:** Investigation, Methodology, Formal analysis. **Carolina Jerez:** Investigation, Methodology, Formal analysis. **Tomas Huerta:** Investigation, Methodology, Formal analysis. **Daniela Elgueta:** Investigation, Methodology, Formal analysis. **René L. Vidal:** Conceptualization, Supervision, Writing – original draft. **Alejandra R. Alvarez:** Conceptualization, Supervision, Writing – original draft, Writing – review & editing. **Gonzalo I. Cancino:** Conceptualization, Supervision, Writing – original draft, Writing – review & editing.

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Declaration of interest

The authors have no competing interests to declare.

References

Agostini, M., Melino, G., Bernassola, F., 2018. The p53 family in brain disease. Antioxid. Redox Signal. 29 (1), 1–14.

Alam, Z.I., Daniel, S.E., Lees, A.J., Marsden, D.C., Jenner, P., et al., 1997. A generalized increase in protein carbonyls in the brain in Parkinson's but not incidental Lewy body disease. J. Neurochem. 69, 1326–1329.

Alvarez, A.R., Sandoval, P.C., Leal, N.R., Castro, P.U., Kosik, K.S., 2004. Activation of the neuronal c-Abl tyrosine kinase by amyloid-β-peptide and reactive oxygen species. Neurobiol. Dis. 17 (2), 326–336.

Benosman, S., Meng, X., Von Grabowiecki, Y., Palamiuc, L., Hritcu, L., Gross, I., Mellitzer, G., Taya, Y., Loeffler, J.P., Gaiddon, C., 2011. Complex regulation of p73 isoforms after alteration of amyloid precursor polypeptide (APP) function and DNA damage in neurons. J. Biol. Chem. 286 (50), 43013–43025.

Berg, D., Lang, A.E., Postuma, R.B., Maetzler, W., Deuschl, G., Gasser, T., et al., 2013. Changing the research criteria for the diagnosis of Parkinson's disease: obstacles and opportunities. Lancet Neurol. 12, 514–524.

Berger, K., Przedborski, S., Cadet, J.L., 1991. Retrograde degeneration of nigrostriatal neurons induced by intrastriatal 6-hydroxydopamine injection in rats. Brain Res. Bull. 26, 301–307.

Bradley, W.D., Koleske, A.J., 2009. Regulation of cell migration and morphogenesis by Abl-family kinases: emerging mechanisms and physiological contexts. J. Cell Sci. 122 (Pt 19), 3441–3454.

Brahmachari, S., Ge, P., Lee, S.H., Kim, D., Karuppagounder, S.S., Kumar, M., Mao, X., Shin, J.H., Lee, Y., Pletnikova, O., Troncoso, J.C., Dawson, V.L., Dawson, T.M., Ko, H.S., 2016. Activation of tyrosine kinase c-Abl contributes to alpha-synuclein-induced neurodegeneration. J. Clin. Invest 126 (8), 2970–2988.

Brahmachari, S., Karuppagounder, S.S., Ge, P., Lee, S., Dawson, V.L., Dawson, T.M., Ko, H.S., 2017. c-Abl and Parkinson's disease: mechanisms and therapeutic potential. J. Park. Dis. 7 (4), 589–601.

Cancino, G.I., Toledo, E.M., Leal, N.R., Hernandez, D.E., Yévenes, L.F., Inestrosa, N.C., Alvarez, A.R., 2008. STI571 prevents apoptosis, tau phosphorylation and behavioural impairments induced by Alzheimer's β-amyloid deposits. Brain 131 (9), 2425–2442.

Cancino, G.I., Perez de Arce, K., Castro, P.U., Toledo, E.M., von Bernhardi, R., Alvarez, A. R., 2011. c-Abl tyrosine kinase modulates tau pathology and Cdk5 phosphorylation in AD transgenic mice. Neurobiol. Aging 32 (7), 1249–1261.

Cancino, G.I., Miller, F.D., Kaplan, D.R., 2013. p73 haploinsufficiency causes tau hyperphosphorylation and tau kinase dysregulation in mouse models of aging and Alzheimer's disease. Neurobiol. Aging 34 (2), 387–399.

