Hubrecht Institute
Developmental Biology and Stem Cell Research

Universiteit Utrecht

Protein tyrosine phosphatases and cancer

Sanne Rijnders

Hubrecht Institute: Developmental biology and stem cell research
Uppsalalaan 8
3584 CT Utrecht
Netherlands
Phone: (+31) 30 212 1800

University of Utrecht
Hijmans van den Berghgebouw
Universiteitsweg 98
3584 CG Utrecht
Netherlands

Protein tyrosine phosphatases and cancer

Master thesis

Student: **Sanne Rijnders**

Student number: 3129616

Master: Biomedical Sciences, Biology of Disease

Project duration: **6 weeks (~ November '08 - December '08)**

Project supervisor: **Prof. Dr. Jeroen den Hertog**

Summary

This thesis describes a complex signaling system that is based on reversible phosphorylation at tyrosine residues mediated by protein tyrosine kinases (PTK) and subsequent dephosphorylation by protein tyrosine phosphatases (PTP). Both enzymes have important regulatory control over cell growth, proliferation and differentiation and for that reason, imbalanced PTK and/or PTP activity has been linked to various disease states. Multiple studies have assessed the involvement of PTPs in disease and here we describe four PTPs that have been found to colocalize with genetic susceptibility loci connected to type II diabetes, one PTP that has been implicated in autoimmunity, 6 PTPs that have been identified in a mutational analysis and that seem to be involved in colorectal cancer and a total of 8 PTPs that have been specifically implicated in breast cancer. Specifically the role of PTP1B is described in breast tumorigenesis, where this protein has been implicated in both tumorsuppressor activities as well as in oncogenic processes. Research on PTP1B function either in PTP1B deficient mice generated through genetic manipulations or through pharmacological inhibition of the protein, showed that PTP1B deficiency resulted in a delay of ErbB2-induced mammary tumorigenesis and protected from lung metastasis creating strong evidence that PTP1B functions as an oncogene in mammary breast tumorigenesis. To date, it often remains unclear what role PTPs play in tumorigenesis: oncogene or tumorsuppressor. In order to discriminate between the two, additional research on the precise function of these proteins is needed.

Table of Contents

Chapter 1: Introduction

It has become clear that many physiological processes are controlled through cellular signaling cascades which are, amongst others subjected to control by phosphorylation. As regulation through phosphorylation is reversible, the system consists of enzymes able to phosphorylate proteins and other organic molecules: protein kinases and another set of enzymes able to dephosphorylate these substrates: protein phosphatases (De-)phosphorylation of substrates occurs specifically at tyrosine, threonine or serine residues [1], with the focus of this thesis especially on tyrosine phosphorylation by protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). After the discovery of protein phosphorylation, the majority of research was focused on protein phosphorylation and protein dephosphorylation was regarded as a irrelevant housekeeping process. However, for the regulation and maintenance of cellular homeostasis (e.g. control cell growth, proliferation and differentiation) eukaryotes have developed a complex system of protein phosphorylation subjected to both PTKs and PTPs activity and thus a dynamic equilibrium must be present between PTK and PTP activity. Within this equilibrium, the role of PTPs is equal to the role of PTK as both are specific and actively involved in setting the levels of protein phosphorylation [2].
The concept of reversible protein phosphorylation at specific residues is present in signaling cascades initiated by growth factors, cytokines, hormones, extracellular matrix components, and cell adhesion molecules. As these signaling cascades have been implicated in cell growth, proliferation and differentiation, tumor suppression, cytoskeletal reorganization, mitotic induction, T cell activation, control of metabolic enzymes, contraction and transport of cells, cell cycle processes, learning and memory and in growth factor, somatostatin and interferon signaling pathways, it can be concluded that reversible protein phosphorylation is of major importance for the maintenance of general cellular homeostasis. Consequently, disruption of the equilibrium between RTKs and PTPs or malfunctioning of either one has been linked to a number of oncogenic and metabolic disease states [3].

Four major PTP families

As protein phosphorylation is a fundamental mechanism for managing eukaryote physiology, it might not come as a surprise that a large part of the eukaryotic genome encodes for phosphoproteins. As much as 30% of the intracellular proteins are phosphoproteins and in addition to this, it is estimated that all genes encoding for protein phosphatases and kinases combined make up 4% of the eukaryotic genome [4]. It is thought that for every kinase, an antagonistic phosphatase is present. So far, this indication seems to be correct: currently 90 genes in the human genome have been found to encode PTKs whereas 107 genes have been found to encode PTP. Out of these 107 PTP genes, 11 are catalytically inactive, 2 dephosphorylate mRNA and 13 dephosphorylate inositol phospholipids. This is resulting in a group of 81 genes that actually have protein phosphatase activity compared to a total of 85 active protein kinases [5]. The 81 PTPs can be divided into four separate families based on the amino acid sequences of their catalytic domains through which each family also obtains specificity for a certain range of substrates.

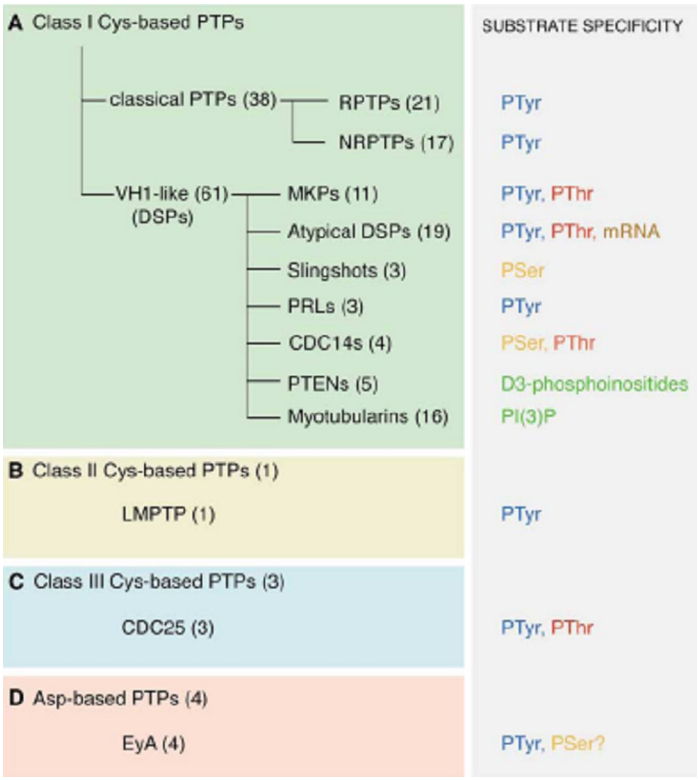

Figure 1: classification and substrate specificity of PTPs [5]

As is evident from figure 1 (which includes both the active as well as the inactive PTPs), the cysteine-based PTPs from class I constitute by far the largest family with 99 members in total. The PTPs from class I can be divided into subfamilies based on the domain architecture and the degree of homology between catalytic domains. Distinction can be made between “classical PTPs” (38 members) and VH1-like “dual specific” PTPs (61 members) [6]. While dual specific PTPs can dephosphorylate tyrosine, threonine and serine residues, classical PTPs are only able to dephosphorylate tyrosine residues. Within the class I PTP subfamily, more distinction can be made: the classical PTPs can be divided into transmembrane receptor-like enzymes (RPTPs) encoded by 21 genes and intracellular non-receptor PTPs (NRPTPs) encoded by 17 genes [6] and the dual specific PTPs be divided into 7 subgroups based upon structure and function of the enzymes (Figure 1). The second class of PTPs contains only one member: a cysteine based, tyrosine specific low molecular weight enzyme. Though the enzyme is able to dephosphorylate a number of tyrosine kinases and their substrates, its physiological function is unclear. The class III cysteine-based PTPs family contains three members which are tyrosine/threonine specific and function as cell cycle regulators by dephosphorylating Cdcks (cell division cycle kinases) at their inhibitory dually phosphorylated N-terminal threonine-tyrosine motifs. Activation of Cdcks is needed to drive progression of cells through the cell cycle [7]. And the class IV family contains PTPs that use a catalytic mechanism that depends on an aspartic acid instead of a cysteine. Currently, class IV only contains 4 EyA genes as the catalytic tyrosine/serine phosphatase activity of these proteins has been established, but it is speculated that many more EyA genes are actually present in the genome [8].

Even though PTPs have been implicated in many physiological processes, the specific function of many PTPs remains currently unknown. Therefore, this thesis will only describe some well studied mammalian classical PTPs as this family of PTPs is the most thoroughly studied and the functions and role of some of these PTPs have been well established in both human health and disease.

Structure of classical PTPs and relationship to enzymatic activity and function

Classical PTPs are characterized by a homologous sequence of ~250 amino acid residues present in the catalytic domains of the protein. Within this sequence homology, an 11-residue signature motif is present; in single letter code, (I/V)H**C**xAGxG**R**(S/T)G [9]. The cysteine and arginine, separated by five arbitrary residues (Cx_5R), represent the core of the active site motif. The active site motif also determines the specificity of the PTPs for phosphorylation of tyrosine residues as the three-dimensional structure of protein tyrosine phosphatase 1B (PTP1B) revealed that only tyrosine residues fit in the depth of this active site cleft and the shorter phosphoserine and phosphothreonine side chains would not reach the phosphate-binding site [10]. And the active-site motif possesses catalytic activity, it is able to remove phosphate groups from phosphorylated amino acids and transfer them to water molecules during the dephosphorylation reaction [11].

Based on examination of the crystal structure of the catalytic domain of human PTP1B, a NRPTP [10] and *Yersinia* PTP (YopH), an active PTP secreted by the causative bacteria of the bubonic plague (*Yersinia pestis*) and other enteric diseases [12], it could be concluded that despite large difference in their DNA sequence (15 – 20% homology [10] [12]), PTPs share a similar catalytic structure which is comprised of a central twisted, mixed β-sheet flanked by α helices. This signature structure includes the majority of the functional groups needed for phosphate binding and catalysis, and thus constitutes the fundamental component of the catalytic site [1]. Within this basic structure, the Cx_5R active site motif residues can be found within a single, central loop which is nestled at the base of a cleft on the surface of the protein [2][11].

Diversity between RPTPs and NRPTPs arises from differences in the structure of regulatory and targeting sequences attached to either the N-terminus or the C-terminus of the shared catalytic domain. In general, the 21 RPTPs possess an extracellular domain, a transmembrane domain, and depending on the type of extracellular domain, the receptors have either one or two intracellular PTP catalytic domains [13]. When a PTP has two intracellular domains, the most proximal domain is usually responsible for the majority of catalytic activity, while the distal domain is completely inactive or (in some cases) weakly active [2]. All RPTPs have a membrane spanning α-helix and must therefore be located in cellular membranes, mostly in the plasmamembrane. Here they interact with the extracellular milieu in a receptor-like fashion. In order to exert a wide array of biological functions, the extracellular domain of the RPTPs is made highly variable due to the presence of specific structural features.

