Benjamin G. Neel
Nicholas K. Tonks *Editors*

Protein Tyrosine Phosphatases in Cancer

Springer

Protein Tyrosine Phosphatases in Cancer

Benjamin G. Neel • Nicholas K. Tonks
Editors

Protein Tyrosine Phosphatases in Cancer

Springer

Editors
Benjamin G. Neel
Laura and Isaac Perlmutter Cancer Center
New York, NY, USA

Nicholas K. Tonks
Cold Spring Harbor Laboratory
Cold Spring Harbor, NY, USA

ISBN 978-1-4939-3647-2 ISBN 978-1-4939-3649-6 (eBook)
DOI 10.1007/978-1-4939-3649-6

Library of Congress Control Number: 2016948122

Printed on acid-free paper

This Springer imprint is published by Springer Nature
The registered company is Springer Science+Business Media New York

Preface

A crucial aspect of signal transduction, the mechanism by which cells respond to environmental cues, is the organization of coordinated networks of protein–protein interactions, a process mediated by protein phosphorylation. Disruption of the normal patterns of phosphorylation results in aberrant regulation of signal transduction and has been implicated in the etiology of a variety of major human diseases, including cancer. The ability to modulate signaling pathways selectively holds enormous therapeutic potential. Although inhibitors of protein tyrosine kinases (PTKs) have yielded some spectacular success stories, such as Gleevec/Imatinib and Herceptin/Trastuzumab, challenges remain. In particular, although initially there may be a dramatic response, patients soon develop resistance to the therapy. Therefore, despite the obvious potential of targeting PTKs, particularly in the context of developments in personalized medicine, it is anticipated that alternative therapies, to target simultaneously different signaling enzymes and events, would be more effective than targeting PTKs alone.

The focus on PTKs for drug development ignores the other major component of phosphorylation-dependent regulation of signaling. Protein phosphorylation is a reversible process, in which the coordinated and competing activities of kinases and phosphatases are important for determining signaling outcome. One of the challenges facing those working on the protein tyrosine phosphatases (PTPs), which function in combination with the PTKs, has been to overcome the prejudice in the field that sees these enzymes dismissed as a family of constitutively active, nonspecific housekeeping enzymes. As will become evident upon reading the reviews assembled in this book, this view of the PTPs is totally without foundation. By contrast, PTPs function as specific regulators of tyrosine phosphorylation-dependent signaling pathways. Furthermore, direct links have been established between the disruption of PTP function and the etiology of major diseases, including metabolic diseases and cancer. As such, the PTPs have been validated as potential therapeutic targets and offer a complementary perspective to the protein kinases through which to develop novel strategies to treat major diseases, including cancer.

In this book, we present a collection of reviews that describe recent developments in our understanding of the function of members of the PTP family, with a

particular focus on their roles in cancer and their potential to form the basis for therapeutic strategies to address this and other major diseases. Our goal is to illustrate that the regulation of signal transduction depends upon integrating the function of PTKs and PTPs, with *both* classes of enzymes serving crucial roles. Contrary to the view of the PTPs as housekeeping enzymes, their structural diversity, their specificity, their ability to function both positively and negatively to regulate signaling, and their links to the etiology of major human diseases, including cancer, all attest to a fundamental role for the PTPs. As further examples of the crucial role of PTPs in the regulation of signaling under normal and pathophysiological conditions are established in the coming years, we hope that these enzymes will be exploited fully for therapeutic development.

New York, NY, USA B.G. Neel
Cold Spring Harbor, NY, USA N.K. Tonks Toronto

Contents

Time to Shine the Spotlight on the Protein Tyrosine Phosphatase Family of Signal Transducing Enzymes

Nicholas K. Tonks

Abstract Although these are exciting times to be working on the protein tyrosine phosphatase (PTP) family of enzymes, they are also challenging times. The detailed characterization of many of the PTPs has revealed fundamentally important roles for these enzymes in the control of cell function. Furthermore, direct links have been established between disruption of PTP function and the etiology of major diseases, including metabolic diseases and cancer. As such, the PTPs have been validated as therapeutic targets and offer a complementary perspective to the protein kinases through which to develop novel strategies to treat major diseases, including cancer. This book presents a collection of reviews that describe recent developments in our understanding of the function of members of the PTP family, with a particular focus on their roles in cancer and their potential to form the basis for therapeutic strategies to address this and other major diseases.

