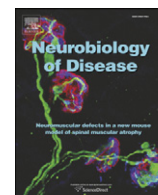
Neurobiology of Disease 40 (2010) 93–101

Contents lists available at ScienceDirect

Neurobiology of Disease

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



Dopaminergic cell damage and vulnerability to MPTP in Pink1 knockdown zebrafish

Ville Sallinen [1], Juha Kolehmainen [1], Madhusmita Priyadarshini, Gabija Toleikyte, Yu-Chia Chen, Pertti Panula *

Neuroscience Center and Institute of Biomedicine/Anatomy, University of Helsinki, Helsinki, Finland

A R T I C L E I N F O

Article history:
Received 4 December 2009
Revised 11 May 2010
Accepted 11 June 2010
Available online 18 June 2010

Keywords:
Parkinson's disease
Park6
Neurodegeneration
Tyrosine hydroxylase
Dopamine
Translation inhibition
Morpholino-oligonucleotide
Diencephalon

A B S T R A C T

The functions of PTEN (phosphatase/tensin homolog)-induced putative kinase (*PINK1*), which is mutated in early-onset Parkinson's disease, are poorly understood. We characterized a PINK1 antibody and found colocalization of PINK1-like immunoreactivity with aminergic markers. We inactivated translation of *Pink1* using morpholino-oligonucleotides (MOs) in larval zebrafish. Dopaminergic neurons consisted of two sets of neuron populations, marked by complementary expression of two tyrosine hydroxylase genes *th1* and *th2*. Translation inhibition of *pink1* resulted in reduction of both *th* mRNA forms until day 5 or 7, respectively. The affected dopaminergic neurons were in one group expressing *th1* and three groups expressing *th2*. Lack of Pink1 sensitized the fish to subeffective doses of MPTP, which caused a locomotor deficit and facilitated loss of *th1* in one diencephalic dopaminergic cell group. Control experiments with *pink1* mRNA and control MO suggested that effects with the splice site targeting MO were specific. Distinct groups of dopaminergic neurons are thus sensitive to loss of *Pink1*. Sensitization of the *pink1* morphant fish to MPTP toxicity suggests that genetic factors play a role in toxin-induced Parkinson's disease.

© 2010 Elsevier Inc. All rights reserved.

Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease among elderly, resulting from the loss of dopaminergic neurons in the substantia nigra (Hornykiewicz and Kish, 1987; Riederer and Wuketich, 1976). Its etiology is unknown, but both genes and environmental factors seem important. Several genes have been identified in PD pathogenesis: alpha-synuclein (Polymeropoulos et al., 1997), leucine-rich repeat kinase 2 (Paisan-Ruiz et al., 2004), parkin (Kitada et al., 1998), DJ-1 (Bonifati et al., 2003), and the more recently found PTEN-induced putative kinase 1 (PINK1) (Valente et al., 2004). PINK1 is a serine–threonine kinase, suggested to span the outer mitochondrial membrane. Currently, the exact localization and cytoplasmic/mitochondrial site of activity of PINK1 are intensely studied, but there is no consensus about these important issues. PINK1 is found in several organs and the distribution of PINK1 in mammalian brain is widespread (Blackinton et al., 2007; Gandhi et al., 2006).

The mechanisms, by which PINK1 mutations lead to clinical manifestations of PD, are poorly known. Loss of PINK1 function leads to increased lipid peroxidation and decreased function of complex 1 of the respiratory chain (Exner et al., 2007), and PINK1 may be neuroprotective by decreasing cytochrome *c* release (Petit et al., 2005). A few targets for PINK1 have been identified: TNF receptor-associated protein 1, which protects against oxidative stress (Pridgeon et al., 2007), and Drp1, which promotes mitochondrial fission (Park et al., 2009; Poole et al., 2008). Interestingly, inactivation of PINK1 leads to dopaminergic cell death, muscle degeneration, photoreceptor loss, and male sterility in *Drosophila*, which can be rescued by overexpressing parkin suggesting a common pathway (Clark et al., 2006; Park et al., 2006; Yang et al., 2006). Loss of dopaminergic cells is not replicated in the vertebrate models of PINK1 deficiency (Kitada et al., 2007; Zhou et al., 2007).