Cohen, G., Heikkila, R.E., 1984. Alloxan and 6-hydroxydopamine: cellular toxins. Methods Enzym. 105, 510–516.

Dötsch, V., Bernassola, F., Coutandin, D., Candi, E., Melino, G., 2010. p63 and p73, the ancestors of p53. Cold Spring Harb. Perspect. Biol. 2, 4887.

Glinka, Y.Y., Youdim, M.B., 1995. Inhibition of mitochondrial complexes I and IV by 6-hydroxydopamine. Eur. J. Pharmacol. 292 (3–4), 329–332.

González-Martín, A., Moyano, T., Gutiérrez, D.A., Carvajal, F.J., Cerpa, W., Hanley, J.G., Gutiérrez, R.A., Álvarez, A.R., 2021. c-Abl regulates a synaptic plasticity-related transcriptional program involved in memory and learning. Prog. Neurobiol. 205, 102122.

Gutiérrez, D.A., Chandía-Cristi, A., Yáñez, M.J., Zanlungo, S., Álvarez, A.R., 2023. c-Abl kinase at the crossroads of healthy synaptic remodeling and synaptic dysfunction in neurodegenerative diseases. Neural Regen. Res. 18 (2), 237–243.

Gutierrez, D.A., Vargas, L.M., Chandia-Cristi, A., de la Fuente, C., Leal, N., Alvarez, A.R., 2019. c-Abl deficiency provides synaptic resiliency against Aβ-oligomers. Front. Cell Neurosci. 13, 526.

Hantschel, O., Nagar, B., Guettler, S., Kretzschmar, J., Dorey, K., Kuriyan, J., Superti-Furga, G., 2003. A myristoyl/phosphotyrosine switch regulates c-Abl. Cell 112, 845–857.

Hruska, K., LaMarca, M.E., Scott, C.R., Sidransky, E., 2008. Gaucher disease: mutation and polymorphism spectrum in the glucocerebrosidase gene (GBA). Hum. Mutat. 29 (5), 567–683.

Hughes, A.J., Daniel, S.E., Kilford, L., Lees, A.J., 1992. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. J. Neurol. Neurosurg. Psychiatry 55, 181–184.

Imam, S.Z., Zhou, Q., Yamamoto, A., Valente, A.J., Ali, S.F., Bains, M., Roberts, J.L., Kahle, P.J., Clark, R.A., Li, S., 2011. Novel regulation of parkin function through c-Abl-mediated tyrosine phosphorylation: implications for Parkinson's disease. J. Neurosci. 31 (1), 157–163.

Karuppagounder, S.S., Brahmachari, S., Lee, Y., Dawson, V.L., Dawson, T.M., Ko, H.S., 2014. The c-Abl inhibitor, Nilotinib, protects dopaminergic neurons in a preclinical animal model of Parkinson's disease. Sci. Rep. 4, 4874.

Kharbanda, S., Ren, R., Pandey, P., Shafman, T.D., Feller, S.M., Weichselbaum, R.R., Kufe, D.W., 1995. Activation of the c-Abl tyrosine kinase in the stress response to DNA-damaging agents. Nature 376 (6543), 785–788.

Killick, R., Niklison-Chirou, M., Tomasini, R., Bano, D., Rufini, A., Grespi, F., Velletri, T., Tucci, P., Sayan, B.S., Conforti, F., Gallagher, E., Nicotera, P., Mak, T.W., Melino, G., Knight, R.A., Agostini, M., 2011. p73: a multifunctional protein in neurobiology. Mol. Neurobiol. 43 (2), 139–146.

Kim, H., Shin, J.Y., Jo, A., Kim, J.H., Park, S., Choi, J.Y., Kang, H.C., Dawson, V.L., Dawson, T.M., Shin, J.H., Lee, Y., 2021. Parkin interacting substrate phosphorylation by c-Abl drives dopaminergic neurodegeneration. Brain 144 (12), 3674–3691.

Klein, A.D., Mazzulli, J.R., 2018. Is Parkinson's disease a lysosomal disorder? Brain 141 (8), 2255–2262.