Based on these differences in the extracellular domain, the RPTPs family can be divided into 5 different subtypes [13]. The type I RPTPs family consists of the CD45 receptor and its multiple isoforms. In the extracellular domain, these receptors have both a cysteine-rich and a highly glycosylated region and their function is essential for B cell and T cell activation as CD45 can dephosphorylate Src family PTKs [11]. The receptors from the

type II family (LAR, PTPδ, PTPκ, PTPμ, PTPρ and PTPσ) contain tandem repeats of immunoglobulin-like and fibronectin type III domains and in addition sometimes have a MAM (meprin-*Xenopus* A5-mu) domain. All domains show similarity to cell adhesion molecules and studies have indicated that the Ig and MAM domains present in PTPμ and PTPκ are participating in directing homophilic interactions. PTPμ and PTPκ do not promote cell adhesion it self, instead they help with signal transduction of signals generated by cell adhesion molecules in a multiprotein complex [2]. Receptors from the type III family (PTPβ DEP-1, SAP1, PTPS31 and GLEPP1) only possess multiple fibronectin type III domains. The fourth family contains two members, PTPα and PTPε which possess small glycosylated segments. And the final family includes PTPζ and PTPγ which both have amino-terminal carbonic anhydrase-like (CA) domains. PTPζ has been implicated in the control of neuronal adhesion as they are expressed in glia cells. The CA-domain forms a binding pocket for a cell recognition molecule (contactin) expressed on neurons, and it is suggested that as neurons bind to this domain, the outgrowth and differentiation of their neurites is induced [2].

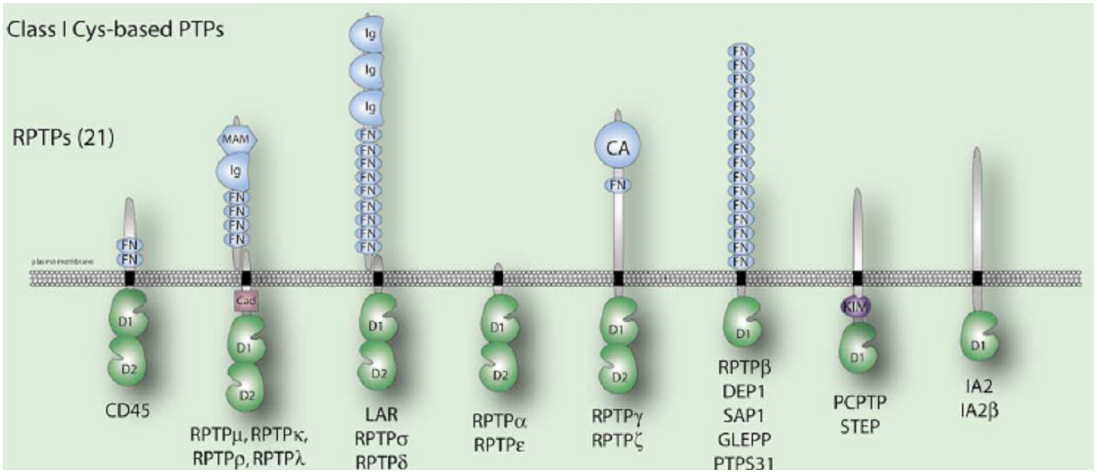

Figure 2: structure of the Class I family of receptor PTPs (adapted from [5])

Like the RPTPs, the 17 NRPTPs also have extra-catalytic domains which in this case are used to direct the proteins to particular locations within the cell in order to regulate their catalytic activity. The regulatory targeting sequences, or domains, attached to the N and C-terminus of the catalytic domain consist mainly of short hydrophobic segments that facilitate protein-protein interactions. There are four different domains known that facilitate the regulation of NRPTPs. The first domain that can be present in addition to the catalytic sequence of NRPTPs is the Src homology (SH)2 domain, a conserved sequence motif of ~100 amino acids. The general function of this SH2 domain is to promote interactions between cytoplasmic signaling molecules and phosphotyrosyl residues on activated growth factor receptors or other signaling molecules in order to bring the appropriate signaling components of the mitogenic pathway together. Examples of NRPTPs containing SH2 domains are SH-PTP1 (later remained SHP1 and it is expressed in hematopoietic cells) and SH-PTP2 (later remained SHP2 and ubiquitously expressed) [14]. The second domain that can be present in NRPTPs is a sequence that is homologous to the band 4.1 family of cytoskeletal proteins and is for example present in

PTP-H1. Presence of this domain suggests there is enzyme targeting to interfaces between the plasmamembrane and the cytoskeleton. Thirdly, NRPTPs can also contain domains related to retinol binding proteins which target these proteins to the endoplasmic reticulum (ER). This restriction to the ER results in a limitation of the availability of these proteins, but cellular stimuli can trigger proteolysis cleaving the C-terminal off the NRPTPs resulting in translocation of the proteins to the cytoplasm where they are catalytically active. Examples of NRPTPs restricted to the ER are PTP1B, TC-PTP, and PTP-PEST. Finally, some NRPTPs contain domains that target these proteins to the nucleus where they might be important in cell cycle regulation and gene transcription [14]. Similar to ER – cytoplasm restriction, these NRPTPs are targeted to a specific site in the cell depending on the specific domain in the extracellular region of the protein, but while ER restriction uses proteolyses to target the proteins, nuclear localization is facilitated through alternative splicing of proteins.

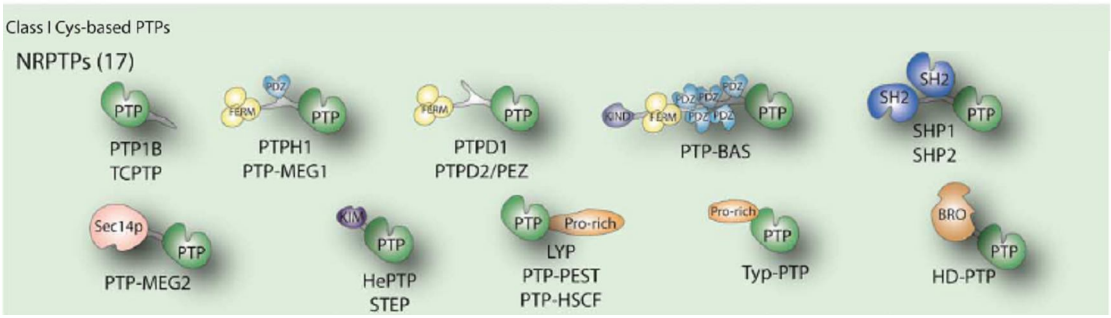

Figure 3: structure of the Class I family of non-receptor PTPs (adapted from [5])

Interplay between RTKs and PTPs

As PTPs can be subdivided into several classes of proteins (with our main focus on RPTPs and NRPTPs) their reversed components, the PTKs, can also be divided into several classes. One large group of PTKs are the receptor tyrosine kinases (RTKs). RTKs are a class of cell surface receptors that exhibit intrinsic tyrosine kinase activity. The family of RTKs comprises of 58 members, notably the epidermal growth factor receptor (EGFR), insulin receptor (IR), vascular endothelial growth factor receptor, fibroblast growth factor receptor, and hepatocytic growth factor receptor subclasses [15]. Like RPTPs, RTKs are comprised of an extracellular domain, transmembrane domain, and a cytoplasmic protein tyrosine kinase domain which has regulatory phosphorylation sites. Ligand binding activates RTKs by inducing a conformational change that enables transphosphorylation of the receptor dimer and initiates downstream signaling cascades [16]. As it is suggested that cell signaling cascades initiated by RTKs are counteracted by PTPs, RTKs thus regulate cellular signaling in the opposite direction of PTP signaling and it can therefore be concluded that like PTPs, RTKs are involved in the regulation of fundamental cellular homeostasis.

The phosphorylation status and signaling activity of RTKs is determined not only by the kinase activity of the enzyme it self, but it is also influenced by the activity of the PTPs that specifically dephosphorylate those kinases. PTPs can regulate both ligand-independent RTK activities by keeping ligand-independent RTKs signaling at low basal levels and they can regulate ligand-activated RTK activity by quickly dephosphorylating ligand-activated receptors. Though more research has been focused on the negative effect

of PTPs on RTK signaling, it has been proposed that certain PTPs can also enhance RTK signaling. This is illustrated by the example of SHP1 and SHP2. Studies have indicated that SHP1 predominantly regulates RTK signaling in a negative manner, while SHP2 positively enhances RTK signaling [2][14]. Models describing the mechanism of action of SHP1 and SHP2 have been made, but still the negative and positive signal-enhancement of RTKs by the PTPs remains poorly understood (reviewed in [14]). This has led to inconclusive findings for PTPs containing a SH2 domain and the possibility has now been raised that from origin, they can both have positive and negative effects on RTK signaling, but that the outcome of the influence depends on the signaling pathway they are active in [2]. Therefore, it is proposed that PTPs may actually interact with PTKs in order to work together with them to regulate the phosphorylation state of downstream signaling molecules in signaling cascades normally activated by RTKs [14].

As intrinsic PTP enzymatic activity is generally two to three orders of magnitude greater than that of the PTKs, and important cellular signaling cascades function under the surveillance of both RTK and PTP activity, the enzymatic activity of PTPs must be tightly regulated and restricted in order to prevent non-specific dephosphorylation of proteins and to make PTK signaling effective [15]. There are two main ways to regulate the activity of kinases through PTPs activity: either the activity of the PTP itself is altered or PTPs are directed to the RTKs by specific recruitment mechanisms.

The first major control mechanism for PTP activity depends on multiple ways to alter the activity of the PTP itself. The most important manner to do this is by posttranslationally modifying the protein through the phosphorylation of serine/threonine residues present in the PTP. Certain PTPs, including PTP1B and SHP1, can also be activated by phosphorylation of tyrosine residues present in the proteins. This is particularly intriguing as it implies a physical and maybe even a functional interaction with PTKs [17]. PTPs can further be posttranslationally controlled by glycosylation, which appears to be restricted to the transmembrane PTPs because only these proteins contain abundant N- and O-linked carbohydrates in their extracellular portions that are needed for the glycosylation process. And lastly, posttranslational control of PTPs can be accomplished through reversible oxidation of the catalytic cysteine residue by oxidants like H_2O_2.

As second manner to control PTP activity involves dimerization. PTPs dimerize at a constant rate regardless of physiological demand and the dimerization can result in either activation or inactivation of the PTP. The outcome of this dimerization depends on the exact composition of the RPTP which is controlled through posttranslational modifications like oxidation or phosphorylation. And posttranslational alterations may shift the dimers from an active to an inactive state or vice versa (reviewed in [18]).

PTP – RTK activity can also be regulated through RKT dimerization. Dimerization of these receptors results in a decrease in the inhibitory properties of PTPs as well as reduced sensitivity of RTKs to dephosphorylation by the PTPs. This might be due to the fact that upon binding of ligand to RTKs, the intrinsic tyrosine kinase activity of these receptor increases, which results in a net increase in tyrosine phosphorylation capacity of the receptor. As a consequence, the enzymatic activity of the PTP is no longer able to counteract the “stronger” RTK [19][20].

