THE MOLECULAR AND CELLULAR CHARACTERIZATION OF A NON-RECEPTOR PROTEIN TYROSINE PHOSPHATASE

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Tyrosine phosphorylation of proteins is a physiologically important post-translational modification catalyzed by tyrosine kinases. The reverse process, dephosphorylation, is catalyzed by protein tyrosine phosphatases (PTPs). Adding or removing a phosphate group from a protein can generate a recognition motif for protein-protein interaction or for protein stability control. From the total percent of phosphorylated proteins (30%) in a cell, only a small percent (0.01–0.05%) are tyrosine phosphorylated (1). This review describes PTPD1, a non-receptor PTP present in normal and cancer cells, involved in cell growth and motility. PTPD1 interacts with Src tyrosine kinase, actin, FAK (focal adhesion kinase), AKAP121 (A-kinase anchor protein 121), BMX/Etk (bone marrow kinase), KIF16B (endosomal Kinesin-like protein 16B) and KIF1C (Kinesin-like protein 1C). All these interactions are demonstrated to involve the FERM domain or the other binding regions in the PTPD1 structure, except the catalytic domain.

Key words: protein tyrosine phosphatase, signal transduction, cytoskeleton, focal adhesions, cancer.

INTRODUCTION

The human genome sequencing revealed more than 100 genes that encode for PTPs. These enzymes are classified into four classes (2, 3) by similarity of their catalytic domains. Three of them have cysteine as catalytic active site (type I, II and III) and the fourth one has aspartate instead of cysteine (type IV). The first class of PTPs contains type I Cys-based PTPs, subdivided into "classical" (dephosphorylates only tyrosine residues) and dual specificity phosphatases (dephosphorylates both tyrosine and serine/threonine residues). The second class of PTPs is type-II cysteine-based and contains low-molecular-mass PTP (LMWPTP). These PTPs are tyrosine-specific and are involved in some cellular events, such as

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cytoskeleton rearrangement, cell growth and modulation of immune response (4) (5). The third class of PTPs contains phosphatases with dual specificity (dephosphorylates tyrosine and threonine) (Cdc25 - like). The fourth class contains tyrosine specific phosphatases (Eya – eyes absent), that are part of the haloacid dehalogenases (HAD) family.

There are 17 types of "classical" PTPs, 9 non-transmembrane and 8 types of receptor-like PTPs. The non-receptor types are known as cytoplasmic PTPs and are named NT1-NT9. The other group, the receptor-like PTPs, is named R1 to R8 and is sub-classified into two subgroups, the first one containing two tandem catalytical domains (R1/6, R2A, R2B, R4, R5) and the second one having only one catalytical domain (R3, R7, R8). The "classical" PTPs contains in the cytoplasmic region some regulatory sequences that perform regulating or targeting function directing these PTPs to different intracellular locations or are important in substrate, phospholipids or ligands binding; for example, the SH2 (Src homology 2) domain is important in phosphotyrosine binding, the SH3 (Src homology 3) domain for binding with proline rich motifs, the FERM (4.1 band/Ezrin/Radixin/Moesin) domain for anchoring to the cytoskeleton or plasma membrane, the PDZ (post-synaptic density-95/ disc large/ zonula ocludens -1) domain for complex receptor organization and lipid recognition, the KIM (kinase interaction motif) domain is important in the interaction with MAPK (mitogen activated protein kinase), the KIND (kinase non-catalytic C-lobe domain) domain has a role in anchoring and supporting PTP-BAS.

The crystal structure of many PTPs is available now and a lot of structural information arises after a closer look over the pdb data. PTP1B is the most studied PTP and most of the data are discussed with its structure as "reference structure". A secondary phosphotyrosine-binding site has been found in the PTP1B structure; this site cannot be predicted by sequence comparisons because it depends on the conformation of the loop connecting two alpha helixes (α2' and α1). The secondary substrate-binding pocket, also named "secondary site", can be accessed from the main active site through a duct named "the gateway region" (6-8). The great variety of structural conformations of the second-site loop made possible a classification of PTPs into five categories (9):

1) "PTP1B-like" available pocket containing a basic residue corresponding to Arg24 from PTP1B
2) PTPs with both the gateway and the second-site loop opened and available
3) PTPs in which the gateway region contains bulky residues that block access to an open secondary site, with an Asp residue instead of Arg24 from PTP1B.
4) PTPs with both the gateway and secondary site blocked and the inaccessible binding cavity with an aromatic residue or a proline in position of Arg24; the second-site loop has a twisted conformation
5) PTPs with the gateway open and available while the secondary pocket is blocked by an aromatic or proline residue from the closed secondary site loop.

This review aims to provide a detailed presentation of a non-receptor PTP from the NT6 subgroup, namely PTPD1.

General presentation and localization of human PTPD1

PTPD1 belongs to the NT6 non-transmembrane group of PTPs that includes PTPD2, and is part of the FERM containing PTPs subfamily, together with PTPH1 (10), PTPMEG (11), PTPBAS/PTP1E/PTPL1/FAP-1 (12), and PTPpez / PTP36 (13-15).

