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Multimerisation of receptor-type protein tyrosine phosphatases PTPBR7 and PTP-SL attenuates enzymatic activity

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Abstract

Dimerisation of receptor-type protein tyrosine phosphatases (RPTPs) represents an appealing mechanism to regulate their enzymatic activity. Studies thus far mostly concern the dimerisation behaviour of RPTPs possessing two tandemly oriented catalytic PTP domains. Mouse gene *Ptprr* encodes four different protein isoforms (i.e. PTPBR7, PTP-SL and PTPPBSγ-42/37) that contain a single PTP domain. Using selective membrane permeabilisation we here demonstrate that PTP-SL, like PTPBR7, is a single membrane-spanning RPTP. Furthermore, these two receptor-type PTPs constitutively formed homo- and hetero-meric complexes as witnessed in chemical cross-linking and co-immunoprecipitation experiments, in sharp contrast to the cytosolic PTPPBSγ-42 and PTPPBSγ-37 PTPRR isoforms. This multimerisation occurs independently of the PTP domain and requires the transmembrane domain and/or the proximal hydrophobic region. Using overexpression of a PTPBR7 mutant that essentially lacks the intracellular PTP domain-containing segment, a monomer-mimicking state was forced upon full-length PTPBR7 immunoprecipitates. This resulted in a significant increase in the enzymatic activity of the PTPRR PTP domain, which strengthens the notion that multimerisation represents a general mechanism to tone down RPTP catalytic activity.
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Keywords: Dimerisation; Hydrophobic region; Phosphatase activity; Protein topology; PTPRR; DifMUP

1. Introduction

Signal transduction via receptor tyrosine kinases (RTKs) emanates from the binding of a ligand to the RTK extracellular domain, which induces subsequently receptor dimerisation, auto-activation via phosphorylation of intracellular tyrosine residues, and recruitment and activation of downstream signalling proteins [1]. In contrast, for most receptor-type protein tyrosine phosphatases (RPTPs) the mechanisms controlling their enzymatic activity remain to be disclosed [2]. Potential reversible mechanisms include PTP inactivation by reactive oxygen species, through oxidation of the catalytic site cysteine [3], and phosphorylation itself, as demonstrated by the increased activity of PTP1B following its tyrosine-phosphorylation by the insulin receptor [4]. Intracellular cleavage of RPTPs that results in the release of the catalytic domain in the cytoplasm, on the other hand, represents an irreversible way to abrogate dephosphorylation of membrane-associated substrates [5], and perhaps the extracellular proteolytic cleavage or ectodomain 'shedding' that is observed for multiple RPTPs should be added to this list [6–8].

The discovery of receptor-type PTPs led to the suggestion that, in analogy with RTKs, these RPTPs may be regulated via ligand-induced multimerisation [2]. Unfortunately, potential ligand proteins have only been identified for a limited number of RPTPs, and thus far ligand-dependent dimerisation and

Abbreviations: DifMUP, difluoromethylumbelliferyl phosphate; EGF, epidermal growth factor; ERK, extracellular signal-regulated kinase; HR, hydrophobic region; KIM, kinase-interacting motif; MAPK, mitogen-activated protein kinase; PEI, polyethylene-imine; RPTP, receptor-type protein tyrosine phosphatase; RTK, receptor tyrosine kinase; TM, transmembrane

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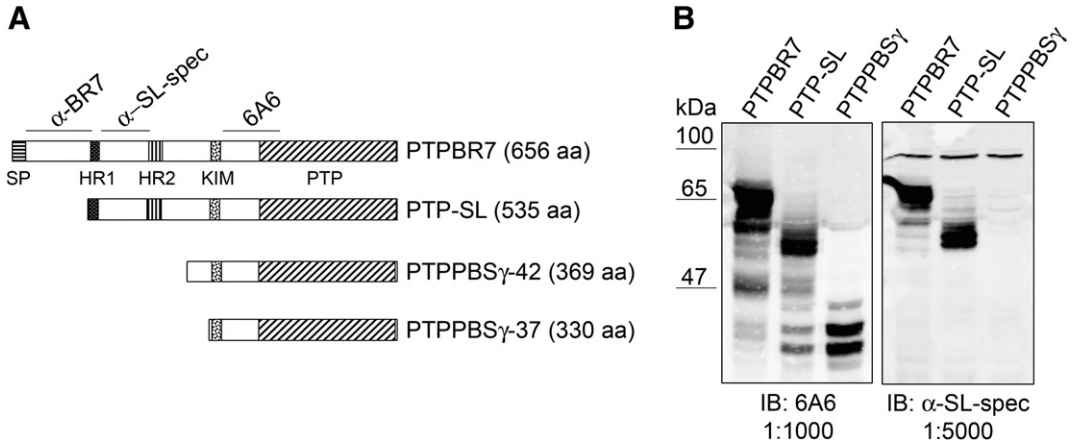