Finally, PTPs can be regulated through specific protein-protein interactions that modulate their activity directly or indirectly by controlling subcellular localization of the enzymes. PTPs are consisting of a modular structure resulting in the presence of different

extracellular domains. These extracellular domains could either directly interact with specific ligands and this is subsequently altering the activity of the PTP. An example of a PTP that can be regulated in this fashion is PTPζ [2]. And on the other hand, the presence of specific domains can direct PTPs to particular subcellular locations like the ER, cellular membranes or the nucleus. By specifically directing the proteins, the substrate specificity and availability of the PTPs can be altered.

Chapter 2: PTPs and disease

As has been described in the introduction, PTP signaling is present in many different signaling cascades and therefore contributes in large part to cellular homeostasis. Through PTP signaling, key elements of this cellular homeostasis like cell growth, proliferation, differentiation, cell cycle progress, metabolism, and cytoskeletal function are controlled and kept in proper boundaries in order to prevent disease. If for some reason defective or inappropriate regulation of PTPs occurs, phosphorylation of tyrosine residues could be altered, possibly contributing to the development of numerous human diseases [15]; summarized in the supplementary table.

Studies relating PTPs to disease

In 2004, Andersen *et al*. [6] conducted a disease association study in which they correlated the mapped human classical PTP genes with known genetic disease markers in order to evaluate if these proteins can be indicated as candidate genes for disease. By using this association study, the investigators were able to identify classical PTPs that map to known susceptibility loci for diseases such as autoimmunity, diabetes, and cancer. As for type II diabetes, it is known that the disease is strongly influenced by genetic background and currently 10 susceptibility loci have been described in which four classical PTPs localize: PTP1B, PTPρ, SHP2 and PCPTP1. As insulin resistance is the main feature of type II diabetes, the identified PTPs form good candidate disease genes as they are involved in the negative regulation of insulin signaling and it is speculated that increased activity of them could contribute to this insulin resistance. So far two independent studies in which PTP1B transgenic mice were generated revealed that the ablation of the gene not only increases insulin sensitivity, but that it also produces resistance to diet-induced obesity due to the removal of a negative inhibitory constraint on insulin and leptin signaling (reviewed in [6]). In addition to this, SHP2 has also been shown to be involved in insulin-mediated signaling pathways. SHP2 does not bind directly to the IR, but instead it binds insulin receptor substrate 1 (IRS-1) via its SH2-domain. This protein plays a key role in transmitting signals from the IR and insulin-like growth factor 1 receptor (IGF-1R) to intracellular downstream effectors among which is phosphatidylinositol 3-kinase (PI3K) with an essential function for insulin-mediated glucose uptake. Disturbance of this insulin – glucose system could possibly be of importance for the occurrence of type II diabetes [21]. In case of diseases involving autoimmunity, CD45 is the PTP of interest as this protein has been indicated in the activation of B and T cell signaling [11]. To better understand CD45 based autoimmunity, CD45 transgenic mice were created. A mutation in the CD45 gene that is potentially over activating the gene (e.g. creating an oncogene) generates mice that display lymphoproliferation, autoantibody production, and severe autoimmune nephritis. Conversely, a knockout mouse (thus removing the activity of the gene) is severely immunodeficient and displays compromised T cell development and reduced B cell responses (reviewed in [6]). Even though the two CD45 mouse-models generate two completely different phenotypes, both models point at an important role for CD45 in mediating antigen receptor signaling and thus both indicate that this PTP might act as a key player in the occurrence of autoimmunity.

Main PTPs involved in cancer

Of more interest to use, classical PTP genes were examined for their involvement in the induction of cancer. Though a variety of RTK genes (like ErbB1 (EGFR), *Neu* (ErbB2), Bcr-Abl) have been linked to tumorigenesis through somatic activating mutations creating potential oncogenes, only a few PTP genes have been implicated to play an important role in malignant cell transformation [3][22]. It is suggested that the majority of PTPs normally function as tumorsuppressor genes as they may be capable of dephosphorylating critical substrates involved in cellular transformational processes [3]. Malignancies derived from PTP genes are thought to occur through (partial) deletion or amplification of the gene: meaning that excessive cell growth can be based on over activation of a PTP, the PTP has become an oncogene or excessive cell growth occurs through the deletion of a tumorsuppressor-PTP, thus removing inhibitory control. So far several PTPs have been linked to multiple cancers as indicated in the supplementary table. Some short summaries will be given of how some PTPs have been found and subsequently how they are involved in tumorigenesis before the focus will be shifted to PTPs specifically involved in breast cancer.

First of all PTPα, a protein that has been implicated in the activation of Src family kinases, is involved in the regulation of integrin signaling, cell adhesion, and growth factor responsiveness [23]. PTPα is also transiently enhanced during neuronal differentiation [24] and the receptor has been implicated in two different types of cancer. In late-stage colorectal tumors increased mRNA levels could be detected and in primary breast carcinomas increased protein levels were detectable. Interestingly, in breast cancer, overexpression of PTPα correlated with reduced tumor aggressiveness while in colorectal cancer, increased levels of PTPα seemed to cause continuous activation of Src subsequently leading to malignant cell transformation. This is thus suggesting that PTPα acts as both a positive and negative regulator of cell signaling cascades involved in tumorigenesis and that the role of PTPα it is most likely depending on the cellular context and type of tumor (reviewed in [6]).

Another example of a PTP involved in tumorigenesis is DEP-1. It is known that multiple chromosomal loci are frequently altered in colon, lung and breast cancer [25]. First in mice and later in men, Andersen *et al.* [6] found that one of these chromosomal loci correlates to the chromosomal locus of DEP-1 making the DEP-1 chromosomal locus function as the underlying gene/region for susceptibility to colon cancer. In some cases of human colon cancer, loss of heterozygosity was observed at the DEP-1 locus and then DEP-1 started to function as an oncogene. This is due to the fact that DEP-1 is a substrate for the RTK Met, which is aberrantly upregulated in several human tumors. Mutations in DEP-1 have been detected in tumors associated with deviating Met signaling, thus indicating that mutated forms of DEP-1 can upregulate RTK signaling. This hypothesis is raising the possibility of a functional interaction between a RTK (Met) and a PTP (DEP-1) in the progression of certain human cancers (reviewed in [6]).

In another study, Wang *et al.* [22] decided to investigate “all” PTPs involved in colorectal cancer. In order to do so they conducted a mutational analysis of the tyrosine phosphatome, meaning they screened colorectal tumor samples for the presence of genes encoding PTPs and subsequently looked for mutations in these genes. This approach led to the identification of 83 somatic mutations in 6 different classical PTPs: the RPTPs LAR, PTPγ and PTPρ, and the NRPTPs PTPH1, PTPBAS, and PEZ. The mutations and

coherent PTPs can be found in 26% of the colorectal cancers and in lesser amount in lung, breast and gastric cancers. A big pitfall of this analysis is that often the function of the found PTP is unknown or very poorly understood making it difficult to indicate the exact role of the found PTP during tumorigenesis.

Though PTPρ is not thought to be important for controlling cellular growth or differentiation as it is normally expressed in the developing central nervous system and in the adult cerebellum, this protein is most frequently mutated out of the 6 proteins found by Wang *et al.* [22]. They were able to indicate the presence of PTPρ in a wide variety of human tissues, including normal colon epithelial as well as cells derived form colorectal cancers. To study the effects of point mutations on the phosphatase activity of PTPρ, Wang *et al.* [22] expressed wild type and mutated variants of the receptor in bacteria and assessed the enzymatic activity of the proteins on substrate. This led to the conclusion that all mutated receptors had a decreased phosphatase activity compared to the wild type receptor and subsequent expression of the receptors into HCT116 and DLD1 colorectal cancer cell lines showed that only the wild type receptor was able to inhibit cell growth, while cells transfected with the mutated receptor were not. All these data are strongly indicating PTPρ as a tumorsuppressor which is in accordance with the general role of PTPs as the inhibitors of kinase activity in various growth-promoting pathways.

The other PTPs are less well studied, but mostly follow the predictions of functioning as tumorsuppressor genes. PEZ and LAR are thought to be important in cell adhesion by regulating tyrosine phosphorylation of adherent junction proteins. Inactivation of these genes through mutations may therefore contribute to the invasive and metastatic capacity of cells as an increase in the phosphorylation level of the adherent junction proteins leads to an increase in cellular motility and migration. For PTPBAS, the largest intracellular enzyme, it is only known that it is active somewhere along the apoptosis signaling pathway and that it functions together with tamoxifen treatment to gain antitumor effects. However, one could suggest that the protein also has oncogenic potential as loss of function of the protein could lead to lesser control/activation of the apoptotic signaling pathway resulting increased cellular survival. Further more, it has been shown that overexpression of PTPH1 possibly inhibits growth of NIH/3T3 cells through interactions with valosin-containing proteins suggesting this PTP is a true tumorsuppressor. And finally it has been found that the locus of the PTPγ gene is often implicated as a genomic region frequently lost in multiple sorts of cancer (lung, renal, early-stage breast) suggesting that this PTP functions as a tumorsuppressor considering loss of the gene is leading to the development of cancer.

Through the last few examples it is becoming clear that many PTPs are actively involved in tumorigenesis, however, much more research is needed. It can be noticed from the supplementary table that many PTPs are implicated in disease, but that only for a few of them real conclusive data has been found describing their exact role in the disease. Additional research is needed to clarify the role of the remaining PTPs and also to identify more PTPs involved in tumorigenesis as it is speculated that the role of PTPs in disease is far greater than currently indicated.

Chapter 3: PTPs associated with breast cancer

In 2007, cancer was considered to be a leading cause of death worldwide as the disease accounted for 7.9 million deaths (~13% of all deaths worldwide). The main types of cancer leading to overall cancer mortality each year are; lung (1.4 million deaths), stomach (866,000 deaths), liver (653,000 deaths), colon (677,000 deaths) and finally with 548,000 deaths a year there is breast cancer, making it the most frequent type of cancer among women [51].

Breast cancer

Control of breast cancer cell proliferation is a complex phenomenon involving multiple factors as for example steroid hormones binding to nuclear receptors and peptide hormones or growth factors acting via tyrosine kinase associated transmembrane receptors [26]. Therefore, when breast cancer is discovered in a patient, a protein and gene expression profile is made by testing the cancerous breast tissue for the presence/expression of specific tumor markers. This includes two good prognostic markers, the estrogen receptor and progesterone receptor and two negative prognostic markers, the EGFR (also known as ErbB1 or HER1) and ErbB2 (also known as HER2 or *Neu* (in rodents)). The tests are usually done by immunohistochemistry and the expression profile helps to predict a patient's prognosis and helps an oncologist in the choice of the most appropriate treatment. Patients expressing the estrogen and progesterone receptor can be treated with hormone therapy while patients expressing ErbB2 (25% of breast cancer cases [27]) will not benefit from this treatment and can better be treated with for example the monoclonal antibody trastuzumab. Unfortunately, all current prognostic indicators for breast cancer are signal transduction molecules (PTKs) while it is known that many other molecules (like PTPs) also contribute to the development and progression of breast cancer making them possible disease markers as well.