We examined the functions of zebrafish Pink1 by inhibiting Pink1 translation by morpholino-modified oligonucleotides (MOs) and the role of Pink1 in 1-methyl-4-phenyl-1,2,3,6-tetrapyradine (MPTP)-induced toxicity in zebrafish. MPTP is used to produce experimental PD in several species (Beal, 2001), including adult and larval zebrafish (Anichtchik et al., 2004; Bretaud et al., 2004; Lam et al., 2005; McKinley et al., 2005; Sallinen et al., 2009b). 1-Methyl-4-phenylpyridinium (MPP+), the toxic metabolite of MPTP, inhibits the mitochondrial respiratory chain complex I leading to oxidative stress and cell death (Nicklas et al., 1987). Our results suggest that Pink1 plays an important role in the development of dopaminergic cell populations and that Pink1 deficiency exacerbates MPTP toxicity. Two non-allelic copies of tyrosine hydroxylase are found in zebrafish (Candy and Collet 2005). They are expressed in 17 (th1) and 4 (th2) clusters in zebrafish brain (Chen et al., 2009). Studies on zebrafish dopaminergic systems should examine both of these genes, as the available antibodies detect only th1 protein (Chen et al., 2009).

While this study was about to be completed, a related approach using zebrafish was published (Anichtchik et al., 2008). The results indicated a severe developmental phenotype following Pink1 translation inhibition.

* Corresponding author.
E-mail address: pertti.panula@helsinki.fi (P. Panula).
[1] Equal contribution.

0969-9961/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.nbd.2010.06.001

In our study, such findings were limited to MOs with strong off-target effects, which were not fully rescued by *pink1* mRNA.

Materials and methods

Fish maintenance

Zebrafish of the Turku line (Kaslin and Panula, 2001) were used and maintained as described earlier (Kaslin et al., 2004; Peitsaro et al., 2003). The permits for the experiments were obtained from the Office of the Regional Government of Southern Finland in agreement with the ethical guidelines of the European convention.

Semi-quantitative RT-PCR

Zebrafish total cDNA was synthesized using SuperScript cDNA synthesis kit (Invitrogen, Carlsbad, CA, USA) starting with 100 ng of cDNA reverse transcribed from the RNA extracted using Trizol (Invitrogen) system or by using RNeasy MinElute Cleanup kit (Qiagen, Hilden, Germany). Synthesized cDNA was amplified with zebrafish forward and reverse primers. Beta-actin expression was used as a control by amplifying cDNA with forward primer *T* and reverse primer (see Supplementary Table 1 for primers).

Quantitative RT-PCR

RNA samples were isolated from pooled embryos (30/sample) by using RNAeasy minikit (Qiagen). RNA was reverse transcribed to produce cDNA using superscript reverse transcriptase-II (Invitrogen) primed with oligo(dT)primers. The SmartCycler® instrumentation (Cepheid, Sunnyvale, CA, USA) with TaqMan® chemistry was used with the SYBR green mix (Takara, Madison, WI, USA) in a total volume of 25 µl. The composition of the reaction mixture was the following: SYBR green mix 12.5 µl, cDNA 1–3 µl, primers at a final concentration of 1 µM (see Supplementary Table 1 for primers). The data were calculated by the comparative method using Ct values of β-actin as the reference control.

Pink1, th1, th2, and dat in situ hybridization

A 1215-bp *pink1* cDNA fragment cloned to pGEM-T easy vector (Promega, Madison, WI, USA) was amplified, purified, and linearized with SalI and ApaI restriction enzymes for synthesizing sense and antisense probes using T7 and SP6 primes included in DIG RNA Labeling Mix (Roche, Mannheim, Germany). *th1* and *th2* probes were synthesized similarly, except Sal1 and Nco1 restriction enzymes were used. The *in situ* hybridizations were carried out as described earlier (Thisse and Thisse, 2008). Specific primers (Supplementary Table 1) were designed to amplify the zebrafish *dat* gene by RT-PCR. The PCR product was cloned into pGEM-Teasy vector and sequenced for synthesizing the cRNA probe for *in situ* hybridization.

