Int. J. Devl Neuroscience 34 (2014) 48–59

Contents lists available at ScienceDirect

International Journal of Developmental Neuroscience

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

Developmental co-expression and functional redundancy of tyrosine phosphatases with neurotrophin receptors in developing sensory neurons

Viktoria Tchetchelnitski[a], Monique van den Eijnden[b], Fanny Schmidt[b], Andrew W. Stoker[a,*]

[a] Institute of Child Health, University College London, 30 Guilford Street, London WC1N 1EH, United Kingdom
[b] MERCK SERONO SA.-Geneva, 9 Chemin des Mines, CH-1202 Geneve, Switzerland

ARTICLE INFO

Article history:
Received 18 October 2013
Received in revised form 22 January 2014
Accepted 22 January 2014

Keywords:
Protein tyrosine phosphatase
PTP
Neurotrophin
TRK signalling
Sensory neuron
DRG

ABSTRACT

Receptor-type protein tyrosine phosphatases (RPTPs) have been implicated as direct or indirect regulators of neurotrophin receptors (TRKs). It remains less clear if and how such RPTPs might regulate TRK proteins in vivo during development. Here we present a comparative expression profile of RPTP genes and Trk genes during early stages of murine, dorsal root ganglion maturation. We find little if any specific, temporal mRNA co-regulation between individual RPTP and Ntrk genes between E12.5 and E14.5. Moreover, a double fluorescent *in-situ* hybridization and immunofluorescence study of seven *Rptp* genes with *Ntrks* revealed widespread co-expression of RPTPs in individual neurons, but no tight correlation with Trk expression profiles. No *Rptp* is expressed in 100% of *Ntrk1*-expressing neurons, whereas at least 6 RPTPs are expressed in 100% of *Ntrk2*- and *Ntrk3*-expressing neurons. An exception is *Ptpro*, which showed very selective expression. Short hairpin RNA suppression of *Ptprf*, *Ptprs* or *Ptpro* in primary, E13.5 DRG neurons did not alter TRK signalling. We therefore propose that TRK signalling may not be simply dependent on rate-limiting regulation by individual RPTP subtypes during sensory neuron development. Instead, TRK signalling has the potential to be buffered by concurrent inputs from several RPTPs in individual neurons.

Crown Copyright © 2014 Published by Elsevier Ltd on behalf of ISDN. All rights reserved.

1. Introduction

Reversible protein phosphorylation plays a key role in cell signalling during neural development, with the neurotrophin receptor family of protein tyrosine kinases (PTKs), the TRKs, being central in controlling neuronal survival, axonogenesis and synaptic plasticity (Chao, 2003; Huang and Reichardt, 2003; Takahashi et al., 2011; Thoenen, 1995). TRK malfunction also underlies diseases such as neuropathies, degenerative disorders and cancers and so it is important that we understand both their positive and negative regulation.

Upon neurotrophin (NT) binding, Trks homodimerize and autophosphorylate conserved intracellular tyrosine (Tyr) residues (Segal and Greenberg, 1996). These represent docking sites for effectors that mediate signalling through phosphatidylinositol-3-kinase (PI3K)/Akt, phospholipase C-γ and the Ras/MAPK pathways (Kaplan and Miller, 2000; Ullrich and Schlessinger, 1990). TRK signalling can also be modulated by tyrosine dephosphorylation, through the actions of protein tyrosine phosphatases (PTPs). Over 100 PTP genes are known, with the classical cysteine-based PTPs categorized into 17 non-transmembrane (NPTPs) and 21 receptor-like PTPs (RPTPs) (Alonso et al., 2004). In the past decade it has become clear that many PTPs can modify PTK signalling, either negatively or positively and can have key roles in development and in disease (Julien et al., 2011; Stoker, 2005; Tonks, 2006).