Fig. 1. Characterisation of the α-SL-specific antiserum. A) Schematic representation of the different mouse PTPRR protein isoforms. Signal peptide (SP), hydrophobic regions (HR1, HR2), kinase-interacting motif (KIM), catalytic phosphotyrosine phosphatase domain (PTP) and the segments recognised by the α-BR7 and α-SL-specific antisera and the monoclonal antibody 6A6 are indicated. HR2 functions as a 'stop-transfer signal' in the receptor-type PTPBR7 isoform. Whether HR2 also is a transmembrane-spanning region in PTP-SL has been addressed in the current study. B) Total lysates of HeLa cells transiently transfected with expression plasmids pSG5/PTPBR7, pSG5/PTP-SL or pSG5/PTPPBSγ were analysed on Western blot using monoclonal antibody 6A6 (left panel) and rabbit polyclonal α-PTP-SL-specific antiserum (right panel). Rabbit polyclonal α-BR7 antiserum only detected the PTPBR7 isoform (data not shown). Please note that the PTPPBSγ mRNA codes for both PTPPBSγ-42 and PTPPBSγ-37 by virtue of alternative translation start codons [21]. Molecular size markers are indicated on the left.

consequent inactivation has only been demonstrated for RPTPζ [9,10]. Nevertheless, for multiple RPTPs, including CD45, RPTPα and DEP-1, the artificial induction of dimerisation clearly affected their enzymatic activity [11–13]. Intriguingly, for quite a number of RPTPs it was found that they constitutively form homo- and heterodimers in cells, presumably in the absence of ligands [14,15]. Current knowledge about the regulation of RPTP activity by dimerisation mostly comes from studies on RPTPs that contain two tandemly oriented phosphatase domains; a catalytic, membrane-proximal domain (D1) and a regulatory, membrane-distal domain (D2) [16]. Interactions between these various PTP domains appeared key determinants in the outcome regarding activity [15,17,18]. A minority of RPTPs contains, like all non-transmembrane PTPs, only a single phosphatase domain that is either active (DEP-1, Glepp1, PTPBR7, PTPOST, RPTPβ, Sap-1, STEP61) or inactive (IA2 and IA2β) [19]. Sap-1 was found to be able to form dimers, but this is independent of its single catalytic PTP domain and rather occurs through extracellular disulfide bond formation. Intriguingly, this multimerisation led to a net decrease in activity [20].

PTPBR7 is one of the RPTPs with a single catalytic domain but, in contrast to Sap-1, it does not contain extracellular cysteines following cleavage of its signal peptide. In the current study we investigated whether mouse PTPBR7 and its family members PTP-SL, PTPPBSγ-42 and PTPPBSγ-37 also may be regulated via multimerisation. These four PTPRR isoforms are all encoded by a single-copy gene, *Ptprr*, but differ in their amino-terminal sequence and, as a consequence, subcellular localisation [21]. PTPBR7 is mainly localised on the plasma membrane, PTPPBSγ-42 and PTPPBSγ-37 are in the cytosol, and PTP-SL is distributed at vesicular structures and the Golgi apparatus. All PTPRR isoforms can specifically bind to the extracellular signal-regulated kinases 1 and 2 (ERK1/2) via a so-called kinase-interacting motif (KIM), and in vitro [22] and in vivo [23] studies established tyrosine-phosphorylated ERK1/2 as bona fide PTPRR substrates. We now demonstrate that PTP-SL, like PTPBR7, is a receptor-type PTP. PTPBR7 and PTP-SL, as opposed to the cytosolic PTPPBSγ isoforms, were found to form homo- and heterodimers constitutively. The multimerisation attenuated PTPRR phosphatase activity, in favour of the model in which RPTP dimerisation negatively regulates PTP signalling.

2. Materials and methods

2.1. Expression constructs

Plasmids pSG5/PTPBR7, pSG8/PTPBR7-VSV, pSG8/PTP-SL-VSV [24], pMyc-ERK2 [25] and pBICD1-HA [26] have been described elsewhere. Plasmid pSG8/PTPBR7-C/S-VSV encoding a catalytically inactive Cys-to-Ser mutant was generated by site-directed mutagenesis of Cys-578 (numbering corresponds to amino acid position in PTPBR7; Acc. No. BAA06696) using the QuickChange protocol (Stratagene, La Jolla, USA). Cloning of a *Sfi*I–*Age*I fragment from pSG5/PTPPBSγ [21] into *Sfi*I–*Age*I digested pSG8/PTPBR7-VSV generated the plasmid pSG8/PTPPBSγ-VSV. In these pSG8-based constructs, a unique *Bgl*II restriction site in fact separates the PTPRR isoform

Fig. 2. PTP-SL is a transmembrane protein. A) HeLa cells selectively permeabilised with digitonin or Triton X-100 were stained with α-PDI antiserum to detect endogenous ER lumenal protein disulphide isomerase (left panels) or with α-Lamp-1 antiserum to detect a cytosolic epitope in lysosome-associated membrane protein-1 (right panels). B) HeLa cells expressing PTPBR7-VSV were permeabilised with the indicated detergents and subsequently co-immunostained with mouse monoclonal α-VSV antibody and rabbit α-BR7 antiserum. Anti-VSV staining always resulted in fluorescent signals at the plasma membrane, vesicular structures and the Golgi apparatus (left panels), as for the α-BR7 antiserum on Triton X-100 permeabilised cells (lower middle panel). In digitonin-treated cells α-BR7 reactivity was only present on the plasma membrane (upper middle panel), confirming that PTPBR7 is a type-I transmembrane protein [24]. C) HeLa cells expressing PTP-SL-VSV were treated with the indicated detergents and then co-immunostained with monoclonal α-VSV antibody and rabbit α-PTP-SL-specific antiserum. For both permeabilisation conditions α-VSV staining revealed Golgi and vesicular structures (left panels), while the α-PTP-SL-specific signal as observed in Triton X-100 treated cells (lower middle panel) is reduced to background levels (as observed in untransfected cells; arrowhead) in digitonin-treated cells.

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