Immunohistochemistry and image analysis

Immunohistochemistry was carried out as described earlier (Kaslin et al., 2004; Sallinen et al., 2009a). Rabbit anti-PINK1 (Cayman Chemicals, Ann Arbor, MI, USA, 1:1000), mouse anti-TH (Diasorin, Stillwater, MN, USA, 1:1000), and mouse anti-ZRF-1(Marcus and Easter, Jr. 1995, 1:500) antibodies were used. To visualize lateral line neuromasts, 5-dpf larvae were incubated in 25 nM Mitotracker Red (Invitrogen) for 15 min at RT, rinsed, fixed in 4% PFA, and incubated with anti-acetylated tubulin antiserum (Sigma, St. Louis, MO, USA) diluted 1:1000.

Immunofluorescence analysis and image processing were carried out as described earlier (Sallinen et al., 2009b). Anatomical structures were named and numbered according to Sallinen et al. (2009b).

Western analysis of PINK1 protein in zebrafish tissues using the same and other antisera did not give consistent results. Thus, it was not possible to use it to verify the effects of MO experiments. Apparently, the antibody detects a protein domain that does not remain intact in lysis buffer. Our conclusion is supported by a thorough analysis of several PINK1 antibodies, including the one used here (Zhou et al., 2008). This report suggests that none of the available antibodies detects endogenous PINK1 protein in human or mouse tissues or human cell lines in Western analysis even when the antigen is identical with PINK1 sequences from these species, supporting the concept that PINK1 protein is either sensitive to lysis, or that the antigenic determinant is not exposed when the protein unfolds. The antigen peptide used to produce the antiserum used in our study is from human PINK1. It has a 61% overall identity with the zebrafish sequence, and there is a 10-amino-acid sequence that is identical (Supplementary Fig. 1). We tested one other PINK1 antibody, which according to the antigen information might have been suitable. However, it produced no reliable staining under any conditions. None of the other available commercial antibodies was made against a relevant antigen for use in zebrafish.

Pink1 morpholino-oligonucleotide (MO) design and injections

Two MOs (Gene Tools, Philomath, OR, USA) were used. One targeted the splice site of exon 3 and intron 3 (MO1, TCACAACCTACCCGTTCAAAGTCAG) and the other targeted the 5′-UTR site (MO2, GAGAGGAAATCTGAAGGCTTTTACG). Control MO was synthesized by mispairing 5 nucleotides in the antisense strand (5mis-*pink1-MO*, GACAGCAAATCTCAAGGGTTTTAGG). However, this MO had non-specific effects at rather low doses, and it could not be used in final experiments. Thus, a standard control MO with no observed adverse effects (ctrlMO, 5′-CCT CTT ACC TCA GTT ACA ATT TAT A 3′) was used in all quantitative RT-PCR experiments.

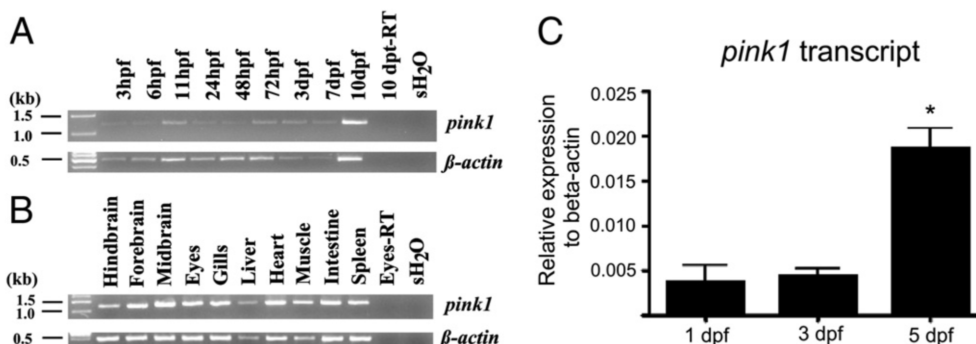


Fig. 1. (A) *pink1* expression during the early zebrafish development. *Pink1* mRNA expression is shown from early embryonic stage (3 hpf) to 10 days larval stage. Only minor differences in *pink1* mRNA expression during the early zebrafish development can be observed. (B) Expression pattern of the zebrafish *pink1*. *Pink1* is ubiquitously expressed. (C) qPCR shows increase in pink1 transcript over time. Note a significant increase at 5 dpf. *Beta-actin* was used as a control to assess equal RNA quantity (applies to A, B, and C).

Download English Version:

https://daneshyari.com/en/article/3069831

Download Persian Version:

https://daneshyari.com/article/3069831

Daneshyari.com