Many RPTPs are expressed selectively and at high levels in the CNS and PNS during neural development. These enzymes have roles in neuronal survival, synaptic plasticity, axon guidance and nerve regeneration (Burden-Gulley and Brady-Kalnay, 1999; Chilton and Stoker, 2000; Ensslen-Craig and Brady-Kalnay, 2004; Johnson and Van Vactor, 2003; Sommer et al., 1997; Stepanek et al., 2001; Sun et al., 2000; Wang and Bixby, 1999). Significantly, the mRNA expression of RPTPs strongly overlaps with Trk gene expression in neural tissues and both protein types are found in neurites and growth cones. Evidence for the regulation of TRKs by PTPs comes from several sources. PTP inhibitors can activate TRK signalling and prevent

Abbreviations: PTP, protein tyrosine phosphatase; RPTP, receptor-like protein tyrosine phosphatase; nrPTP, Non-receptor protein tyrosine phosphatase; DRG, dorsal root ganglion; shRNA, short hairpin RNA; PTK, protein tyrosine kinase; NT, neurotrophin.

* Corresponding author. Tel.: +44 207 905 2244; fax: +44 207 831 4366.
E-mail address: a.stoker@ucl.ac.uk (A.W. Stoker).

0736-5748/$36.00 Crown Copyright © 2014 Published by Elsevier Ltd on behalf of ISDN. All rights reserved.
http://dx.doi.org/10.1016/j.ijdevneu.2014.01.005

cell death in hippocampal neurons (Gerling et al., 2004), suggesting that PTPs can hold TRK signalling in check. Indeed a number of RPTPs directly or indirectly interact with TRK proteins in vitro and can alter their signalling properties in cultured cells. Downregulation of LAR signalling augments NGF-induced neurite outgrowth and activation of TrkA in PC12 cells (Tisi et al., 2000; Xie et al., 2006). LAR regulates TrkB signalling in hippocampal neurons, possibly indirectly through activation of pp60Src, which then activates TrkB (Yang et al., 2006, 2005). RPTPσ, a close relative of LAR, is expressed widely and dephosphorylates all three TRKs in cultured cells (Faux et al., 2007). Over-expression of RPTPσ in chick sensory neurons also suppresses NGF-dependent neurite outgrowth, without affecting cell survival (Faux et al., 2007). Interestingly, RPTPσ-deficient mice suffer from defects in proprioception, supporting the hypothesis of a possible interaction of RPTPσ with TrkC (Batt et al., 2002; Elchebly et al., 1999; Meathrel et al., 2002; Wallace et al., 1999). RPTP-BK shows very specific expression in subgroups of neurons in DRGs and has been suggested to control the differentiation and axonogenesis of NT-3 and NGF-dependent neurons (Beltran et al., 2003). RPTP-BK-deficient mice show nociceptive sensory neurons deficits and abnormalities in axonal guidance from proprioceptors and nociceptors within the spinal cord (Gonzalez-Brito and Bixby, 2009). Moreover, it has been shown that RPTPζ and RPTPγ have a differential ability to affect TRK signalling and NGF-dependent neurite outgrowth in PC12 cells (Shintani et al., 2001). Whereas RPTPγ-deficient mice do not show any abnormalities in NGF-induced neurite outgrowth, RPTPζ dephosphorylates Tyr residues in the activation loop of TrkA (Shintani and Noda, 2008) and there is elevated TrkA-phosphorylation in RPTPζ-deficient mice, with subtle nociceptive deficits (Lafont et al., 2009). RPTP-BR7 interacts with TrkA and dephosphorylates it, possible affecting its maturation (Noordman et al., personal communication). Lastly, non-receptor PTPs such as SHP1 can also control TRK activity (Marsh et al., 2003). There is therefore compelling evidence for regulatory interactions between PTPs and TRKs from cell culture studies. However, although these studies suggest specific interactions of particular PTPs with TRKs, it is not clear whether such specificity occurs or is required *in vivo* in developing neurons. This is exemplified by the fact that major perturbations in TRK activities, as judged by changes in neuronal numbers for example, either do not occur or are very subtle in mice deficient for single RPTP genes. It is not clear if this is due to functional redundancy in RPTPs. To begin to address this, we define for the first time the precise dynamics of RPTP and Trk gene expression in dorsal root ganglia (DRGs), during a critical period of neuronal development in vivo. DRGs contain populations of at least 20 different subclasses of neurons that depend on various combinations of TRK for their survival and differentiation (Buchman and Davies, 1993; Liebl et al., 1997). We analyzed E12.5 to E14.5 in the mouse, a window encompassing key periods of neurogenesis for TrkA nociceptive, thermoceptive and pruriceptive neurons, following from earlier production of proprioceptive TrkC+ neurons and mechanoreceptive TrkB+ neurons (Huang and Reichardt, 2003; Kramer et al., 2006; Marmigere and Ernfors, 2007; Phillips and Armanini, 1996). We studied co-expression of PTP and TRK genes using both a QPCR analysis and co-*in situ* analysis. We also experimentally suppressed the expression of *Pptrf, Ptprs* and *Ptpro* in cultured E13.5 DRG neurons and examined biochemical responses downstream of TRK signalling.