8 PTPs implicated in breast cancer

Based on two different strategies, the role of various PTPs in breast cancer signaling has been assessed. The first strategy, which is based on a comparison between mRNA/protein expression of PTPs in normal or cancerous breast tissue, led to the identification of 5 important PTPs: PTPγ, LAR, PTPα, PTP1B and SHP1. A second strategy, which was focused on identifying PTPs regulated in tumors induced by oncogenes in transgenic animal models or by treatments influencing growth or apoptosis in breast cancer cell lines, led to the identification of three additional PTPs: PTPε, DEP-1 and PTPBAS [26]. All these PTPs are mentioned in the supplementary table. And from this table it can be concluded that most PTPs are not just involved in one specific disease, but rather, they might actually be important for the development of multiple different diseases.
One of the PTPs found is PTPγ, this protein has been implicated as a tumorsuppressor in lung and kidney cancer [28] and in addition to this, Zheng *et al.* [29] have found downregulation of this protein in breast cancer. mRNA levels of PTPγ in normal breast tissue, cancerous breast tissue and breast cancer cell lines were detected and quantified by using RT-PCR and a RNAse protection assay in order to compare the expression profiles. From these assays it could be concluded that PTPγ mRNA was expressed in all

investigated tissues but that it was expressed more highly in normal breast tissue then in cancerous tissue or in cancer cell lines indicating PTPγ thus functions as a tumorsuppressor. Next to the assessment of mRNA levels, Zheng *et al*. [29] also looked at the regulation of PTPγ mRNA expression in these cell types. They found that PTPγ mRNA expression is inhibited by the presence of 17 beta-estradiol in a dose-dependent manner in primary cultured breast cells. 17 beta-estradiol – estrogen receptor complexes can initiate/control gene transcription and therefore these findings led the investigators to suggest that PTPγ is a potential estrogen-regulated tumorsuppressor gene in human breast cancer which may play an important role in neoplastic processes of human breast epithelium.

So far PTPγ is the only PTP that has been found with a decreased expression level in cancer, all other PTP are expressed more abundantly in cancer, thus in contrast to PTPγ, an increased expression level of LAR has been found in breast cancer [30]. By using Northern and Western blots, Yang *et al*. [30] were able to show that LAR expression is increased by 10-fold in breast cancer cell lines and breast cancerous tissue when compared to normal breast tissue. RT-PCR indicated there was one specific neuronal splice variant most dominantly overexpressed making this variant a good tumor marker. As it is known that type II PTPs contain cell adhesion motifs in their extracellular domain they are thought to be important for cell adhesion and could therefore contribute to the invasive and metastatic capacity of cells. This principle was investigated by Levea *et al*. [31] and they confirmed a high correlation between LAR expression and metastatic potential of cells. Expression levels of LAR were high in 13762NF rat mammary adenocarcinoma cells (a cell line that is highly metastatic) while expression of the protein was low in non-metastatic MTC cells. In accordance to this, they also found this relation in human breast cancer samples where LAR expression was strongly positive in 50% of metastatic cases but in only 21% of 'non-metastatic' cases. This is making overexpression of LAR a negative indicator for breast cancer status as it is associated with metastatic capacity of cells and it can thus be named an oncogene.

Another PTP involved in breast cancer is PTPα. As briefly discussed before, PTPα has been implicated in late-stage colorectal tumors and in primary breast carcinomas where PTPα acts as an important regulator of Src kinase activity. The tyrosine-protein kinase activity of Src is normally inhibited by phosphorylation by c-Src kinase as this induces a conformational change leading to an inactive form of the kinase while activation of Src occurs trough dephosphorylation by PTPα. The effects of PTPα on Src activity were shown by Zheng *et al*. [32] in fibroblasts and independently in other cells as well by den Hertog *et al*. [24]. PTPα overexpressing cells exhibit elevated level of Src activity while cells in absence of PTPα had reduced Src activity. Though it was expected that these elevated levels of Src activity would have pro-oncogenic consequences, Ardini *et al*. [23] showed that in primary human breast cancer samples the level of PTPα strongly varied among different tumor samples, and in 29% of the samples the protein was significantly overexpressed. This correlated with low tumor grade and positive estrogen receptor status. Subsequently, Ardini *et al*. [23] showed that expression of PTPα in breast carcinoma cells led to growth inhibition, associated with an increased portion of cells in the G0/G1 phase of the cell cycle and delayed tumor growth and metastasis. These results therefore indicate that the overall high level of kinase and phosphatase activity in breast cancer is

for a large part due to high Src activity and thus related to overexpression of PTPα making PTPα a tumorsuppressor gene.
Then there is the NRPTP SHP1, this protein is mainly expressed in hematopoietic cells and studies have indicated that SHP1 predominantly regulates RTK signaling in a negative manner. In most leukemia and lymphoma cells SHP1 protein levels are diminished or completely missing indicating this protein as an important tumorsuppressor. However, the negative regulatory role of this PTP in hemopoietic cell signaling may not apply to all cancers. In 2000, Yip *et al.* [33] were able to link SHP1 to breast cancer. They assessed the expression of SHP1 mRNA in human breast cancer cell lines and primary breast cancers tissue and were able to indicate mRNA in a total of 19 breast cancer cell lines examined, and in 42 of the 72 primary breast cancers samples SHP1 mRNA expression was increased 2-12 fold relative to normal breast epithelial cells. Yip *et al.* [33] were also able to positive correlate the expression of SHP1 mRNA to GRB2 mRNA expression, and since these proteins can bind to each other and regulate MEK/MAP kinase activation, their simultaneous upregulation may amplify tyrosine kinase signaling in breast cancer cells making SHP1 a positive enhancer of tyrosine kinase signaling pathways in breast cancer and it might thus function as an oncogene.
By using the second approach to identify PTPs involved in breast tumorigenesis, PTPε was indicated as a potential candidate for mammary gland hyperplasia. Not much is known about PTPε. It belongs to the same subfamily as PTPα and two mRNA variants of the protein exist: a full length transmembrane form (PTPεM) and a smaller variant only consisting of the intracellular part of the protein (PTPεC). Elson and Leder [34] demonstrated that PTPεM was expressed in normal mouse tissue during pregnancy, whereas PTPεC was expressed only during the mammary gland involution period. In addition they looked for PTP expression in transgenic mice that overexpress either *Ras*, *Myc*, *Neu* or *int*-2 proto-oncogenes in the mammary epithelium as overexpression of these proteins leads to the formation of breast tumors. They found that PTPεM (and not PTPεC) was highly expressed in murine mammary tumors initiated by *Ras* and *Neu*, but not in mammary tumors initiated by *Myc* or *int*-2. Further more, it was suggested that PTPε expression is mammary tumor specific as it has not yet been found in other *Ras*-based tumors and cell lines. As all these results still did not clarify the role of PTPε, Elson and Leder [34] developed specific transgenic mice expressing altered levels of PTPε. In the mouse model they found that overexpression of PTPε or an imbalance between PTPεM and PTPεC either plays a role in *Ras*- and *Neu*-mediated transformation of mammary epithelium or marks mammary epithelial cells particularly susceptible to transformation by these oncogenes. In addition to making transgenic mice overexpressing PTPε, Gil-Henn *et al.* [35] made transgenic mice possessing *Neu*-induced mammary tumors and lacking the PTPε gene. This led to the discovery that normally PTPε activates Src. When PTPε is lacking, Src activity is reduced. This is resulting in less transformed mammary epithelial tumors and reduced proliferation rates indicating that absence of PTPε is working as a tumorsuppressive impulse.
Another important PTP is DEP-1 as it has been associated with colon, lung and breast cancer [25]. In breast cancer cell lines, Keane *et al.* [36, 37] analyzed the expression of PTPs involved in cellular differentiation and growth inhibition. By using RT-PCR, 31 out of the 42 analyzed PTPs were found to be regulated in differentiating ZR75-1 (estrogen dependent) breast cancer cells, and among these 31 PTPs was DEP-1. Next to regulation

in estrogen dependent cells, DEP-1 was also regulated in differentiating, non-estrogen dependent SKBr-3 cells. The investigators decided to focus specifically on the role of DEP-1 in growth inhibition or induction of differentiation in breast cancer cells and they transfected ZR75-1, SKBR-3, and MCF-7 breast cancer cell lines with a DEP-1 cDNA construct under control of a constitutively active cytomegalovirus promoter. As these cells started to overexpress DEP-1, resistant colonies were selected with G418. The data revealed that overexpression of DEP-1 inhibited the development of resistant colonies by 3- to 5-fold in all three lines compared to mock transfection. And thus, overexpression of DEP-l in these breast cancer cell lines inhibits colony formation and could therefore be considered as a tumorsuppressor gene.

Currently the least is known about the importance of PTPBAS in the development of breast cancer. Freiss *et al.* [37] were able to indicate very low expression of this protein in breast cancer cell lines, and showed that expression of PTPBAS mRNA is upregulated in the presence of non-steroidal or steroidal antagonists of estrogen in MCF-7 breast cancer cells possessing the human estrogen receptor. Upregulated levels of PTPBAS mRNA caused inhibition of growth factor signaling in these cells and correlated with an increase in membrane PTP activity. The authors therefore concluded that PTPBAS was a key mediator for the antiestrogen-inhibitory action on the growth factor pathway thus making PTPBAS functioning as a tumorsuppressor in breast cancer cell growth.

Chapter 4: The role of PTP1B in breast cancer

As most PTPs involved in breast cancer have now been discussed, and their role in mammary tumorigenesis has briefly been explained, our focus will shift towards PTP1B. PTP1B was the first mammalian PTP identified and purified [38]. It is a ubiquitously expressed, classical NRPTP that has been indicated as the prototype for the superfamily of PTPs as it often holds true that features found for this PTP tend to extend to other PTPs as well. PTP1B has 435 amino acids and a molecular weight of 50 kDa [39]. The structure consists of a catalytic domain at the C-terminus followed by two tandem proline-rich motifs that allow the interaction with SH3-domain-containing proteins. In the C-terminus is a hydrophobic tail anchor sequence present which directs the protein to the cytoplasmic face of the ER [40].

Regulation of PTP1B

In the introduction the general methods to control PTP activity have been described. Though these mechanisms also hold true for the regulation of PTP1B, the regulation of this particular protein will be discussed in more detail. Regulation of PTP1B can occur at the gene transcription level and an example of this regulatory mechanism comes from the transcription factor Y-box-binding protein 1 (YB-1) as the promoter of the PTP1B gene contains an enhancer sequence that serves as a binding site for YB-1. Besides this, a positive correlation exists between the expression of PTP1B and that of YB-1 in cancer cell lines and animal models of type II diabetes.

The amount of PTP1B can also be controlled posttranslationally, thus at the protein level. Depending on the presence of the ER-anchor sequence, PTP1B exists in an ER bound form or in a plasmamembrane bound form. Cleavage of the anchor releases the enzyme from the ER and generates a 42 kDa cytoplasmic protein. This protein has enhanced specific activity and dephosphorylates a set of cellular substrates different from those encountered by the ER-targeted enzyme, thus indicating that subcellular localization is spatially and temporally regulating the accessibility of PTP1B to its substrates. Next to this, protein activity can also be altered more directly. In first place this occurs through Akt mediated phosphorylation of serine residues present in the catalytic domain of the protein. This results in a decreased ability of PTP1B to dephosphorylate the IR. Next to this, through EGF stimulation, PTP1B can be phosphorylated at tyrosine residues resulting in an increase in its catalytic activity indicating that phosphorylation can have multiple effects on PTP1B signaling. Another posttranslational control mechanism of PTP1B activity is based on reversible oxidation: reactive oxygen species produced in response to insulin or other extracellular stimuli can inhibit the activity of the protein by selectively modifying the active site cysteine residue.

