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Commentary

Checks and balances: Interplay of RTKs and PTPs in cancer progression

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ABSTRACT

In recent years, targeted therapies for receptor tyrosine kinases (RTKs) have shown initial promise in the clinical setting for the treatment of several tumors driven by these oncogenic signaling pathways. Unfortunately, clinical relapse due to acquired resistance to these molecular therapeutics is common. An improved understanding of how tumors bypass the inhibitory effects of RTK-targeted therapies has revealed a rich myriad of possible mechanisms for acquired resistance. Protein tyrosine phosphatases (PTPs) can function as oncogenes or tumor suppressors to either enhance or suppress RTK signaling. Recent studies suggest that the loss or gain of function of PTP's can significantly impinge on RTK signaling during tumor progression. Here we review the interplay between RTKs and PTPs as an emerging mechanism for acquired resistance to RTK-targeted therapies, that may aid in the design of improved therapies to prevent and overcome resistance in treatments for cancer patients.

1. Introduction

Tyrosine kinases function as key signaling molecules to influence several physiological processes. Considerable evidence indicates that the hyperactivation of tyrosine kinases enhances the activation of downstream signaling cascades in human malignancies, promoting potent oncogenic signaling and neoplastic growth. A search of the human genome for tyrosine kinase motifs identified 90 unique kinase genes, which can be classified into two groups: non-receptor versus receptor tyrosine kinases [1]. Of these genes, 32 genes represent non-receptor tyrosine kinases, a diverse family of intracellular proteins that function as critical transducers of cell growth, survival and migration. Examples of non-receptor tyrosine kinases that are overexpressed or constitutively activated in human cancers include Jak (reviewed in [2]), Src (reviewed in [3]), focal adhesion kinase (FAK) and Bcr-Abl (reviewed in [4,5]). This commentary focuses on the regulation of RTK signaling by PTPs to drive tumorigenesis; further discussions of non-receptor tyrosine kinases are only included for context. The remaining 58 genes represent receptor tyrosine kinases (RTKs), a family of single pass transmembrane proteins that are able to transduce extracellular signals into intracellular responses. Ligand binding to the extracellular domain drives receptor dimerization and cross-phosphorylation of docking sites for intracellular proteins that relay signals to regulate protein function, protein–protein interactions and gene expression. Increased RTK signaling correlates with the etiology of several human solid tumors and as such represents an attractive target for therapeutic intervention. Examples include members of the epidermal growth factor family of receptor tyrosine kinases such as (ErbBs)EGFR/ErbB1 and ErbB2/Her2/(*Neu*, in rodents), insulin-like growth factor receptor-1 (IGF-R1), vascular endothelial growth factor receptor (VEGFR) and Met, the receptor for hepatocyte growth factor (HGF). Several strategies for targeting these RTKs have been developed and tested in clinical trials, the most successful being small molecule tyrosine kinase inhibitors and humanized monoclonal antibodies, that counter the activities of RTKs involved in cancer (reviewed in [6,7]). However, the finding of acquired resistance to many of these drugs has spurred studies aimed at understanding molecular mechanisms used by tumor cells to bypass their inhibitory effect.

Abbreviations: Cag A, cytotoxin associated antigen A; DUSP, dual specificity phosphatase; EGF, epidermal growth factor; EGFR, EGF receptor; ErbBs, epidermal growth factor receptor family of receptor tyrosine kinases; FAK, focal adhesion kinase; FGFR, fibroblast growth factor receptor; HGF, hepatocyte growth factor; IGF-1R, type 1 insulin-like growth factor receptor; MEF, mouse embryonic fibroblast; Met, HGF receptor; PI3K, phosphatidylinositol 3-kinase; PIP2, phosphatidylinositol 4,5,-bisphosphate; PIP3, 3,4,5,-triphosphate; PTEN, phosphatase and tensin homolog deleted on chromosome 10; PTP, protein tyrosine phosphatases; NSCLC, non-small cell lung carcinoma; PDGF, platelet derived growth factor; PDGFR, PDGF receptor; RTK, receptor tyrosine kinase; SH2, src homology 2; shRNA, small hairpin RNA; SNP, single nucleotide polymorphisms; STAM2, signal transducing adaptor molecule 2; TKI, tyrosine kinase inhibitor; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.