Our data indicate extensive, overlapping expression of many PTPs in DRGs, with individual RPTP genes extensively overlapping with Trk gene expression. However, there is little evidence of tight, developmental co-regulation of individual RPTPs and TRKs, although Ptpro is the most selectively co-expressed with TrkC and TrkB at E13.5. Lack of alteration in TRK signalling after individual RPTP suppression suggests that TRK proteins are likely buffered in their regulation by the actions of multiple, co-expressed RPTPs.

2. Materials and methods

2.1. RNA preparation

DRGs from E12.5, E13.5 and E14.6 CD-1 mouse embryos (48–65 DRG per embryo) were dissected and RNA extracted using the RNeasy® Lipid Tissue Kit (Qiagen) following manufacturer's instructions. RNA purity (260/280 ratio) and concentration were measured using a NanoDrop ND-1000 Spectrophotometer and integrity was tested with an Agilent Bioanalyzer and the RNA 6000 Nano LabChip Kit. 1 μg of mRNA was reverse transcribed into cDNA using the iScript™cDNA Synthesis Kit (BioRad) following manufacturer's instructions.

2.2. Quantitative RT real-time PCR (qPCR) screen

qPCR was performed in technical duplicates with biological triplicates in a 384-well-plate format. Each reaction contained 1.25 ng of cDNA, 2.5 μl primer pair and 5 μl of 2× QuantiTect® SYBR Green PCR Master mix (Qiagen) including HotStart Taq DNA polymerase, QuantiTect® SYBR Green PCR buffer, dNTP mix, SYBR Green I, ROX passive reference dye and RNase-free H_2O. A LightCycler (ABI 7900HT Fast Real-Time PCR System) was used as follows: 95 °C for 5 min; 40× (10 s at 95 °C, 30 s at 60 °C). Primer specificities were verified by dissociation curve analysis. The primers detected 94 PTP genes, *Ntrk1, Ntrk2* and *Ntrk3* and three housekeeping genes (HKGs): *Psmb2, Hprt1* and *Gps1* (primer sequences are available upon request). For relative global gene expression analysis, $2^{-\Delta CT}$ values were calculated with $\Delta Ct = Ct$ (sample) – Mean Ct (average of three HKGs (*Psmb2, Hrpt1* and *Gps1*)). NormFinder, a publicly available Microsoft® Excel® Visual Basic Application, was used to identify the optimal reference genes (*Psmb2, Hrpt1* and *Gps1*) (Andersen et al., 2004; Schmittgen and Livak, 2008). The results were displayed as percentage of expression compared to the set of HKGs ($\% 2^{-\Delta CT}$). For comparison and evaluation of global gene expression, the mean of all three stages was calculated. Statistical analysis was performed with Prism 4 (GraphPad Software) using one-way analysis of variance (ANOVA) with the Tukey's Multiple Comparison post-test. Data were categorized according to their p values as non-significant (ns) with $p > 0.05$, significant (*) with $0.01 < p < 0.05$, very significant (**) with $0.001 < p < 0.01$ and extremely significant (***) with $p < 0.001$.