The gene encoding for PTPD1 protein maps on chromosome 14 at 14q31.3, according to Entrez Gene (http://www.ncbi.nlm.nih.gov/gene/11099). Alternatively it is called PTPN21 (protein tyrosine phosphatase, non-receptor type 21) or PTPRL10.

PTPD1 is a cytosolic non-receptor PTP expressed both in normal and cancer cells (13, 16, 17). In normal tissues it can be found highly expressed in human placenta, skeletal muscle and lung, but is absent in the spleen. A low level of expression can be observed in the colon and kidney, but the very less quantity was found to be in the stomach and liver (13).

Microarray data show that PTPD1 is also expressed in several other organs, such as trigeminal ganglia, testis, adrenal gland, heart, skeletal muscle, uterus, eyes, cerebellum and esophagus; in cancer cells it was observed to be highly expressed in RD (ATCC CCL 136) and A673 (ATCC CRL 1598) cell lines of rhabdomyosarcoma (13), MCF-7 (breast cancer), PC3 (prostate cancer), T84 (lung carcinoma), BeWo (placental), LS174T (colon cancer), SW48 (colon cancer), HCT116 (colon cancer), SK (neuroblastoma) (17), J82, RT4, 5637, HT-1376 (bladder cancer) (17), HeLa (cervical cancer) and A549 (alveolar adenocarcinoma) cell lines (http://www.ebi.ac.uk/s4/summary/molecular/gene;jsessionid=1D66F6EF8FB0C82 3721B8B60541D17D5?term=PTPN21).

PTPD1 can be found in mitochondria (18) and is also partially localized along actin filaments (19), at adhesion plaques (19) and with the endocytic vesicles (17).

The structure of PTPD1

PTPD1 is a 133281 Da protein encoded by 1174 aminoacids that are forming mainly two major domains: FERM (Four point one (4.1) band/Ezrin/Radixin/ Moesin) and PTP domain united through an intermediate region.

The FERM domain is located at N-terminus (1-339 aa) and is important for PTPD1 binding to different membranes. The C-terminal PTP domain (921-1174 aa) is the catalytic domain that contains the active cysteine. Between these two domains there are some small regions that are important for ligand binding: two putative SH2 domains, one putative SH3 domain, one acidic region and five proline rich motifs. Both SH2 putative regions $Y_{158}ESQ_{161}$ and $Y_{217}GEE_{220}$ are located in the FERM domain. The putative SH3 domain (PPPPYPPPRP) is localized after FERM domain (566-575 aa) and the acidic region

(EEEEDEDFEEE) is located near PTP domain (712-722 aa). The five sequences PYX (where X is a non-polar aminoacid: A, P, V or L) are situated at the C-terminus of FERM domain (336-338, 354-356, 408-410) and in the middle of intermediate region (523-527).

The tertiary structure showed that PTPD1 is part of the PTPs category that has the gateway and the secondary pocket blocked (9). This category also includes the non-receptor PTPs along with receptor type PTPs (PTPH1, PTPMEG1, PTPD2, CD45, RPTPm, RPTPk, RPTPr, RRPTPa and RPTP3), and an aromatic residue or a proline in position of Arg24 from PTP1B. The second-site loop adopts a twisted conformation in this category of phosphatases.

PTPD1 catalytic activity

In 2009, Barr *et al*. performed a large scale analysis on numerous PTPs and PTPD1 was completely inactive against 40 peptides derived from tyrosine phosphorylated proteins, even at high enzyme concentrations, but presented a low phosphatase activity on DiFMUP (a synthetic fluorescent substrate) (9). This may have an explanation about how specific the PTPD1 acts on its substrate and that, if a more comprehensive peptide analysis was done, maybe some peptides would have been positive, PTPD1 being catalytically active.

Roles of PTPD1

Analysis of the homologous gene in rodents proposed the possible roles of this PTP in liver regeneration and spermatogenesis (16).

PTPD1 has been previously shown to be involved in loss of heterozygosity (LOH), tumor progression (20) and colorectal cancer (21). In colorectal cancers with microsatellite instability (21), PTPD1 shows the highest mutation frequency from all the six genes identified with frameshift mutations.

A recent proteomic study revealed that PTPD1 is found to be oxidized in A431 carcinoma cells (22). PTP oxidation is a common event in cancer cells and is seems to contribute to the cellular transformation, not to be only a secondary effect of it.

In the PTPD1 sequence, 1772 single-nucleotide polymorphisms (SNPs) have been shown. Two of these non-synonymous SNPs, rs2274736 (Val936Ala) and rs2401751 (Leu385Phe), showed a significant association with schizophrenia, which may be caused by a combination of risk genes (23).

Interactions of PTPD1 with different signaling molecules and its involvement in cellular functions

PTPD1 forms a complex with actin (24) and was demontrated to interact with Src tyrosine kinase (13), FAK (focal adhesion kinase) (24), KIF1C (kinesin family member 1C) (25), KIF16B (kinesin family member 16B) (17), AKAP121 (A-kinase anchor protein 121) (18) and Etk (epithelial tyrosine kinase) (19).