And finally, the activity of PTP1B can also be modified by small ubiquitin-like modifier (SUMO) proteins. SUMOs are a family of small proteins that are covalently attached to and detached from other proteins to modify their function. Sumoylation of protein results in increased protein stabilization and/or redirection of the subcellular localization of the protein (reviewed in [41][42]).

There have been several studies implicating PTP1B signaling in the regulation of a wide variety of cellular events. It has been shown that PTP1B can dephosphorylate, and thus inactivate, various PTKs including the EGFR, platelet derived growth factor receptor

(PDGFR), IR, IGF-1R, p210Bcr-Abl, Janus kinase 2 (JAK2), and TYK2. But on the other hand, PTP1B can also positively regulate kinase signaling through dephosphorylation of Src and Ras mediated signaling (reviewed in [43]). From these findings it becomes clear that cellular activity regulated through PTP1B is very complex: PTP1B can either positively enhance cellular activity or it can inhibit cellular activity implicating PTP1B signaling in many different cellular functions. This functional diversity can be demonstrated by multiple studies that have implicated PTP1B in antagonizing insulin and insulin-like growth factor 1 (IGF-1) signaling, antagonizing signaling by the oncoprotein p210bcr-abl, suppressing transformation by *Neu*, *Crk*, *Src*, and *Ras*, and regulating integrin signaling (Reviewed in [44]). Based on these results, PTP1B does not have one specific function making it very difficult to suggest what would happen if PTP1B expression is altered. Overexpression of the protein can be beneficial for cells in a way that the protein functions as a tumorsuppressor by inhibiting cellular activity, but overexpression might as well upregulate cellular activity thereby making it a potential oncogene.

PTP1B has influence on many different receptors (see above) and can therefore be implicated in many different diseases, but it can be said that the contribution of PTP1B to the occurrence of metabolic disorders has been the most extensively studied. As it has been well established that PTP1B acts as a negative regulator of insulin signaling and helps cells to respond to metabolic stress, it does not come as a surprise that the phosphatase is involved in the occurrence of type II diabetes and obesity. The main feature of the disease is resistance to insulin and therefore PTP1B forms a good candidate disease gene as it is speculated that increased activity of PTP1B could contribute to the insulin resistance. This is making PTP1B a very attractive dug target for both type II diabetes and obesity and for that purpose, the pharmaceutical industry is attempting to make good PTP1B-inhibitors (reviewed in [6]).

It is known that PTP1B dephosphorylates multiple different kinases like EGFR, JAK2 and TYK2 involved in the control of cell growth, proliferation and cytokine signaling (response to interferon stimulation), so many investigators also started to assess the contribution of PTP1B to oncogenesis (and in particular to mammary tumorigenesis). One of the first things that needed to be investigated was what would happen to cells/animals if PTP1B would be absent. If PTP1B is a true tumorsuppressor, one would expect large amounts of tumors to occur in the absence of the protein. But if PTP1B functions as an oncogene, absence of the protein would lead to delay in tumor formation.

To elucidate this problem, in the late 1990s, two independent groups generated transgenic mice lacking the gene for PTP1B, *Ptpn1*$^{-/-}$ or PTPB1-null mice and as it turned out, the animals displayed a very striking phenotype. The mice were healthy but they did have enhanced insulin sensitivity due to the increased phosphorylation level of the IR. However, it was interesting to notice that the mice did not exhibit increased tumorigenesis, which is indicating that abolishment of PTP1B is negatively affecting cellular growth. Thus as deletion of PTP1B is correlated to the non-occurrence of tumors, it could be suggested that presence of PTP1B might be involved in tumorformation. And in that case, PTP1B would function as an oncogene (reviewed in [42]).

In a follow-up study, the effect of PTP1B abolishment has been assessed for its influence of RTK signaling in fibroblasts isolated from wild type and *Ptpn1*$^{-/-}$ mice. RTK signaling is important for tumor formation as overexpression or inappropriate activation of RTKs

results in higher levels of activated RTKs thereby influencing cell growth, differentiation, migration and survival. RTK signaling occurs through activation of multiple signaling cascades including the Ras/Erk and PI3K/Akt cascades [15]. The studies on fibroblasts revealed that in case of EGFR, PDGFR and IGF-1R signaling, absence of PTP1B leads to abolishment of dephosphorylation, resulting in an increased phosphorylation level of the receptors after stimulation by their ligands. It is intriguing to notice, however, that the downstream signaling cascades initiated by the increased activity of the kinases are different for the various receptors. In response to IGF-1 or PDGF, PTP1B-null fibroblasts exhibit increased Akt activation (promoting cellular survival) but decreased Erk activation while wild type fibroblasts treated with PDGF or EGF show an increase in Erk activation (promoting cellular proliferation) combined with a decrease in Akt activation. These results indicate that regulation of downstream RTK signaling through PTP1B is very complex. This has both advantages as this complexity will help to prevent spurious mitogenic signaling and it will make sure that the system can not be overruled by a deletion in a single PTP. But, a large disadvantage is the fact that this complexity makes it very difficult to determine the exact role of PTP1B in tumorigenesis (reviewed in [42]). Again, based on the data given above, PTP1B is most likely acting as an oncogene causing tumorigenesis but it can not be excluded that PTP1B might also exert some tumorsuppressor activity as it might lower RTKs activity and reduce cell growth.

Assessing the precise role of PTP1B in breast tumorigenesis

For the first time in 1993, Zhai *et al*. [45] discovered the presence of PTP1B in epithelial cancer. Later it became clear that PTP1B expression is modulated in various other types of cancer as well. In some types of cancer like esophageal cancer, PTP1B mRNA levels are decreased while in various other types of cancer cells, such as ovarian, breast and Bcr–Abl transformed chronic myelogenous leukemia cancer cells, overexpression of PTP1B has been reported [46]. Ever since, interest in PTP1B as a potential gene involved in breast tumorigenesis began to rise and several groups assessed the role of PTP1B in this process. And for the first time, in 1994, Wiener *et al*. [47] identified the presence of PTP1B in breast cancer. They assessed whether PTP1B was overexpressed in human mammary tumors and investigated if this overexpression is associated with the overexpression of ErbB2 as ErbB2 overexpression has been reported in 25% of the patients with breast cancer [27].

A short introduction into the ErbB family of receptors; the family consists of four closely related members: the EGFR, ErbB2, ErbB3 (HER3) and ErbB4 (HER4) which are involved in aspects of cell growth and division. Upon activation by a subset of potential growth factor ligands, the receptors are capable of forming homodimers, heterodimers, and possibly higher order oligomers. Though EGFR is able to signal independently as a homodimer, ErbB2 and ErbB3 must form heterodimers as they are non-functional on their own. In the case of ErbB2 this is due to the fact that there is no activating ligand for the receptor and thus it can only function in context of a heterodimer with a ligand bound receptor. While in case of ErbB3, the receptor is still able to bind ligand, but due to substitutions in critical residues in its catalytic domain the receptor has become catalytically inactive. Interestingly, though the ErbB2 and ErbB3 receptors have functional limitations making them unable to function on their own, an ErbB2 – ErbB3 signaling complex forms the most potent signaling module of the ErbB receptor family in

terms of cell growth and transformation. Signaling via the complex is normally involved in organ morphogenesis including cardiac, glial and neuronal, and mammary gland development. But the complex also has a high oncogenic potential. Ligand activated ErbB2 – ErbB3 complexes signals very potently through MAPK (proliferation), PI3K (apoptosis or cell growth), phospholipase-Cγ, protein tyrosine kinase C, and the Janus Kinase. As cancer does not necessarily arise only from an increase in cellular proliferation, the ErbB2 – ErbB3 complex can contribute to oncogenesis as signaling through the complex can disrupt the delicate balance between cell division and apoptosis thereby accelerating tumor formation. It is currently unknown if ErbB2 expression alone can transform cells, but it is well established that ErbB2 overexpression can be found in 25% of the patient's with breast cancer [27]. Therefore, ErbB2 can be categorized as an oncogene resulting in an aggressive form of breast cancer with a poor prognosis for patients (reviewed in [48]). So far it is not known how PTP1B and ErbB2 influence each other but it can be assumed they do; PTPs balance PTK activity during normal growth-regulation pathways, and PTP1B is involved in the linkage between different signaling cascades and may interface with inappropriate PTK activity in transformed cells, thus maybe also in ErbB2-induced breast cancer.

Wiener *et al*. [47] took a closer look into this intriguing, complicated relation between PTPs and RTKs as ErbB2 seems to be a key mediator in the occurrence of breast cancer and PTP1B might be involved as a potential cancer suppressor. By immunohisto-chemical staining, the presence of both proteins was assessed in normal human breast tissue and mammary tumor samples. It was found that the PTP1B protein was overexpressed in ~73% of the tumor samples compared to normal epithelium and statistical analysis revealed a significant association between this PTP1B overexpression and breast cancer ($P<.038$) but also between PTP1B overexpression and ErbB2 overexpression ($P<.006$). And finally, the investigators use Northern blot analysis to confirm that PTP1B overexpression was due to increased transcription of the PTP1B gene. From the experiments it was subsequently concluded that due to increased PTP1B transcription, overexpression of the protein is often found in human breast cancers and that this overexpression is associated with overexpression of ErbB2. But the authors were not able to discriminate whether PTP1B overexpression is balancing RTK activity, thus functioning as a tumorsuppressor, or whether PTP1B overexpression is the actual cause of the tumor formation.

Then in 2000, Bjorge *et al.* [44] investigated the cause of elevated c-Src tyrosine kinase activity in several types of human breast cancer cell lines. It was known that this elevated level could be due to elevated c-Src expression levels, increased c-Src specific activity or an activating mutation in c-Src, but instead, Bjorge *et al.* found that some cell lines with elevated c-Src levels also possess elevated phosphatase activity directed against the C-terminal negative regulatory domain of Src family kinases. They identified this phosphatase as PTP1B and suggested a regulatory role of this phosphatase in the control of c-Src kinases activity meaning that PTP1B could possibly function as an oncogene as its overexpression leads to upregulation of c-Src activity.