Klein, A., Maldonado, C., Vargas, L.M., Gonzalez, M., Robledo, F., Perez de Arce, K., Zanlungo, S., 2011. Oxidative stress activates the c-Abl/p73 pro-apoptotic pathway in Niemann-Pick type C neurons. Neurobiol. Dis. 41 (1), 209–218.

Ko, H.S., Lee, Y., Shin, J.H., Karuppagounder, S.S., Gadad, B.S., Koleske, A.J., Pletnikova, O., Troncoso, J.C., Dawson, V.L., Dawson, T.M. 2010. Phosphorylation by the c-Abl protein tyrosine kinase inhibits Parkin's ubiquitination and protective function. Proc. Natl. Acad. Sci. U. S. A. 107 (38), 16691–16696. (https://doi.org/10.1073/pnas.1006083107).

Kook, Y.H., Ka, M., Um, M., 2011. Neuroprotective cytokines repress PUMA induction in the 1-methyl-4-henylpyridinium (MPP++) model of Parkinson's disease. Biochem. Biophys. Res. Commun. 411 (2), 370–374.

Kumar, S., Bharti, A., Mishra, N.C., Raina, D., Kharbanda, S., Saxena, S., Kufe, D., 2001. Targeting of the c-Abl tyrosine kinase to mitochondria in the necrotic cell death response to oxidative stress. J. Biol. Chem. 276 (20), 17281–17285.

Kwon, S.H., Kim, S., Park, A.Y., Lee, S., Gadhe, C.G., Seo, B.A., Park, J.S., Jo, S., Oh, Y., Kweon, S.H., Ma, S.X., Kim, W.R., Kim, M., Kim, H., Kim, J.E., Lee, S., Lee, J., Ko, H. S., 2021. A novel, selective c-Abl inhibitor compound 5 prevents neurodegeneration in Parlkinson's disease. J. Med Chem. 64 (20), 15091–15110.

Lee, S., Kim, S., Park, Y.J., Yun, S.P., Kwon, S.H., Kim, D., Kim, D.Y., Shin, J.S., Cho, D.J., Lee, G.Y., Ju, H.S., Yun, H.J., Park, J.H., Kim, W.R., Jung, E.A., Lee, S., Ko, H.S., 2018. The c-Abl inhibitor, Radotinib HCl, is neuroprotective in a preclinical Parkinson's disease mouse model. Hum. Mol. Genet 27 (13), 2344–2356.

Mahul-Mellier, A.L., Fauvet, B., Gysbers, A., Dikiy, I., Oueslati, A., Georgeon, S., Lamontanara, A.J., Bisquertt, A., Eliezer, D., Masliah, E., Halliday, G., Hantschel, O., Lashuel, H.A., 2014. c-Abl phosphorylates alpha-synuclein and regulates its degradation: implication for alpha-synuclein clearance and contribution to the pathogenesis of Parkinson's disease. Hum. Mol. Genet 23 (11), 2858–2879.

Maiani, E., Diederich, M., Gonfloni, S., 2011. DNA damage response: the emerging role of c-Abl as a regulatory switch? Biochem. Pharmacol. 82 (10), 1269–1276.

Mantovani, F., Piazza, S., Gostissa, M., Strano, S., Zacchi, P., Montovni, R., Blandino, G., Del Sal, G., 2004. Pin1 links the activities of c-Abl and p00 in regulating p73 function. Mol. Cell 14 (5), 625–636.

Marín, T., Dulcey, A.E., Campos, F., de la Fuente, C., Acuña, M., Castro, J., Pinto, C., Yañez, M.J., Cortez, C., McGrath, D.W., Sáez, P.J., Gorshkov, K., Zheng, W., Southall, N., Carmo-Fonseca, M., Marugán, J., Alvarez, A.R., Zanlungo, S., 2022. c-Abl Activation Linked to Autophagy-Lysosomal Dysfunction Contributes to Neurological Impairment in Niemann-Pick Type A Disease. Front. Cell Dev. Biol. 10, 844297.