Keywords Signal transduction • Protein tyrosine phosphatase • Protein tyrosine kinase • Drug discovery • Oncogene • Tumor suppressor • Cancer • Diabetes and obesity • Cell adhesion • Redox

The reversible addition and removal of phosphate to proteins, which is termed protein phosphorylation, is the central feature of the mechanism of signal transduction—the process by which cells respond to stimuli in their environment. The activities of the enzymes that mediate the addition (kinases) and removal (phosphatases) of phosphate groups are coordinated in signal transduction pathways to mediate the cellular response to environmental stimuli and the function of these enzymes is frequently disrupted in major human diseases, including cancer. The ability to modulate such signal transduction pathways selectively with drugs holds enormous

N.K. Tonks (✉)
Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, NY 11724, USA
e-mail: tonks@cshl.edu

B.G. Neel, N. Tonks (eds.), *Protein Tyrosine Phosphatases in Cancer*,
DOI 10.1007/978-1-4939-3649-6_1

therapeutic potential. Drugs that target the protein kinases represent breakthroughs in cancer therapy. For example, the HER2 protein tyrosine kinase is amplified and/or overexpressed in several cancers, in particular in ~25 % of breast cancer, where it is associated with poor prognosis. The humanized HER2-directed antibody, Herceptin (Trastuzumab), is an example of a "rational cancer therapy" for treatment of HER2-positive metastatic breast tumors. It targets HER2 as a unique marker of the cancer cell. Although Herceptin is used frequently and is presented as a frontline treatment of choice, the overall success rate is low and patients develop resistance to the therapy. This problem of resistance, both de novo and acquired, has become an obstacle to the successful application of kinase-directed therapies in general. Therefore, despite the obvious potential, it is anticipated that new alternative therapies, administered alone or in combination with kinase-directed drugs, will represent the way forward. The challenge is to identify such alternative therapies. In light of the intimate cooperation between kinases and phosphatases in the regulation of signal transduction under normal and pathophysiological conditions, it would seem prudent also to consider the protein phosphatases in this context. The protein tyrosine phosphatases (PTPs), in particular, have been garnering attention as potential therapeutic targets; nevertheless, they remain a largely untapped resource for drug development. The purpose of this book is to present a collection of reviews that describe recent developments in our understanding of the function of members of the PTP family, with a particular focus on their roles in cancer and their potential to form the basis for therapeutic strategies to address this and other major diseases.

A misconception that still pervades the field is the view of PTPs as a family of constitutively active, broad specificity, housekeeping enzymes. Furthermore, the dismissal of phosphatases as "erasers" in systems biology models of signal transduction only serves to reinforce such prejudice. In contrast, myriad studies have established that structural and functional diversity is a hallmark of the PTP family that matches such diversity within the protein tyrosine kinases. In fact, the function of PTKs and PTPs are integrated such that both classes of enzymes are critical regulators of signal transduction. Furthermore, disruption of both classes of enzymes has been shown to underlie major human diseases, including cancer.

The PTP family comprises 107 genes in humans [1]; this structural diversity alone points to functional specificity beyond a general housekeeping role. Of these, 37 encode "classical" pTyr-specific enzymes (Fig. 1). Within these classical PTP genes, 21 encode transmembrane, receptor-like proteins (RPTPs), the structures of which suggest the potential for modulation of signaling events directly through ligand-regulated protein tyrosine *dephosphorylation*. Again this is indicative of a direct role in signaling, beyond a housekeeping function. Similar to the PTKs, the diversity in the extracellular segments of the RPTPs presumably reflects an equivalent diversity in the nature of the ligands to which they respond. As discussed in the chapter by Andrew Stoker, however, the identity and function of such ligands remain a largely unresolved issue in the field.