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The intensity and duration of RTK signaling is countered in part by the action of protein tyrosine phosphatases (PTPs), a family of proteins encoded by 107 genes in the human genome [8,9]. Among these, 38 genes encode tyrosine-specific PTPs (classical PTPs) and are further sub-classified as receptor- (21 members) or non-receptor- (17 members) PTPs [9]. The remaining members of the PTP superfamily are dual specificity phosphatases (DUSP's) that can dephosphorylate serine/threonine residues, tyrosine residues or lipids [9]. In this review, we will focus on the classical PTPs and use examples to illustrate how they interplay with RTKs to modulate the cancer phenotype. Depending on the cellular context, PTPs can serve as either tumor promoters or tumor suppressors to directly control RTK activation or indirectly to modulate signaling pathways downstream of RTKs. Of the classical PTPs, 12 show potential oncogenic functions while 22 show tumor suppressor functions in human cancers [9]. As with RTKs, PTPs are regulated at multiple levels in cancer: (1) overexpression, (2) mutation, (3) gene amplification, (4) allelic loss, (5) transcriptional repression, (6) post-transcriptional regulation and (7) post-translational modification [8]. A growing body of literature suggests that the gain or loss of function of some PTPs can exacerbate the effects of oncogenic RTKs serving to amplify and in some cases, bypass RTK-dependent activation of key growth, survival and pro-invasive signals. Here, we focus on three select PTPs: PTP1B (PTPN1), SHP2 (PTPN11) and PTP-PEST (PTPN12). It should be acknowledged that while other classical PTPs not discussed here play important roles in cancer progression, studies on PTPN1, PTPN11 and PTPN12 fulfill important criteria relevant to this review. All three PTPs have been examined in human cancer patient tissues, they have been shown to directly act on RTK's and their role in cancer progression has been tested in xenograft or orthotopic mouse models of cancer. We use these PTPs to illustrate how the gain or loss of function of PTPs interplays with oncogenic RTK signaling pathways to control some hallmarks of cancer cells. We then discuss how PTP-driven activities may contribute to acquired resistance to targeted-RTK inhibitors. Finally, we consider future therapeutic prospects for PTPs and PTP-regulated pathways in cancers. Due to space limitations, readers are referred to several outstanding, recent reviews on the regulation and function of PTPs in human cancer for additional details [8–10].

2. Mechanisms of acquired resistance to anti-cancer treatments

Molecular targeted therapeutics such as tyrosine kinase inhibitors (TKIs) and humanized monoclonal antibodies represent a specific strategy for treating cancers compared to general cell loss using standard cytotoxic treatments. Unfortunately, many tumors (including tumors of the breast, colon, lung, and head and neck among others) treated with these reagents acquire genetic alterations to overcome the inhibitory effects of these drugs (acquired resistance) resulting in patient relapse. Extensive studies indicate that acquired resistance to RTK-targeted therapies can be the result of any combination of the following molecular mechanisms: (1) secondary RTK mutations acquired during therapy; (2) RTK gene amplification; (3) cross-talk with other RTK signaling pathways; (4) environmental factors including inflammatory agents and infection; and (5) receptor-independent activation of downstream signaling cascades or loss of regulatory mechanisms [11].

The hypothesis that classical PTPs contribute to acquired resistance is based in part on recent findings with PTEN (phosphatase and tensin homolog deleted on chromosome 10). The lipid phosphatase PTEN functions to convert 3,4,5,-triphosphate (PIP3) back to phosphatidylinositol 4,5,-bisphosphate (PIP2), and as such counters phosphoinositide 3-kinase (PI3K)/Akt prosurvival signaling. PTEN functions as a tumor suppressor since point mutations, loss of heterozygosity and epigenetic changes leading to functional loss enhance tumorigenesis. For example, knockdown of PTEN in ErbB2 overexpressing breast tumor cells results in trastuzumab resistance *in vitro* and *in vivo* [12]. Trastuzumab is a humanized monoclonal antibody that is highly selective against the extracellular domain of ErbB2. Acquired resistance to the EGFR TKI erlotinib/Tarceva in A431 breast cancer cells requires down regulation of PTEN [13]. In cell lines derived from non-small cell lung carcinoma (NSCLC) with acquired resistance to gefitinib/IRESSA (a TKI directed against EGFR), decreased PTEN protein expression as well as increased constitutive Akt phosphorylation was detected when compared with parental cell lines [14]. Similarly, acquired resistance to cetuximab, a neutralizing EGFR monoclonal antibody, is mediated by decreased PTEN stability in HCC827 NSCLC cells [15]. In light of these findings, it is tempting to speculate that additional members of the phosphatase superfamily could play a role in acquired resistance towards targeted anti-cancer therapies. To this end, a recent study shows that decreased PTP1B expression (via siRNA) leads to resistance of leukemia cells to the TKI, STI571, an inhibitor of abl tyrosine kinase [16]. Since PTP1B targets BCR-ABL, loss of PTP function would enhance the oncogenic activity of BCR-ABL [17].