2.3. Single and double in-situ hybridization and immunofluorescence

Single chromogenic and fluorescent ISH was performed on embryo sections. Embryos were fixed in 4%-paraformaldehyde/PBS, cryo-protected, embedded either in Cryo-M-bed O.C.T. (Bright Instrument Co.) or gelatine and sectioned at 11 μm. RNA probes were synthesized according to manufacturer's instructions using DIG- or FITC labelled kits (Roche). Probes were denatured in pre-warmed (65 °C) hybridization buffer (0.2 M NaCl, 5 mM EDTA, 10 mM Tris-HCl pH 7.5, 5 mM $NaH_2PO_4{\cdot}2H_2O$, 5 mM Na_2HPO_4, 50% deionized formamide, 0.1 mg/ml yeast tRNA, 10% dextran sulphate, 1× Denhardt's solution). The slides were incubated with the probe at 65 °C overnight, and then washed in MABT (100 mM maleic acid, 150 mM NaCl, 0.1% Tween-20, pH 7.5), then stringent 65 °C washes for 30 min (0.3 M sodium citrate, 3 M NaCl, 50% formamide, 0.1% Tween-20). Slides were blocked (2% blocking reagent (Roche), 10% heat inactivated sheep serum, MABT) for 1 h, before binding with anti-DIG or anti-FITC antibody (Roche) overnight at 4 °C. Alkaline phosphatase-conjugated-anti-DIG/FITC Fab fragments (Roche) and horseradish peroxidase-conjugated anti-DIG/FITC Fab fragments (Roche) were used for chromogenic or fluorescent detection, respectively. Slides were washed with MABT or PBS-T (PBS with 0.1% Triton X-100) for chromogenic or fluorescent detection, respectively. For chromogenic detection, the slides were equilibrated in developing buffer (100 mM Tris pH 9.8, 100 mM NaCl, 50 mM $MgCl_2$) and incubated with NBT and BCIP (Roche) in developing buffer containing 5% polyvenylalcohol for 1 hour to overnight. For fluorescent detection the Tyramide Signal Amplification plus fluorescent system kit (PerkinElmer Life Sciences) was used following manufacturer's instructions. For double fluorescent hybridization, DIG- and FITC-labelled RNA probes were hybridized together to the specimen. After the first colour reaction the reporter enzyme was deactivated for 30 min in 3% H_2O_2-PBS, before the second colour reaction. Slides were mounted in VectaMount™ or Vectashield® containing DAPI (Vector Laboratories) for chromogenic or fluorescent slides, respectively. Polyclonal TrkA-antibody (Upstate # 06-574), polyclonal TrkB-antibody (R&D; AF1494), polyclonal TrkC-antibody (R&D; AF1404), anti-goat Alexa Fluor 488 (Invitrogen; A-11055) and FluoroLink™Cy™3-labelled streptavidin (Amersham Biosciences; PA43001) were used. Pictures were recorded with a Zeiss Axiophot and Zeiss Imager.Z1 ApoTome. For standard immunofluorescence studies, slides were first blocked with 1% BSA (Fraction V, Sigma Aldrich)/PBS/0.05% Triton X-100 for 15 min. Primary and biotinylated-secondary antibodies were added in the same buffer for 30–60 min each, followed by streptavidin-conjugated fluorophores. Manual cell counting was performed on digital images, focusing on neurons identified by their large, round nuclei (DAPI-stained). Between 213 and 1510 neurons on 2–13 different pictures of randomly selected DRG regions from several embryos were counted for the Trk/Trk combinations and the RPTP/Trk combinations (Numbers are shown in Supplementary Table 1). The percentage of cells unambiguously positive for RPTP and/or Trk receptors was determined and SDs calculated.

Download English Version:

https://daneshyari.com/en/article/2785942

Download Persian Version:

https://daneshyari.com/article/2785942

Daneshyari.com