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Proteolytic processing of the protein tyrosine phosphatase α extracellular domain is mediated by ADAM17/TACE

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ABSTRACT

The receptor protein tyrosine phosphatase alpha (PTPα) is involved in the regulation of tyrosine kinases like the Src kinase and the insulin receptor. As with other PTPs, its function is determined by alternative splicing, dimerisation, phosphorylation and proteolytical processing. PTPα is cleaved by calpain in its intracellular domain, which decreases its potential to dephosphorylate Src kinase. Here, we demonstrate that PTPα is also processed in the extracellular domain. Extracellular processing was exclusively found for a splice variant containing an extra nine amino acid insert three residues amino-terminal from the transmembrane domain. Processing was sensitive to the metalloprotease-inhibitor Batimastat, and CHO-M2 cells lacking a disintegrin and metalloproteinase 17 (ADAM17; tumor-necrosis-factor α converting enzyme) activity were not able to cleave PTPα. After transient overexpression of ADAM17 and PTPα in these cells, processing was restored, proving that ADAM17 is involved in this process. Further characterization of the consequences of processing revealed that dephosphorylation of the insulin receptor or activation of Src was not affected but focus formation was reduced. We conclude that extracellular proteolytic processing is a novel mechanism for PTPα regulation.

Introduction

The currently known 37 human protein tyrosine phosphatases (PTPs) are involved in the regulation of diverse physiological processes like growth, differentiation or metabolism. Structurally, they can be grouped into transmembrane, receptor-like and non-transmembrane forms. The receptor phosphatases (R-PTPs) have either one or two intracellular phosphatase domains, with the membrane distal domain typically being catalytically inactive (Andersen et al., 2001; Tonks, 2006). PTPα is a receptor phosphatase with two intracellular phosphatase domains (Kaplan et al., 1990; Krueger et al., 1990; Matthews et al., 1990), however, its membrane distal domain has some residual phosphatase activity (Buist et al., 1999). Substrates of PTPα include Src family kinases (Bhandari et al., 1998; Harder et al., 1998; Zheng et al., 1992), the insulin receptor (Møller et al., 1995), the potassium channels Kv1.1, Kv1.2, and Kv2.1 (Gil-Henn et al., 2001; Imbrici et al., 2000; Tsai et al., 1999) as well as the focal adhesion protein p130cas (Buist et al., 2000b). Amongst those substrates, Src family kinases are most prominent and still highly investigated. Mice lacking PTPα have an impaired Src activity (Ponniah et al., 1999; Su et al., 1999) whereas overexpression of PTPα leads to activation of the Src kinase by dephosphorylation of its carboxyl-terminal tyrosine residue and eventually to cell transformation or differentiation (den Hertog et al., 1993; Zheng et al., 1992). More recently, PTPα was shown to regulate integrin-stimulated focal adhesion kinase (FAK) autophosphorylation (Chen et al., 2006; Zeng et al., 2003), the lipid raft protein Cbp/PAG (Maksumova et al., 2005; Vacaresse et al., 2008), and paxillin (Vacaresse et al., 2008) in a Src family kinase dependent manner. In addition, PTPα together with Src is involved in interleukin-1 induced calcium-signaling (Wang et al., 2009).

The extracellular domains of PTPα and the related PTPε are short and heavily glycosylated, which is in contrast to other R-PTPs that have large extracellular domains with a variety of motifs including immunoglobulin or fibronectin type III modules (Tonks, 2006). However, the role of these cell adhesion features is only poorly understood. For many R-PTPs, structural diversity of the extracellular domain is created by alternative splicing. Splice variants of CD45 (Thomas, 1989), LAR (O'Grady et al., 1994), PTPδ (Pulido et al., 1995) and PTPσ (Yan et al., 1993) are expressed in a tissue specific way, but functional differences are still a matter of debate (for CD45, see Tchilian and Beverley, 2006). In addition to a splice variant of PTPα that generates a stop codon in the membrane proximal

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phosphatase domain, two isoforms varying in the extracellular domain were reported (Kaplan et al., 1990; Krueger et al., 1990; Matthews et al., 1990). The proteins differ in a stretch of nine amino acids located in the external membrane proximal stalk region, and are referred to as PTPα132 and PTPα123, respectively. The functional consequence of this structural diversity is a reduced Src activation of the shorter form (PTPα123), as determined in a focus formation assay using Src overexpressing cells and a PTPα containing a mutated carboxyl-terminal tyrosine residue (Kapp et al., 2007). In a different experimental setting, only the non-mutated short form generated foci upon overexpression (Tremper-Wells et al., 2010). Thus, the role of the extracellular domain of PTPα for its efficiency to activate Src is not yet clear.

In addition to generating variance by alternative splicing, several R-PTPs are proteolytically processed. The PTPs LAR, σ, δ, μ and κ occur as two non-covalently linked fragments, which result from the cleavage by a furin-like protease (Aicher et al., 1997, and references therein). Subsequently, some of these PTPs can be further processed by other proteases like ADAM (a disintegrin and metalloproteinase) 10 or 17 and γ-secretase (Anders et al., 2006; Ruhe et al., 2006). IA-2 and IA-2β known as major autoantigens in diabetes mellitus type 1, are also extracellularly cleaved by a furin-like protease (Seidah and Chretien, 1997). More recently, PTPBR7 (Dilaver et al., 2007), and CD45 (Kirchberger et al., 2008) were reported to be processed, and PTPζ is cleaved by plasmin and/or a combination of a metalloproteinase/γ-secretase (Chow et al., 2008a, b). Finally, PTPα and PTPε are cleaved by calpain in the cytoplasmic part. Upon cleavage, the activity to dephosphorylate the membrane associated Src kinase or the potassium channel Kv2.1 is lost or severely impaired, because of translocation of PTPα from the membrane to the cytoplasm (Gil-Henn et al., 2001).