Initial studies of the prototypic receptor PTP, CD45, revealed another important facet of the function of members of the PTP family—their ability to function positively as well as negatively in the regulation of cell signaling. This reinforces further

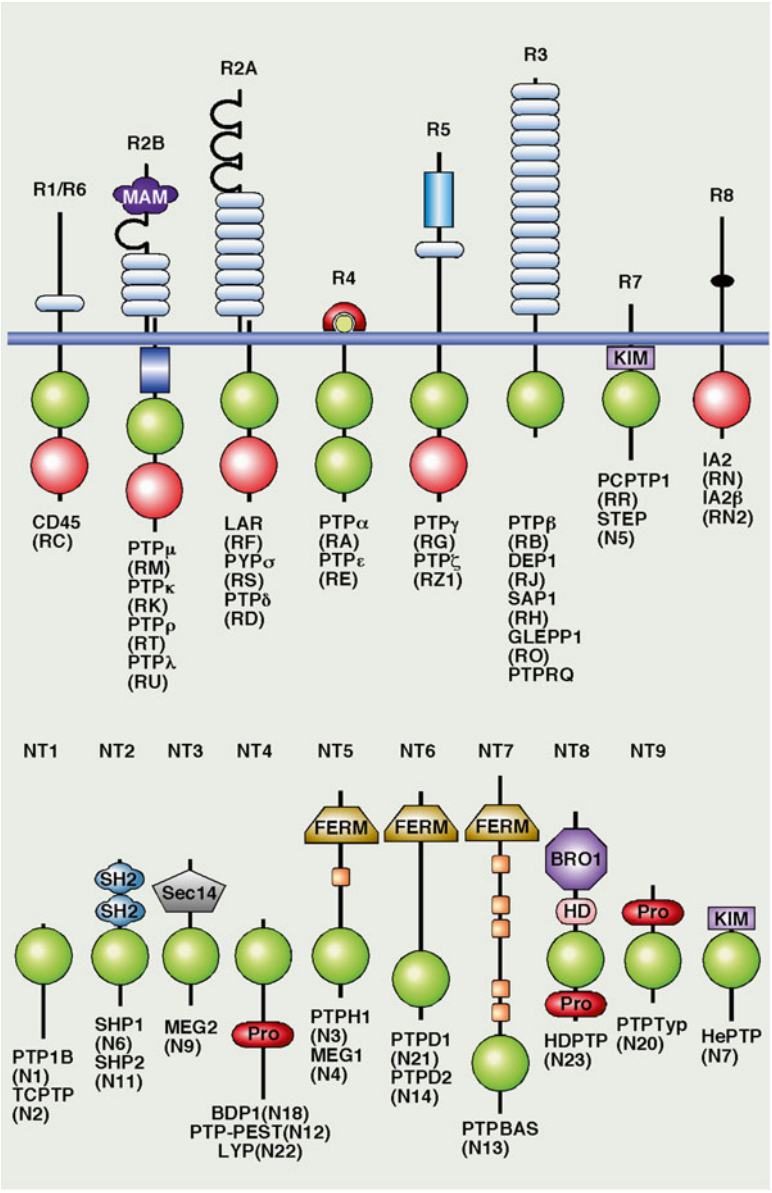

Fig. 1 The classical, pTyr-specific members of the PTP family

the concept that PTPs may play an important direct role in switching on signaling pathways, rather than simply acting as passive antagonists of PTK function (Fig. 2).

The remaining 16 classical PTPs are nontransmembrane, cytoplasmic enzymes, in which the catalytic domain is flanked by sequences that may serve a regulatory function (Fig. 1). This includes SH2 domains, which, as described by Chan and Neel, can

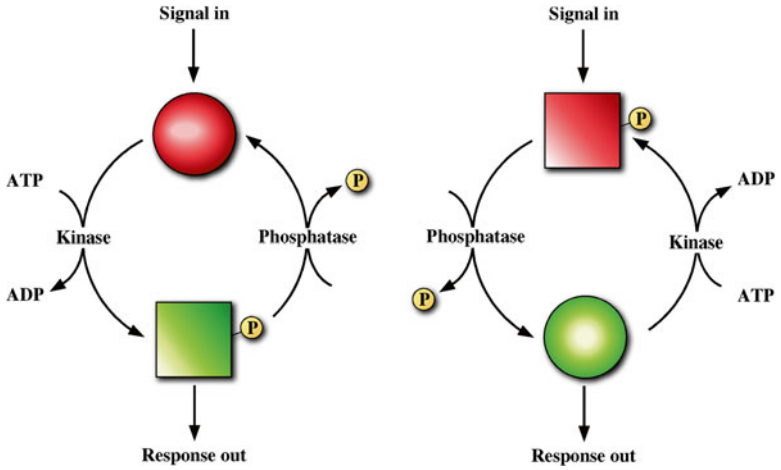

Fig. 2 The PTP family can serve as either positive or negative regulators of signal transduction. Members of the PTP family have the potential to act negatively in the regulation of signaling, by dephosphorylating autophosphorylation sites in the PTKs themselves and/or phosphorylation sites in downstream PTK targets. Importantly, PTPs may also play a positive role in promoting signaling by dephosphorylating inhibitory sites in other signaling proteins, such as by dephosphorylating the inhibitory site at the C-terminus of SRC family PTKs, thereby activating the kinase and promoting its signaling function