2.1. PTP1B/PTPN1—a dual role in cancer?

PTP1B (PTPN1) was the first PTP to be isolated and has been extensively characterized at the cellular, biochemical and structural level [18]. It has been implicated in a wide array of physiological processes including cell proliferation, survival and motility as well in human pathologies including diabetes and cancer [9,10,18]. PTP1B is reported to suppress the activation of several RTK signaling pathways including EGFR, Met, platelet derived growth factor (PDGF) receptor and IGF-1R through at least two mechanisms: direct dephosphorylation of tyrosine residues in the intracellular domain and control of RTK endocytosis [19,20]. Substantial evidence from overexpression, knockout or knockdown studies and *in vitro* biochemical studies, supports the direct dephosphorylation of activated EGFR and Met by PTP1B. Dephosphorylation of STAM2 (signal transducing adaptor molecule 2) by PTP1B promotes efficient trafficking of EGFR to late endosomes, a process critical for RTK degradation [19,20]. Since the translocaton of EGFR to late endosomes terminates receptor signaling, PTP1B might function as a tumor suppressor to limit the duration of signaling from either internalized EGFR [21] or prevent EGFR recycling back to the plasma membrane. In addition PTP1B is reported to stabilize cell-cell adhesion in epithelial cells [22] and endothelial cells [23], suggesting its activity may suppress motility or vascular permeability to cell invasiveness. Finally, decreased PTP1B expression may increase resistance to apoptotic stimuli and cytotoxic agents [24] and cause a decrease in ER stress response [25]. Although these findings point to a potential tumor suppressive function for PTP1B, PTP1B knockout mice do not develop spontaneous tumors. Rather, the dominant phenotype of PTP1B knockout mice is linked to obesity and diabetes [18] suggesting that the role of PTP1B in tumorigenesis is likely context dependent.

In contrast, extensive studies suggest a tumor promoting role PTP1B [26]. For example, PTP1B−/− mouse embryonic fibroblasts (MEFs) show decreased proliferation in response to PDGF and impaired integrin-mediated cell motility [27]. PTP1B exerts its effect on proliferation through modulation of Ras/ERK activity [28] and controls motility through a number of potential targets including p130cas, c-src, FAK and cortactin [26]. In both colon cancer cells and breast cancer cells, PTP1B is required to promote invasion and formation of invadopodia through c-src dependent mechanisms [29,30]. In fact, PTP1B can directly activate c-src

through dephosphorylation of its C-terminal inhibitory tyrosine residue at amino acid 529 [26]. Perhaps the most compelling evidence that PTP1B plays a pro-oncogenic role comes from studies examining the expression level of PTP1B in human tumor samples. PTP1B expression in breast and ovarian tissues increases in proportion to increased histological abnormalities such as carcinoma *in situ* and invasive carcinoma, and enhanced expression of ErbB2 [31,32]. Moreover, increased staining intensity of PTP1B was detected at the invasive fronts of tumors, which may suggest a role for PTP1B in regulating events at the leading edge of migrating cells [31,32]. PTP1B is required for ErbB2 transformation of breast cancer cells *in vitro*, in a c-src dependent mechanism [33]. In a series of elegant studies, the role of PTP1B in ErbB2-driven breast cancer progression was tested using murine breast cancer models. Genetic or pharmacological ablation of PTP1B in ErbB2/*Neu* murine breast cancer models (MMTV/*Neu*NT or MMTV/NDL2) resulted in delayed tumor onset and suppression of lung metastasis [34,35]. These effects were accompanied by increased apoptosis and changes in Ras/ERK and PI3K/Akt activity [34]. Interestingly, the effect of PTP1B on tumorigenesis was selective to ErbB2-driven mammary tumors since the Polyoma middle T antigen mouse model, was refractory to PTP1B levels [34,35]. To further demonstrate the pro-oncogenic role of PTP1B, overexpression of PTP1B via MMTV-driven expression in mouse mammary glands resulted in spontaneous tumor formation [34].