Proteases of the ADAM family are activated by inhibitors of PTPs like peroxovanadate (POV) and phenylarsine oxide (PAO), and by activators of protein kinase C (PKC) like phorbol esters (Merlos-Suarez and Arribas, 1999; Vecchi et al., 1998). We have previously reported about a vanadate induced proteolytic processing of PTPα (Lammers et al., 2000). Here, we have further characterized this processing and found that only the splice variant containing the additional nine amino-acid insert (PTPα132) was cleaved. The ADAM family member ADAM17/TACE (TNF-α converting enzyme) was identified as the responsible protease. Processing of PTPα132 had no effect on regulation of insulin signaling but reduced the potential to activate the Src kinase.

Materials and methods

cDNA constructs and site-directed mutagenesis

cDNAs of the two PTPα splice variants containing (PTPα132) or lacking (PTPα123) a nine amino-acid insert in the extracellular membrane proximal stalk region were cloned into the cytomegalovirus immediate early promoter based expression vector pRK5. Site directed mutagenesis of PTPα132, mutating serines 189 and 213 to alanine, was performed by the single-stranded DNA method of Kunkel et al. (1987) using the oligonucleotides S189A: 5′ GGATAAGCGGAAAGCATTGGAATGGCTCCC and S213A: 5′ TTTCCTGTTGGTGGCTGGGGATCTGGCCAG. The double mutant was generated using standard cloning procedures, and mutations were confirmed by sequence analysis. ADAM17 was cloned from a murine cDNA-library using the primers ADAM17-132: 5′ GCACCTGCTAAGTTGCTTCC and ADAM17-2759: 5′ AGGTTTCCCAGAGAGGTGGT. Mutation of the carboxy-terminal tyrosine or the catalytic cysteine residue has been described earlier (Lammers et al., 1998). The cDNA encoding PTPαΔNT was generated by PCR using the oligonucleotide 5′-XbaI site – haemagglutinin-tag – GGTATTCTGACTCGAAG and cloned into the pRK5 vector. This yields a protein starting with the antibody tag followed by the 9 amino-acids insert of the PTPα132 splice variant in the extracellular domain. Because of the lack of a signal peptide, this protein is located intracellularly. The construct PTPαintra was generated by PCR using the oligonucleotide 5′-BglII-site – GCCATGGCAAGGTTTAAGAAATACAAGCA and cloned into the expression vector pRK5. This generates a PTPα protein starting with the juxtamembrane sequence (M-A-) RFKKY-(PTPα).

Antibodies and cell lines

Mouse monoclonal antibodies 29 and 45 are directed against the first phosphatase domain of PTPα (kind gift of N.P.H. Møller, Bagsværd, Denmark). ADAM17 antibodies were from Santa Cruz (sc-6416). Horseradish peroxidase-coupled secondary antibodies for use in Western blot experiments were from Sigma. BHK, CHO-T, 293, BOSC23 and GP+E cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and 2 mM glutamine. Prior to stimulation with the indicated substances, the cells were made quiescent by changing the serum content from 10% to 0.5% for 18–24 h.

Transient expression experiments and protein analysis

Transient expression was performed employing the method of Chen and Okayama (1987). Eighteen hours after the addition of DNA precipitates, cells were washed once and supplied with fresh medium containing 0.5% fetal calf serum. The next day, cells were lysed in 200 μl of lysis buffer/6-well-dish (50 mM HEPES, pH 7.5; 150 mM NaCl; 1.5 mM $MgCl_2$; 1 mM EGTA; 10% (v/v) glycerol; 1% (v/v) Triton X-100; 100 mM NaF; 10 mM $Na_4P_2O_7$; 1 mM Na_3VO_4; 1 mM phenylmethylsulfonyl fluoride; 10 μg/ml aprotinin), and the lysates pre-cleared by centrifugation at 13,000 × *g* for 4 min at 4 °C. After addition of sample buffer, proteins were boiled for 5 min, size separated by SDS-PAGE, transferred to nitrocellulose filters and analyzed by Western blotting. Bound antibodies were visualized using the ECL system (Amersham Pharmacia Biotech). Alternatively, antibody (1.5–2 μg) and Protein A–sepharose were added to the lysate supernatant, incubated for at least 4 h at 4 °C on a turning wheel, the sepharose beads collected, washed with HNTG (20 mM HEPES, pH 7.5; 150 mM NaCl; 0.1% Triton X-100; 10% glycerol; 10 mM NaF; 1 mM sodium orthovanadate) and subjected to immunoanalysis as described above.

In vitro phosphatase activity assay

Lysates from transiently transfected 293 cells were used to dephosphorylate *para*-nitrophenylphosphate as described (Kapp et al., 2007). The phosphatase activity was corrected for the amount of PTPα present in the lysates.

Focus formation assays

BOSC23 cells were transfected with pLXSN plasmids containing the PTPα or ADAM17 cDNAs and the supernatants used to infect GP+E cells. After geneticin selection, cell pools were expanded, supernatants harvested and filter sterilized. To determine the titer, NIH3T3 cells were infected and selected for G-418 resistance. Cell colonies were stained with crystal violet and counted. For focus formation assays, 75,000 NIH3T3 cells overexpressing a moderate amount of c-Src were seeded into a six-well dish and 16 h later infected for 6–7 h with equal amounts of retroviruses (5×10^5–1×10^6) in the presence of 6 μg/ml polybrene. Infection volume was 1.5 ml, equalized with medium and retroviruses without donor DNA. 48 h later, cells were trypsinized and seeded into a