modulate the activity of the PTP both directly by occluding the active site in the basal state and indirectly by controlling its subcellular distribution by targeting it to defined signaling complexes. Overall, this illustrates a general principle that subcellular targeting is an important component of the regulation of PTP function. Nevertheless, it is important also to stress that the PTPs are *not* simply a collection of nonspecific enzymes, the activity of which is regulated indirectly by tethering. As described by Hendriks and Bohmer, there is clear evidence of gene duplication in the nontransmembrane PTPs, for example, giving rise to PTP1B and TCPTP, as well as SHP1 and SHP2. Although these pairs have a high degree of sequence identity, they display distinct, nonredundant functions, consistent with specificity. A detailed review of TCPTP, which is encoded by the *PTPN2* gene in humans, is presented by Tony Tiganis. This PTP can impact important signaling pathways through its recognition of various PTKs as substrate, including RPTKs, JAKs and SRC, as well as the STAT transcription factors. It exists in two distinct spliced forms: TC48, which is targeted to the cytoplasmic face of ER membranes, and TC45, which has the ability to shuttle in and out of the nucleus. These isoforms, which share the same catalytic domain, nonetheless have the ability to act on distinct substrates, or to act on common substrates in a spatially and temporally distinct manner. This review focuses on the identification of TCPTP as a tumor suppressor in T cell acute lymphoblastic leukemias, as well as its potential tumor suppressive role in breast cancer, and discusses the intriguing possibility that alterations in the distribution of TCPTP between its two spliced isoforms may have an impact in cancer.

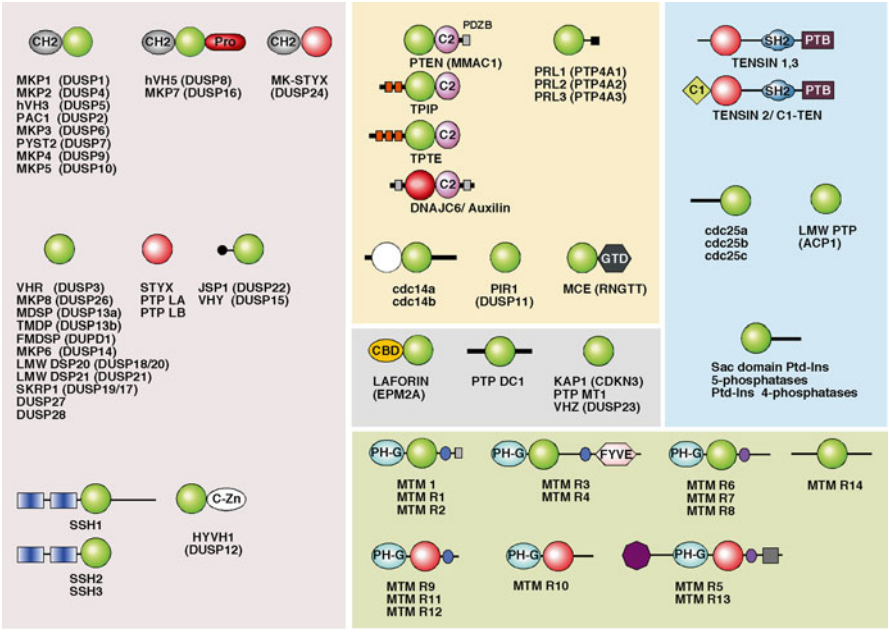

Fig. 3 The dual specificity phosphatases (DUSPs)