Finally, in 2007, Julien *et al.* [49] were able to provide grounded evidence that PTP1B functions as a oncogene as they were able to show that deficiency or inhibition of PTP1B delays ErbB2-induced mammary tumorigenesis and that it also protects from lung metastasis. In their search to elucidate the function of PTP1B, Julien *et al*. [49] used

several different approaches. First of all, they used a genetic approach. Many different "standard" transgenic mice expressing known mutations leading to specific (breast cancer) phenotype exist and it is therefore of great importance to use the model most closely resembling the origin. One type of transgenic mouse strain is based on an activating mutation in ErbB2. ErbB2 is often amplified in breast cancer and amplification of this gene can either be due to point mutations in the region of the gene encoding the transmembrane domain or through deletions or insertions in the extracellular domain. Transgenic mice have been made that carry a transgenic form of ErbB2 containing a five amino acid in-frame deletion in the extracellular domain caused under the control of mouse mammary tumor virus (MMTV) promoter. The animals are named '*Neu* deletion in extracellular domain 2' (NDL2) transgenic mice and they overexpress the gene specifically in their mammary glands. In females, this leads to the development of multiple mammary tumors and the mice frequently also have lung metastasis lesions. Use of this specifically mutated mouse-model is rather convenient as it relatively closely resembles human breast cancer tumorigenesis: the ErbB2/*Neu* alteration in the NDL2 transgenic mice has also been detected in human breast cancer and as the disease is progressing, expression of the gene in mice is associated with an increase in the level of phosphorylated ErbB2 and ErbB3, a feature frequently also seen in human breast cancer. And it has been established that 90% of all breast tumors overexpress both ErbB2 and PTP1B at the same time (reviewed in [49]).

In order to assess the function of PTP1B in ErbB2-dependent breast cancer tumorigenesis, Julien *et al*. [49] crossed NDL2 transgenic mice with *Ptpn1*$^{-/-}$ mice. Their first observation was that NDL2 *Ptpn1*$^{+/+}$ mice developed tumors at an average age of 147.5 days whereas NDL2 *Ptpn1*$^{-/-}$ mice did not develop tumors at this stage. Instead, their tumor growth was delayed by ~85 days compared to the NDL2 *Ptpn1*$^{+/+}$ mice. As a control NDL2 *Ptpn1*$^{+/-}$ mice were generated and these animals showed an intermediate profile of tumor occurrence, the first tumors could be detected ~35 days after NDL2 *Ptpn1*$^{+/+}$ mice (Figure 4, left). These results gave the first hint in the function of PTP1B in breast cancer occurrence – it provides genetic evidence that PTP1B contributes to ErbB2-induced mammary tumor occurrence and malignancy and there are indications that the absence of one allele of *Ptpn1* is sufficient to somewhat delay the occurrence and malignancy of breast cancer.

As was described before, ErbB2-induced breast cancer is often accompanied by lung metastasis and thus the role of PTP1B in the occurrence of these tumors was also assessed. As it turns out, 4 weeks after mammary tumor onset lung metastasis could be detected in NDL2 *Ptpn1*$^{+/+}$ mice. But in the absence of PTP1B or with reduced levels of PTP1B the occurrence of lung metastasis could not be detected 4 weeks after the onset of mammary tumors, and even 6 weeks later the level of lung metastasis was still reduced compared to the PTP1B expressing mice (figure 4, right). Again these results are indicating that in the absence of PTP1B, breast cancer malignancy is delayed and counteracted as the level of secondary metastasis is decreased.

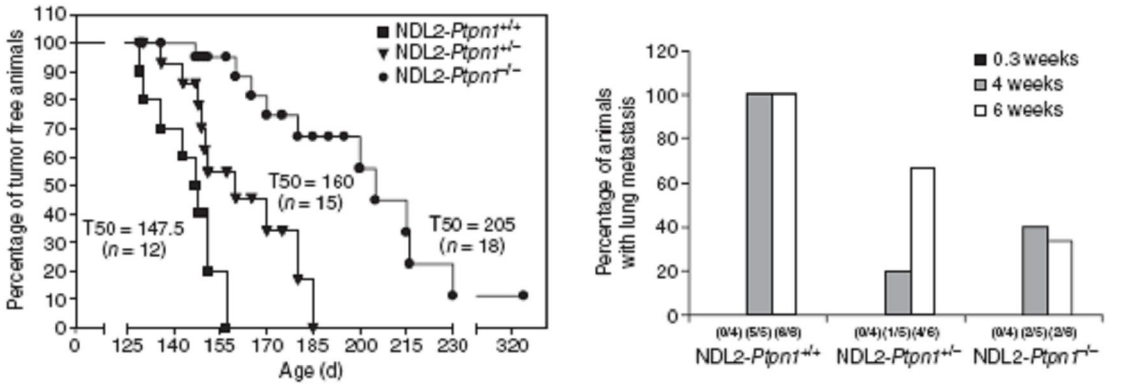

Figure 4: reduction in the rate of tumor development (left) and occurrence of lung metastases (right) in NDL2 *Ptpn1*$^{-/-}$ mice (Adapted from [49])

Left, Kaplan-Meier kinetic analysis of tumor occurrence in NDL2 *Ptpn1* female transgenic mice: after weaning of the second litter the mother-mice were examined twice a week in order to detect mammary tumors. The curves were drawn and analyzed using Prism software. Log rank tests of survival plots of the data indicated a statistically significant difference between each survival curve: NDL2 *Ptpn1*$^{+/+}$ versus NDL2 *Ptpn1*$^{+/-}$ (P = 0.004); NDL2 *Ptpn1*$^{+/+}$ versus NDL2 *Ptpn1*$^{-/-}$ (P = 0.0001); and NDL2 *Ptpn1*$^{+/-}$ versus NDL2 *Ptpn1*$^{-/-}$ (P = 0.001). Right, Lung metastases were monitored by histopathological analysis from hematoxylin and eosin–stained lung sections from all animals presented in the left graph, and the (numbers) represent the frequency of lung metastases. (n) = number of animals analyzed for each genotype, (T50) = median time to tumor onset.

Next to the genetic approach, a pharmacological approach was also used to assess the role of PTP1B in tumorigenesis. The genetic results provided strong evidence that PTP1B has a critical and positive role in human ErbB2-induced breast cancer and from this, it has been indicated that PTP1B inhibition might be beneficial in the treatment of the disease. However, before speculations can be made on PTP1B as a potential drug target, it needs to be determined if PTP1B (besides the genetic inhibition) can be inhibited pharmacologically. To assess if pharmacological inhibition of PTP1B leads to the same results as genetic PTP1B inhibition, Julien *et al*. [49] treated NDL2 *Ptpn1* mice with an oral phosphatase inhibitor specific for PTP1B and they monitored the effect in animals by measuring the glucose levels during the 21 days of treatment (it is known that PTP1B deficiency enhances insulin sensitivity and decrease blood glucose levels, thus forming an easy measurable parameter). The results showed that treatment of NDL2 *Ptpn1*$^{+/+}$ transgenic mice with the PTP1B inhibitor resulted in lowering of blood glucose over the time course of the treatment to the levels comparable to those observed in NDL2 *Ptpn1*$^{-/-}$ mice. Treatment of NDL2 *Ptpn1*$^{-/-}$ mice with the inhibitor had no effect on blood glucose levels indicating specific target engagement. From these results it can be suggested that the pharmacological inhibitor has the same effect on PTP1B deficiency as NDL2 *Ptpn1*$^{-/-}$ mice, and therefore the formation of tumors can be assessed after treatment with the inhibitor as it seems that it is specific.

NDL2 *Ptpn1* transgenic mice were treated with either a vehicle or the PTP1B inhibitor and they were followed in a time course in order to monitor the onset of mammary tumor formation after the therapy was stopped. This approach led to the discovery that the onset of mammary tumor formation was significantly delayed in NDL2 *Ptpn1*$^{+/+}$ mice treated with the inhibitor (T50 = 75 days after treatment stop) compared to similar mice treated with a vehicle (T50 = 28 days after treatment stop) (Figure 5) and that vehicle and

PTP1B-inhibitor treatment did not have any effect on NDL2 *Ptpn1*$^{-/-}$ mice. Therefore, these results strongly indicate that PTP1B might represent a valuable therapeutic target for anticancer treatment.

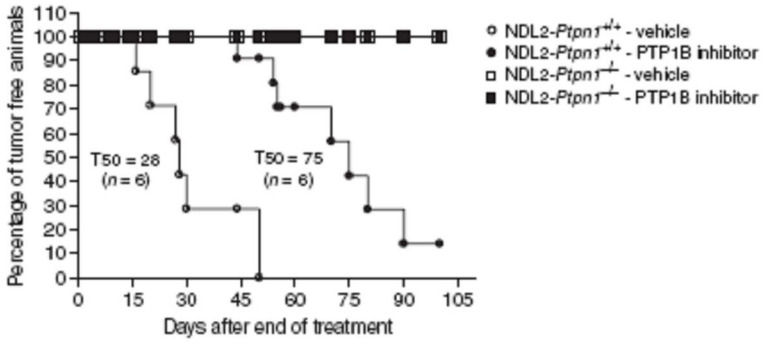

Figure 5: Kaplan-Meier kinetic analysis showing a delay in the onset of mammary tumor development in NDL2 *Ptpn1*$^{+/+}$ female transgenic mice treated with a PTP1B inhibitor (Adapted from [49])
Mice were examined twice per week after the end of treatment in order to detect mammary tumors. The curves were drawn and analyzed using Prism software. Log rank tests of survival plots of the data indicate a statistically significant difference between NDL2 *Ptpn1*$^{+/+}$ receiving the inhibitor and NDL2 *Ptpn1*$^{+/+}$ mice receiving the vehicle ($P < 0.001$). (n) = number of animals analyzed for each genotype, (T50) = median time to tumor onset.

As Julien *et al*. [49] had found strong indications that PTP1B actually functions as an oncogene, causing formation of mammary tumors and contributing to the malignancy of these tumors (potential to cause metastasis), the investigators also assessed if overexpression of PTP1B in healthy breast tissue would lead to tumor initiation and progression. For this purpose transgenic mice were created that, under the control of the MMTV promoter, express wild type PTP1B fused with enhanced green fluorescence protein (PTP1B-EGFP) in their mammary gland. Because the expression of PTP1B-EGFP was under the MMTV promoter, the level of transgenic protein expression directly correlated with increasing numbers of litters. Next to this, it was also noticed that at the same time, the levels of PTP-EGFP increased and that mammary tumors were formed. The formation of the tumors related to the number of litters delivered by the mother. After two pregnancies mice presented normal mammary ductal hyperplasia, but after seven pregnancies mice showed induced adenocarcinoma infiltrating the fat pad. These results indicate that PTP1B is able to drive tumor formation and it can therefore be concluded that PTP1B possesses oncogenic properties or that it at least contributes positively to the process of cellular transformation.

Chapter 5: Discussion

In this thesis classical receptor and non-receptor PTPs have been discussed for their involvement in health and disease. Unlike very early assumptions (PTPs are housekeeping genes) the enzymes turned out to be important mediators of cellular homeostasis and clear associations have been established between altered signaling as a result of change in PTP activity and disease. Out of the 99 class I PTPs known to exist in man, only the 38 classical PTPs have been discussed and the 69 remaining PTPs were not included as there is only limited data available on these proteins. As we are gaining more knowledge on classical PTPs, we are discovering that they exert many more functions then assumed so far. And with the discovery of these additional functions, the proteins can also be linked to more disease phenotypes. This is finally resulting in an increase in the importance of PTPs for disease incidence and thus additional research on PTPs needs to be encouraged. Next to the fact that the role of many PTPs is currently unknown, the role of the investigated PTPs is often inconclusive or suggestive stressing the need for additional attention on these proteins as well.