PTPD1 interacts with actin filaments through its FERM domain and plays a role in regulation of actin cytoskeleton remodeling and cell-to-matrix contact sites (24). Evidence for this argument was the expression of an inactive mutant of PTPD1, which disassembled filaments of actin and reduced the adhesion plaques numbers in fibroblasts.

PTPD1 can be found at adhesion sites; it interacts with FAK (focal adhesion kinase) and modulates signaling by regulation of FAK autophosphorylation. FAK is a scaffold protein kinase essential for both turnover of focal adhesions and cellular motility. PTPD1 interacts with FAK through a domain next to the FERM domain. This "FAK binding domain" is required to localize PTPD1 at focal adhesions sites (24).

FAK is activated by growth factors or components of extracellular matrix by multiple phosphorylations. The autophosphorylation of Tyr 397 is necessary for interacting with Src. The complex between Src-FAK induces phosphorylation of some tyrosine substrates (p130Cas, paxillin and p190RhoGAP) and activates different downstream signaling cascades (26-28). Active PTPD1 is necessary for signaling and stimulation of EGF by FAK autophosphorylation (24). The same study showed that, if Src is inhibited, PTPD1 signaling is suppressed. In this respect, the authors enounced a model whereby PTPD1 activates and enrolls Src close to FAK, in response to EGF signaling.

Src and FAK cooperate to induce cell scattering and migration in response to EGF signaling (29). In 2008, Carlucci *et al*. (24) showed that PTPD1 promotes growth factor-dependent cell motility.

FAK is implicated in a wide variety of signaling pathways that promote cell migration and proliferation, both of them correlated with tumorigenesis (30). Because of the relationship between PTPD1 and FAK, some authors (24) hypothesized that PTPD1 could participate in tumor invasiveness and expressed the inactive mutant of PTPD1 in human breast cancer cells (MCF-7) and observed that it inhibited extracellular matrix invasion and cell migration.

PTPD1 is recruited into endosomes by interaction with KIF16B after EGF stimulation of cells. KIF16B is a kinesin family motor protein that regulates intracellular transport of early endosomes along microtubules and is involved in controlling the stability and signaling of membrane receptors (17). KIF16B overexpression enhances recycling of internalized receptors to the cell membrane, whereas down-regulation of KIF16B impairs endocytic cargo movement and promotes receptor degradation (31). Carlucci *et al*. (17) observed that PTPD1 has a synergistic role with KIF16B in favoring recycling of internalized EGFR through the endocytic pathway.

PTPD1 also interacts with another KIF family member, KIF1C, through its FERM domain (25). KIF1C is involved in membrane trafficking from the Golgi to the endoplasmic reticulum (25).

Another interactor of PTPD1 is AKAP121, which binds to the acidic region of PTPD1 and redirects it to the outer membrane of mitochondria (18). AKAP121 with Src, PKA and PTPD1 are forming a complex *in vivo*. This complex is necessary for maintaining the mitochondrial membrane potential and the ATP synthesis (18). The inactive PTPD1 does not influence the activity of c-Jun N-terminal kinase 1/2 and p38_ mitogen-activated protein kinase (18). When PTPD1 is not in complex with other molecules, EGFR signaling is directed to the nucleus, where it activates gene transcription/ translation through ERK1/2 or Elk1 (18).

The region 726–848 aa of PTPD1 is responsible for specific binding Etk, another interaction partner. The interaction between these two partners stimulates the kinase activity of Etk and modulates the activation of Stat3 involved in cellular signaling (19).

PTPD1 involvement in cancer development

PTPD1 expression was analyzed in human bladder lesions (17). The level of expression of PTPD1 is increasing with tumor stage, from low in benign lesions to high in malignant tumors. PTPD1 is accumulated in cell during the transition from normal tissues to tumor lesions, but its expression level is decreased during the development of the tumor. Thus, in an early stage of the bladder cancer there are approximately 60% of cells expressing PTPD1 than in an intermediate stage, where there are only 35%, or in an advanced disease stage, with 23%. This correlation between PTPD1 expression and cancer stage can be explained by a necessity of cells developing into malignant to become more invasive and proliferative. PTPD1 silencing had an inhibitory effect against cell growth and motility of bladder cancer cell lines (J82, RT4, 5637, HT-1376) through down-regulating EGFR level and downstream signaling (17).

Together with the fact that EGF and EGFR are also implicated in different aspects of bladder cancer development, it seems that PTPD1 is acting in the first period of the tumor development, when EGF signaling is up-regulated and cell growth, invasivity and spreading of the cells are more elevated.

Endogenous PTPD1 silencing reduces cell motility (24) and growth of human bladder cancer cells (17). The same effect is obtained when PTPD1 inactive mutants or PTPD1 lacking FERM domain are expressed. However, overexpression of PTPD1 promoted cell scattering and migration. In conclusion, complete and active PTPD1 is necessary for cell motility.

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