The largest portion of the PTP family is classified under the global heading of "dual specificity phosphatases," also known as DUSPs (Fig. 3). These enzymes have the capacity to dephosphorylate Ser/Thr residues in proteins, as well as pTyr proteins; in addition, as discussed below, they can control signal transduction via the dephosphorylation of nonprotein substrates, such as inositol phospholipids. Many of the DUSPs have been implicated in the control of fundamentally important physiological processes, including the cell cycle and cytoskeletal function. Kidger and Keyse review the role of MAP kinases (ERK, JNK, and p38) in cancer, and focus on those DUSPs that serve as MAP Kinase Phosphatases (MKPs). They review how the function of the MKPs is controlled at the level of expression, the stimuli that lead to their induction, their subcellular location (particularly nuclear vs cytoplasmic distribution), and their intrinsic specificity for particular MAP kinases. In addition, they describe how abnormal regulation of MKP function is encountered in a wide variety of tumors. In short, the complex roles of the MAP kinases in cancer are matched by the complexity in function of the DUSP MKPs that control them.

Considering the established role of PTKs as drivers of tumorigenesis, it was thought originally that the PTPs would serve predominantly as tumor suppressors. Nevertheless, the identification and characterization of the first PTP tumor suppressor took a long time, and when it was published it included some surprises. In 1997, three groups identified independently the tumor suppressor from the chromosome 10q23 locus. Although this discovery is primarily associated with the work of Wigler/Parsons (PTEN) and Steck (MMAC1), it is important

not to overlook the contribution of Hong Sun's lab, who contemporaneously identified it as TEP1, a TGF-β-regulated phosphatase [2]. PTEN is one of the most frequently lost or mutated tumor suppressors in human cancer and is generally associated with advanced and metastatic disease. Furthermore, germline mutations in *PTEN* are also associated with a number of autosomal dominant cancer predisposition syndromes, known as PTEN Hamartoma Tumor Syndromes. Disruption of PTEN has also been implicated in various nonneoplastic diseases, highlighting its diverse roles in vivo and emphasizing the importance of understanding the mechanisms underlying its effects.

PTEN exerts its tumor suppressive effects via dephosphorylation of the three position in the inositol sugar of phosphatidylinositol derivatives, such as PIP3, and the consequent regulation of PI 3-kinase-dependent signaling, thus expanding the repertoire of potential PTP substrates to include nonprotein phosphatidylinositol phospholipids. Nowak and Trotman present an overview of PTEN function and its implications for cancer. In particular, they highlight the fact that PTEN is haploinsufficient; there is frequent deletion of one copy of the gene, but although mutation of the remaining allele is rarely found, it is not sufficient to maintain a wild type condition. Loss of both copies of the *PTEN* gene triggers senescence, which highlights the importance also of understanding the control of PTEN expression and function through transcriptional and posttranslational mechanisms.

PTEN is a complex protein that features the PTP domain in its N-terminal segment; this forms an extensive interface with a C2 domain, which serves to bind phospholipid membranes. The C-terminus of the protein is characterized by the presence of multiple phosphorylation sites and a binding motif for PDZ domain-containing regulatory proteins. PTEN has an unusual architecture to its active site, which is sufficiently large to accommodate the sugar head-group of inositol phospholipids as a substrate; however, it displays intrinsic phosphatase activity towards both protein and lipid substrates. Furthermore, tumor-derived mutations are not restricted to the catalytic core of PTEN, but have been identified throughout the coding sequence. This suggests that there may also be phosphatase-independent functions of PTEN, a topic that is reviewed by Papa and Pandolfi. In particular, they highlight mechanisms for nuclear import of PTEN and discuss a scaffolding role of the protein in the control of chromosome stability and DNA repair. The authors also highlight examples of the covalent modification of PTEN, which includes not only phosphorylation, but also ubiquitylation and sumoylation. As described also by others, such covalent modifications are turning about to be an important feature of the mechanisms by which PTP function is regulated in vivo.

Subsequent to the identification of PTEN, a large body of evidence now supports tumor suppressive roles for other members of the PTP family, including the classical pTyr-specific enzymes. Andrew Stoker focuses on the receptor-like PTPs in this regard. Interestingly, RPTPs are subject to a range of mutations in a wide variety of cancers, including in their extracellular segments, which highlights the potential importance of ligand binding for the normal regulation of their function. Furthermore, there are RPTPs that serve potential oncogenic functions. For example, expression of RPTPα alone was sufficient to transform fibroblasts, with the