Today, various PTPs have been associated with tumorigenesis but it is often unclear whether they functions as an oncogene or as tumorsuppressors. This can be illustrated by the fact that alterations in PTP signaling have very diverse effects on cellular signaling. Upregulation of PTP activity results in increased dephosphorylation of RTKs. For some RTKs this leads to inhibition of their activity while others are activated through dephosphorylation. This indicates that increased PTP activity can result in both oncogenic as well as tumorsuppressive potentials. When PTP activity is decreased, the same mechanism holds true: some RTKs will be over activated while others will be downregulated. Based on the intriguing interplay between RTK – PTP activity and the effects this interplay has on cellular functioning, it can not be excluded that some PTPs actually possess both oncogenic as well as tumorsuppressive capacities.

In order to discriminate between oncogenic and tumorsuppressive potential of PTPs various approaches can be followed. They include research into when and where the PTP-gene is transcribed, how gene and protein levels are regulated, which substrates are subject to the activity of the protein, what happens to the substrates when dephosphorylated (activation or inhibition) and which downstream signaling cascades are initiated/inhibited and what the consequence is of this signal initiation. These questions can be addressed by studying cell cultures, where genes can be overexpressed or deleted and effects of these alterations can be indicated in changes in cellular growth and morphology. But most importantly, the PTPs also need to be studied in whole organisms (e.g. mouse models) as the alteration of PTP activity might influence broad aspects of cellular signaling having major effects on normal functioning of organisms.

For a long time, strong indications existed that PTP1B was involved in the occurrence of breast cancer, but it was inconclusive whether the protein possessed oncogenic or tumorsuppressive activity. Finally, in 2007 Julien *et al.* [49] came with a break through. Both genetic and pharmacological methods were used to assess the contribution of PTP1B to breast tumorigenesis and they found that the protein functions as an oncogene: deletion of the gene delays primary and secondary tumor formation while expression of the protein in non-carcinogenic healthy tissue is initiating the formation of tumors. To exclude the possibility that the observed effects were an artifact of transgenesis, Julien *et*

al. [49] did control experiments. They observed that the pharmacological inhibition resulted in the same phenotype as was observed in genetically deficient mice demonstrating that the observations in these mice are caused by the specific genetic mutation and that PTP1B truly functions as an oncogene.
So far, the presence of PTP1B has only been confirmed in breast cancer and implicated in ovarian cancer (supplementary table) but as PTP1B is a ubiquitously expressed protein it might be possible that it can promote oncogenic signaling in various other tissues as well. Therefore, the contribution of this protein to the occurrence of other types of cancer should be assessed more extensively. Nowadays this can be done fairly easily by using good genetic tools which enable researchers to perform PTK and PTP expression profiling in human cancers. This profiling can be done in tissue samples derived from healthy and diseased individuals but also in (cancerous) cell lines. In 2004 Wang *et al.* [22] performed a mutational analysis of the tyrosine phosphatase gene superfamily to assess which PTPs are involved in colon cancer. With their screen, they were able to identify 6 phosphatases affecting 26% of the colorectal cancers and in smaller amount these PTPs also affected lung, breast and gastric cancers. This is a strong indication that genetic screenings are very useful tools for assessing the role of PTPs in the occurrence of cancers and a tool that could be used more often. With our expanding knowledge of PTPs, it is worthwhile to repeat some of the previous genetic screenings. For the largest part the test results will be comparable, but there is a chance that new/additional PTPs or different mutations in PTPs will be found. This could in part be due to improvements in the assay itself and improved practical skills but new methods have also been developed to analyze the results leading to a better understanding of the results. Besides technical advantages, our general knowledge on PTP – RTK signaling has been improved which is also beneficial for the interpretations of the screening results. The functions of individual PTPs start to become clear and suggestions of involvement of these proteins in particular diseases can now be assessed with the genetic screens.
As we are beginning to better understand the complete and complex role of normal PTP functioning, we are also gaining better insight in the role of PTPs during disease and PTPs subsequently have become important drug targets. The most profound example comes from the role of PTP1B in type II diabetes and obesity. Many different biochemical, genetic and pharmacological studies have provided evidence that inhibition of the protein could result in an improved glucose tolerance and insulin sensitivity, validating the suggestion that PTP1B inhibition could be a beneficial treatment. Though various different pharmacological compounds are available that inhibit PTP1B (reviewed in [50]), the pharmaceutical industry has not succeeded yet in making a potent and selective PTP1B inhibitor that is orally available and has the desirable physicochemical properties and in vivo efficacies. However, they continue to search for such a product and it has frequently been suggested that those PTP1B inhibitors may also be beneficial drugs for breast cancer patients as the drug may help to reduce breast cancer growth and delay (or even prevent) the development of metastasis.

References

1. Barford, D., *Protein phosphatases*. Curr Opin Struct Biol, 1995. **5**(6): p. 728-34.
2. Neel, B.G. and N.K. Tonks, *Protein tyrosine phosphatases in signal transduction*. Curr Opin Cell Biol, 1997. **9**(2): p. 193-204.
3. Cool, D.E. and E.H. Fischer, *Protein tyrosine phosphatases in cell transformation.* Semin Cell Biol, 1993. **4**(6): p. 443-53.
4. Hunter, T., *Protein kinases and phosphatases: the yin and yang of protein phosphorylation and signaling*. Cell, 1995. **80**(2): p. 225-36.
5. Alonso, A., et al., *Protein tyrosine phosphatases in the human genome*. Cell, 2004. **117**(6): p. 699-711.
6. Andersen, J.N., et al., *A genomic perspective on protein tyrosine phosphatases: gene structure, pseudogenes, and genetic disease linkage.* Faseb J, 2004. **18**(1): p. 8-30.
7. Honda, R., et al., *Dephosphorylation of human p34cdc2 kinase on both Thr-14 and Tyr-15 by human cdc25B phosphatase.* FEBS Lett, 1993. **318**(3): p. 331-4.
8. Tootle, T.L., et al., *The transcription factor Eyes absent is a protein tyrosine phosphatase.* Nature, 2003. **426**(6964): p. 299-302.
9. Walton, K.M. and J.E. Dixon, *Protein tyrosine phosphatases.* Annu Rev Biochem, 1993. **62**: p. 101-20.
10. Barford, D., et al., *Purification and crystallization of the catalytic domain of human protein tyrosine phosphatase 1B expressed in Escherichia coli.* J Mol Biol, 1994. **239**(5): p. 726-30.
11. Fauman, E.B. and M.A. Saper, *Structure and function of the protein tyrosine phosphatases.* Trends Biochem Sci, 1996. **21**(11): p. 413-7.
12. Stuckey, J.A., et al., *Crystal structure of Yersinia protein tyrosine phosphatase at 2.5 A and the complex with tungstate.* Nature, 1994. **370**(6490): p. 571-5.
13. Tonks, N.K., et al., *Signal transduction and protein tyrosine dephosphorylation.* Adv Second Messenger Phosphoprotein Res, 1993. **28**: p. 203-10.
14. Mourey, R.J. and J.E. Dixon, *Protein tyrosine phosphatases: characterization of extracellular and intracellular domains*. Curr Opin Genet Dev, 1994. **4**(1): p. 31-9.
15. Blume-Jensen, P. and T. Hunter, *Oncogenic kinase signalling.* Nature, 2001. **411**(6835): p. 355-65.
16. Meakin, S.O. and E.M. Shooter, *Molecular investigations on the high-affinity nerve growth factor receptor*. Neuron, 1991. **6**(1): p. 153-63.
17. Mustelin, T. and T. Hunter, *Meeting at mitosis: cell cycle-specific regulation of c-Src by RPTPalpha.* Sci STKE, 2002. **2002**(115): p. PE3.
18. den Hertog, J., A. Ostman, and F.D. Bohmer, *Protein tyrosine phosphatases: regulatory mechanisms.* Febs J, 2008. **275**(5): p. 831-47.
19. Ostman, A. and F.D. Bohmer, *Regulation of receptor tyrosine kinase signaling by protein tyrosine phosphatases.* Trends Cell Biol, 2001. **11**(6): p. 258-66.
20. Schaapveld, R., B. Wieringa, and W. Hendriks, *Receptor-like protein tyrosine phosphatases: alike and yet so different.* Mol Biol Rep, 1997. **24**(4): p. 247-62.
21. Matozaki, T. and M. Kasuga, *Roles of protein-tyrosine phosphatases in growth factor signalling.* Cell Signal, 1996. **8**(1): p. 13-9.

22. Wang, Z., et al., *Mutational analysis of the tyrosine phosphatome in colorectal cancers*. Science, 2004. **304**(5674): p. 1164-6.
23. Ardini, E., et al., *Expression of protein tyrosine phosphatase alpha (RPTPalpha) in human breast cancer correlates with low tumor grade, and inhibits tumor cell growth in vitro and in vivo*. Oncogene, 2000. **19**(43): p. 4979-87.
24. den Hertog, J., et al., *Receptor protein tyrosine phosphatase alpha activates pp60c-src and is involved in neuronal differentiation*. Embo J, 1993. **12**(10): p. 3789-98.
25. Ruivenkamp, C.A., et al., *Ptprj is a candidate for the mouse colon-cancer susceptibility locus Scc1 and is frequently deleted in human cancers*. Nat Genet, 2002. **31**(3): p. 295-300.
26. Freiss, G. and F. Vignon, *Protein tyrosine phosphatases and breast cancer*. Crit Rev Oncol Hematol, 2004. **52**(1): p. 9-17.
27. Hutchinson, J.N. and W.J. Muller, *Transgenic mouse models of human breast cancer*. Oncogene, 2000. **19**(53): p. 6130-7.
28. LaForgia, S., et al., *Receptor protein-tyrosine phosphatase gamma is a candidate tumor suppressor gene at human chromosome region 3p21*. Proc Natl Acad Sci U S A, 1991. **88**(11): p. 5036-40.
29. Zheng, J., et al., *17 beta-estradiol-regulated expression of protein tyrosine phosphatase gamma gene in cultured human normal breast and breast cancer cells*. Anticancer Res, 2000. **20**(1A): p. 11-9.
30. Yang, T., et al., *Leukocyte common antigen-related tyrosine phosphatase receptor: increased expression and neuronal-type splicing in breast cancer cells and tissue*. Mol Carcinog, 1999. **25**(2): p. 139-49.
31. Levea, C.M., et al., *PTP LAR expression compared to prognostic indices in metastatic and non-metastatic breast cancer*. Breast Cancer Res Treat, 2000. **64**(2): p. 221-8.
32. Zheng, X.M., Y. Wang, and C.J. Pallen, *Cell transformation and activation of pp60c-src by overexpression of a protein tyrosine phosphatase*. Nature, 1992. **359**(6393): p. 336-9.
33. Yip, S.S., et al., *Up-regulation of the protein tyrosine phosphatase SHP-1 in human breast cancer and correlation with GRB2 expression*. Int J Cancer, 2000. **88**(3): p. 363-8.
34. Elson, A. and P. Leder, *Protein-tyrosine phosphatase epsilon. An isoform specifically expressed in mouse mammary tumors initiated by v-Ha-ras OR neu*. J Biol Chem, 1995. **270**(44): p. 26116-22.
35. Gil-Henn, H. and A. Elson, *Tyrosine phosphatase-epsilon activates Src and supports the transformed phenotype of Neu-induced mammary tumor cells*. J Biol Chem, 2003. **278**(18): p. 15579-86.
36. Keane, M.M., et al., *Chemotherapy augments TRAIL-induced apoptosis in breast cell lines*. Cancer Res, 1999. **59**(3): p. 734-41.
37. Freiss, G., C. Puech, and F. Vignon, *Extinction of insulin-like growth factor-I mitogenic signaling by antiestrogen-stimulated Fas-associated protein tyrosine phosphatase-1 in human breast cancer cells*. Mol Endocrinol, 1998. **12**(4): p. 568-79.