Melino, G., Bernassola, F., Ranalli, M., Yee, K., Zong, W.X., Corazzari, M., Knight, R.A., Green, D.R., Thompson, C., Vousden, K.H., 2004. p73 Induces apoptosis via PUMA transactivation and Bax mitochondrial translocation. J. Biol. Chem. 279 (9), 8076–8083.

Murray-Zmijewski, F., Lane, D.P., Bourdon, J.C., 2006. p53/p63/p73 isoforms: an orchestra of isoforms to harmonise cell differentiation and response to stress. Cell Death Diff 13 (6), 962–972.

Nakagawa, T., Takahashi, M., Ozaki, T., Watanabe, K.I., Todo, S., Mizuguchi, H., Nakagawara, A., 2002. Autoinhibitory regulation of p73 by ΔNp73 to modulate cell survival and death through a p73-specific target element within the ΔNp73 promoter. Mol. Cell. Biol. 22 (8), 2575–2585.

Niklison-Chirou, M.V., Killick, R., Knight, R.A., Nicotera, P., Melino, G., Agostini, M., 2016. How does p73 cause neuronal defects? Mol. Neurobiol. 53 (7), 4509–4520.

Papa, S., De Rasmo, D., 2013. Complex I deficiencies in neurological disorders. Trends Mol. Med. 19 (1), 61–69.

Perez de Arce, K., Varela-Nallar, L., Farias, O., Cifuentes, A., Bull, P., Couch, B.A., Koleske, A.J., Inestrosa, N.C., Alvarez, A.R., 2010. Synaptic clustering of PSD-95 is regulated by c-Abl through tyrosine phosphorylation. J. Neurosci. 30 (10), 3728–3738.

Ramadan, S., Terrinoni, A., Catani, M.V., Sayan, A.E., Knight, R.A., Mueller, M., Kramer, P.H., Melino, G., Candi, E., 2005. p73 induces apoptosis by different mechanisms. Biochem. Biophys. Res. Commun. 331 (3), 713–717.

Schlatterer, S.D., Acker, C.M., Davies, P., 2011. c-Abl in neurodegenerative disease. J. Mol. Neurosci. 45 (3), 445–452.

Sian, J., Dexter, D.T., Lees, A.J., Daniel, S., Agid, Y., Javoy-Agid, F., Jenner, P., Marsden, C.D., 1994. Alterations in glutathione levels in Parkinson's disease and other neurodegenerative disorders affecting basal ganglia. Ann. Neurol. 36, 348–355.

Simuni, T., Fiske, B., Merchant, K., Coffey, C.S., Klingner, E., Caspell-Garcia, C., Lafontant, D.E., Matthews, H., Wyse, R.K., Brundin, P., Simon, D.K., Schwarzschild, M., Weiner, D., Adams, J., Venuto, C., Dawson, T.M., Baker, L., Kostrzebski, M., Ward, T., Rafaloff, G., Parkinson Study Group NILO-PD Investigators and Collaborators, 2021. Efficacy of nilotinib in patients with moderately advanced parkinson disease: a randomized clinical trial. JAMA Neurol. 78 (3), 312–320.

Steckley, D., Karajgikar, M., Dale, L.B., Fuerth, B., Swan, P., Drummond-Main, C., Poulter, M.O., Ferguson, S.S.G., Strasser, A., Cregan, S.P., 2007. Puma is a dominant regulator of oxidative stress induced Bax activation and neuronal apoptosis. J. Neurosci. 27 (47), 12989–12999.

Sun, X., Majumder, P., Shioya, H., Wu, F., Kumar, S., Weichselbaum, R., Kharbanda, S., Kufe, D., 2000. Activation of the cytoplasmic c-Abl tyrosine kinase by reactive oxygen species. J. Biol. Chem. 275 (23), 17237–17240.

Sunday, O.P., Adekunle, M.F., Temitope, O.T., Richard, A.A., Samuel, A.A., Olufunminyi, A.J., Elizabeth, O.O., 2014. Alteration in antioxidants level and lipid peroxidation of patients with neurodegenerative diseases {Alzheimer's disease and Parkinson disease}. Int. J. Nutr. Pharmacol. Neurol. Dis. 4, 146–152.