38. Tonks, N.K., C.D. Diltz, and E.H. Fischer, *Characterization of the major protein-tyrosine-phosphatases of human placenta*. J Biol Chem, 1988. **263**(14): p. 6731-7.
39. Chernoff, J., et al., *Cloning of a cDNA for a major human protein-tyrosine-phosphatase.* Proc Natl Acad Sci U S A, 1990. **87**(7): p. 2735-9.
40. Frangioni, J.V., et al., *The nontransmembrane tyrosine phosphatase PTP-1B localizes to the endoplasmic reticulum via its 35 amino acid C-terminal sequence*. Cell, 1992. **68**(3): p. 545-60.
41. Bourdeau, A., N. Dube, and M.L. Tremblay, *Cytoplasmic protein tyrosine phosphatases, regulation and function: the roles of PTP1B and TC-PTP.* Curr Opin Cell Biol, 2005. **17**(2): p. 203-9.
42. Stuible, M., K.M. Doody, and M.L. Tremblay, *PTP1B and TC-PTP: regulators of transformation and tumorigenesis.* Cancer Metastasis Rev, 2008. **27**(2): p. 215-30.
43. Dube, N. and M.L. Tremblay, *Involvement of the small protein tyrosine phosphatases TC-PTP and PTP1B in signal transduction and diseases: from diabetes, obesity to cell cycle, and cancer*. Biochim Biophys Acta, 2005. **1754**(1-2): p. 108-17.
44. Bjorge, J.D., A. Pang, and D.J. Fujita, *Identification of protein-tyrosine phosphatase 1B as the major tyrosine phosphatase activity capable of dephosphorylating and activating c-Src in several human breast cancer cell lines.* J Biol Chem, 2000. **275**(52): p. 41439-46.
45. Zhai, Y.F., et al., *Increased expression of specific protein tyrosine phosphatases in human breast epithelial cells neoplastically transformed by the neu oncogene*. Cancer Res, 1993. **53**(10 Suppl): p. 2272-8.
46. Dube, N., A. Cheng, and M.L. Tremblay, *The role of protein tyrosine phosphatase 1B in Ras signaling*. Proc Natl Acad Sci U S A, 2004. **101**(7): p. 1834-9.
47. Wiener, J.R., et al., *Overexpression of the protein tyrosine phosphatase PTP1B in human breast cancer: association with p185c-erbB-2 protein expression.* J Natl Cancer Inst, 1994. **86**(5): p. 372-8.
48. Citri, A., K.B. Skaria, and Y. Yarden, *The deaf and the dumb: the biology of ErbB-2 and ErbB-3*. Exp Cell Res, 2003. **284**(1): p. 54-65.
49. Julien, S.G., et al., *Protein tyrosine phosphatase 1B deficiency or inhibition delays ErbB2-induced mammary tumorigenesis and protects from lung metastasis.* Nat Genet, 2007. **39**(3): p. 338-46.
50. Zhang, S. and Z.Y. Zhang, *PTP1B as a drug target: recent developments in PTP1B inhibitor discovery*. Drug Discov Today, 2007. **12**(9-10): p. 373-81.
51. WHO, fact sheetº297, July 2008.

Appendixes

Supplementary table

Name		Chr Location	PubMed and OMIM Search	References	Comments
PTP	Gene	UCSC View			
LyPTP	PTPN22	1p13.2	Region associated with rearrangements in solid and hemopoietic tumors, SNP polymorphism in type I diabetes	Cohen *et al.*, Hill *et al.*, Botinni *et al.*, 2004	Expressed in lymphoid tissues, associates with Grb2 and oncogene CBL, negative regulator of T-cell signaling
LAR	PTPRF	1p34.2	Frequently deleted in human neuroblastoma, small cell lung cancer (coamplification of LAR and MYCL1), increased expression in breast cancer	Jirik *et al.*, Harder *et al.*, Zabolotny *et al.*, Yang *et al.* 1999, Levea *et al.*2000	Tumor suppressor? Insulin resistance?
PTPλ	PTPRU	1p35.2	Parkinson, onset		
CD45	PTPRC	1q31.3	Autoimmunity, Combined Immune Deficiency Syndrome (SCID) and Multiple sclerosis Susceptibility to HIV-1 infection	Jacobsen *et al.*, Vorechovsky *et* al., Barcellos *et al.*, Kung *et al.*, Tchilian *et al.*	
HePTP	PTPN7	1q32.1	Non-Hodgkin lymphomas		
PTPIA2	PTPRN	2q35	Autoantigens in type I insulin-dependent diabetes mellitus	Cui *et al.*, Solimena *et al.* 1996	
PTPγ	PTPRG	3p14.2	3p14.2 hot spot for alterations in lung, colorectal, renal and early-stage breast cancer	LaForgia *et al.* 1991, Pitterle *et al.*, Panagopoulos *et al.*, Wang *et al.* Zheng *et al.* 2000	Candidate tumor suppressor gene in renal and lung carcinoma, 1 PTP allele was lost in 3 of 5 renal carcinoma cell lines and in 5 of 10 lung carcinoma tumor samples tested
PTPBAS	PTPN13	4q21.3	4q21.3 frequently deleted in liver and ovarian cancers, low expression in breast cancer cell lines	Inazawa *et al.*, Freiss 1998	Putative tumor suppressor genes
PTPκ	PTPRK	6q22.33	Tumor supressor, frequently deleted in primary CNS lymphomas	Zhang *et al.*, Nakamura *et al.*	Putative tumor suppressor gene region 6q22.2-q22.3
PEST	PTPN12	7q11.23	Aberrant transcripts in colon cancer, important for cell adhesion and motility, and sequence variations have been found primary human breast and kidney tumor samples	Takekawa *et al.*, Streit *et al.* 2006	Aberrant transcripts found in colorectal carcinoma cell line (DLD-1) including a missense point mutation, a 77 bp and 173 bp deletion

Supplementary table, continued

PTPIA2β	PTPRN2	7q36.3	Autoantigens in type I insulin-dependent diabetes mellitus	Cui *et al.*, Kawasaki *et al.* 1996	Autoantibodies to IAR are significantly more predictive of disease than those to IA2
DEP-1	PTPRJ	11p11.2	Colon cancer somatic mutations, susceptibility to colon cancer-1, indicated in lung and breast Cancer	Ruivenkamp *et al.* 2002, Watanabe *et al.* 2002	Ptprj is a candidate for the mouse colon-cancer susceptibility locus Scc1 and is frequently deleted in human cancers
SH-PTP1	PTPN6	12p13.31	Sezary syndrome, leukemogenesis, experimental autoimmunity encephalomyelitis, and severe congenital neuropenia (Kostmann's syndrome)	Beghini *et al.*, Leon *et al.*, Deng *et al.*, Tidow *et al.*, Oka T *et al.*, Andersen *et al.* 2004, Yip 2000	Aberrant splicing within the N- SH2 domain, abberant splicing was lower in CD117(+)-AML bone marrow mononuclear cells at remission than at diagnosis
PCPTP1	PTPRR	12q15	Type II diabetes?	Bektas *et al.*	
SH-PTP2	PTPN11	12q24.13	Noonan syndrome 1, cardiofaciocutaneous syndrome, AML, type II diatebes?	Tartaglia *et al.*, Kavamura *et al.*	Ubiquitously expressed
SAP1	PTPRH	19q13.42	Overexpressed in colorectal cancer. Not a Peutz-Jeghers syndrome candidate	Seo *et al.*, Marneros *et al.*	
PTPρ	PTPRT	20q12-q13.11	Myeloproliferative disorders, colorectal, gastric and lung cancer? Type II diabetes?	Wang *et al.* 2004	
PTP1B	PTPN1	20q13.13	Insulin resistance, obesity quantitative trait loci, breast carcinomas, ovarian cancer	Echwald, Dipaola, Gu, Lee, Hunt, Lembertas, Zodervan, Tanner Kononen, Montagne, Andersen	
PTPα	PTPRA	20p13	Primary breast carcinomas, late-stage colorectal Tumors	Ardini *et al.* 2000, Tabiti *et al.* 1995, Su 1999	
PTP-meg2	PTPN9	15q24.2	Autism	Smith *et al.* 2000	
PTEN	PTEN	10q23.3	Bannayan-Zonana, Cowden syndrome, Lhermitte-Duclos disease	Marsh *et al.* 1997, Liaw *et al.* 1997	
PEZ	PTPN14	1q32.2	Colorectal cancers	Wang *et al.* 2004	
PTPH1	PTPN3	9q31	Colorectal cancers	Wang *et al.* 2004	
PTPε	PTPRE	10q26	Breast cancer, mammary gland hyperplasia	Elson and Leder 1995, Gill-Henn *et al.* 2003	

Table is made based on a table provided by Andersen *et al.* [6] (Supplementary table 4) and is extended with information from a table by Alonso *et al.* [5] (table 3). Further more, the combined table is extended with information found during the literature study. All references are put together in the fourth column of the final table.

List of abbreviations

B cell	B lymphocyte
CA	Carbonic anhydrase-like
Cdcks	Cell division cycle kinases
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor, also known as HER-1 or ErbB1
ER	Endoplasmic reticulum
IR	Insulin receptor
IGF-1	Insulin-like growth factor 1
IGF-1R	Insulin-like growth factor 1 receptor
JAK2	Janus kinase 2
MAM	Meprin-*Xenopus* A5-mu
MMTV	Mouse mammary tumor virus
NDL2	*Neu* deletion in extracellular domain 2
NRPTPs	Non-receptor protein tyrosine phosphatase
PDGFR	Platelet derived growth factor receptor
PO_4	Phosphate group
PTK	Protein tyrosine kinase
PTP	Protein tyrosine phosphatase
PTPεM	Transmembrane protein tyrosine phosphatase ε
PTPεC	Intrecellular protein tyrosine phosphatase ε
PTP1B	Protein tyrosine phosphatase 1B
PTP1B-EGFP	PTP1B fused with enhanced green fluorescence protein
RPTP	Transmembrane receptor-like protein tyrosine phosphatase
RTK	Receptor tyrosine kinase
SH (domain)	Src homology (domain)
SH-PTP1	Src homology-protein tyrosine phosphatase 1
SH-PTP2	Src homology-protein tyrosine phosphatase 2
SUMO	Small ubiquitin-like modifier
T cell	T lymphocyte
YB-1	Transcription factor Y-box-binding protein 1