Tanabe, A., Yamamura, Y., Kasahara, J., Morigaki, R., Kaji, R., Goto, S., 2014. A novel tyrosine kinase inhibitor AMN107 (Nilotinib) normalizes striatal motor behaviors in a mouse model of Parkinson's disease. Front. Cell. Neurosci. 8, 50.

Tomasini, R., Secq, V., Pouyect, l, Thakur, A.K., Wilhelm, M., Nigri, J., Vasseur, S., Berthezene, P., Calvo, E., Melino, G., Mak, T.W., Iovanna, J.L., 2013. Tap73 is required for macrophage-mediated innate immunity and the resolution of inflammatory response. Cell Death Differ. 20 (2), 293–301.

Troncoso-Escudero, P., Parra, A., Nassif, M., Vidal, R.L., 2018. Outside in: unraveling the role of neuroinflammation in the progression of Parkinson's disease. Front. Neurol. 9, 860.

Troncoso-Escudero, P., Sepulveda, D., Pérez-Arancibia, R., Parra, A.V., Arcos, J., Grunenwald, F., Vidal, R.L., 2020. On the right track to treat movement disorders: promising therapeutic approaches for Parkinson's and Huntington's disease. Front. Aging Neurosci. 12, 571185.

Tsai, K.K., Yuan, Z.M., 2003. c-Abl stabilizes p73 by a phosphorylation-augmented interaction. Cancer Res 63 (12), 3418–3424.

Vidal, R.L., Sepulveda, D., Troncoso-Escudero, P., Garcia-Huerta, P., Gonzalez, C., Plate, L., Jerez, C., Canovas, J., Rivera, C.A., Castillo, V., Cisternas, M., Leal, S., Martinez, A., Grandjean, J., Sonia, D., Lashuel, H.A., Martin, A.J.M., Latapiat, V., Matus, S., Sardi, S.P., Wiseman, R.L., Hetz, C., 2021. Enforced dimerization between XBP1s and ATF6f enhances the protective effects of the UPR in models of neurodegeneration. Mol. Ther. 29 (5), 1862–1882.

Werner, M.H., Olanow, C.W., 2022. Parkinson's disease modification through Abl kinase inhibition: an opportunity. Mov. Disord. 37 (1), 6–15.

Wu, J., Xu, X., Zheng, L., Mo, J., Jin, X., Bao, Y., 2021. Nilotinib inhibits microglia-mediated neuroinflammation to protect against dopaminergic neuronal death in Parkinson's disease models. Int. Immunopharmacol. 99, 108025.

Wu, R., Chen, H., Ma, J., He, Q., Huang, Q., Liu, Q., Li, M., Yuan, Z., 2016. c- Abl-p38α signaling plays an important role in MPTP-induced neuronal death. Cell Death Differ. 23 (3), 542–552.
Yañez, M.J., Campos, F., Marín, T., Klein, A.D., Futerman, A.H., Alvarez, A.R., Zanlungo, S., 2021. c-Abl activates RIPK3 signaling in Gaucher disease. Biochim. Biophys. Acta Mol. Basis Dis. 1867 (5), 166089.
Yuan, Z.M., Shioya, H., Ishiko, T., Sun, X., Gu, J., Huang, Y.Y., Lu, H., Kharbanda, S., Weichselbaum, R., Kufe, D., 1999. p73 is regulated by tyrosine kinase c-Abl in the apoptotic response to DNA damage. Nature 399 (6738), 814–6817.
Zeevalk, G.D., Razmpour, R., Bernard, L.P., 2008. Glutathione and Parkinson's disease: is this the elephant in the room? Biomed. Pharmacother. 62, 236–249.
Zhu, J., Wang, J.Y., 2004. Death by Abl: a matter of location. Curr. Top. Dev. Biol. 59, 165–192.
Zukerberg, L.R., Patrick, G.N., Nikolic, M., Humbert, S., Wu, C.L., Lanier, L.M., Gertler, F. B., Vidal, M., Van Etten, R.A., Tsai, L.H., 2000. Cables links Cdk5 and c-Abl and facilitates Cdk5 tyrosine phosphorylation, kinase upregulation, and neurite outgrowth. Neuron 26 (3), 633–646.