How can we reconcile the opposing roles of PTP1B in cancer progression? PTP1B could be differentially regulated, either through compartmentalization to distinct subcellular sites or by post-translational modification [19]. For example, PTP1B is normally tethered to the endoplasmic reticulum through a C-terminal tail. Cleavage by the Ca^{++}-dependent protease, calpain releases PTP1B into the cytosol or to the plasma membrane, a process that serves to enhance PTP1B catalytic activity [14]. Thus, PTP1B may be restricted in its access to targets depending on its subcellular localization. PTP1B can be inactivated by serine phosphorylation, modification by ubiquitin-like molecule, SUMO, or by ROS-mediated oxidation of the active site [26,36,37]. Interestingly, PTP1B tyrosine phosphorylation and catalytic activity is enhanced in response to EGF [38]. Thus overexpression of cytosolic PTP1B may favor its oncogenic functions. This view is supported by the fact that overexpression of calpain-resistant PTP1B promoted invadopodia formation in breast cancer cells [29]. Alternatively, inactive PTP1B may serve to prolong RTK signaling from endosomes as a consequence of delayed trafficking to subsequent degradation compartments [19,20]. Finally, it is important to note that PTP1B can be controlled by integrin-mediated mechanisms that promote cellular growth, survival and invasion, independently of RTK signaling [27].

2.2. SHP2/PTPN11—a tumor promoter?

SHP2 encoded by the *PTPN11* gene in humans, is a cytosolic PTP that acts downstream of most if not all ligand activated RTKS including EGFR, Met, fibroblast growth factor receptor (FGFR), IGF-1R in numerous systems [10]. To date, SHP2 is the only PTP designated an "oncogene". SHP2 possesses two src homology 2 (SH2) domains, followed by C-terminal catalytic domain that is autoinhibited through intermolecular interaction of the N-terminal SH2 domain with the catalytic domain [10]. Upon RTK activation, SHP2 is recruited via the adaptor proteins Gab1 or Gab2 [39]. Following recruitment of Gab1 or Gab2 to RTK signaling complexes, phosphorylated tyrosine residues on Gab1 or Gab2 serve as docking sites for the two SH2-domains of SHP2. This interaction relieves autoinhibition of SHP2, thus activating the PTP activity. SHP2's primary biological role is to activate the Ras/ERK pathway, although the mechanism of activation is not completely defined. One possible mechanism involves dephosphorylation of p120Ras [40]. SHP2 also has effects on c-src activation, possibly through mediating crosstalk of β4 integrin with Met [41]. Several studies implicate SHP2 in control of integrin mediated cell motility and activation of FAK and RhoA [42,43]. Also independent of RTK activation, SHP2 can activate src kinase downstream of α6β4 integrin [44]. Similarly, SHP2 can activate PI3K-Akt signaling and Rho GTPase activity, depending on the RTK or cell type.

The identification of activating SHP2 mutations in cancers such as childhood juvenile myelomonocytic leukemia and lymphoma prompted the search for similar SHP2 mutations in cancers of epithelial origin. These mutations, which typically reside in the N-terminal SH2 domain, render SHP2 in a conformationally active state [10]. Approximately 85% of patients with childhood juvenile myelomonocytic leukemia have activating mutations in either SHP2, Ras or homologous loss of the neurofibromotosis type I gene (*NF1*), which encodes a ras GTPase activating protein [45]. Although these mutations are mutually exclusive in juvenile myelomonocytic leukemia, these findings indicate the dependence of this cancer on activated ras signaling. Conversely, activating SHP2 mutations in epithelial cancers such as lung adenocarcinoma and neuroblastoma occur infrequently [10]. However, these data support the tenet that hyperactivation of SHP2 appears important for the pathogenesis of breast and gastric carcinomas. For example, SHP2 is often hyperactivated in breast cancer, particularly ErbB2-positive invasive breast cancer [46], through a mechanism involving interactions with the signaling adaptor Gab2. Overexpression of Gab2 in MCF10A cells increases cell proliferation [47]. Moreover, cooperation of Gab2 with ErbB2-driven cell invasion requires intact SHP2 binding sites on Gab2 [48]. Expression of dominant negative SHP2 or siRNA targeting SHP2, blocked the *in vitro* effects of Gab2 overexpression on cell transformation. Further, Gab2 overexpression enhances tumorigenesis in NeuNT murine model of breast cancer while Gab2 knockout prevents tumorigenesis and metastasis [48]. Inhibition of SHP2 by overexpression of a catalytically inactive mutant or shRNA-mediated depletion blocks anchorage-independent growth and restores normal epithelial morphology in 3D cultures [49]. These studies provide promising evidence that targeting SHP2 directly may help to suppress the oncogenic effects of ErbB2-addicted cancers. In support of this, in NSCLC harboring activating EGFR mutations, SHP2 activation (and ERK) is reduced, making the cells more sensitive to gefitinib treatment [50]. This suggests that SHP2 activation may play a role in the responsiveness of tumor cells to TKIs. Hyperactivation of SHP2 has been recently implicated in *Helicobacter pylori* driven gastric cancer. The indirect activation of SHP2 following introduction of the virulence factor cytotoxin-associated antigen A (CagA) in *H. pylori* infected cells enhanced gastric epithelial cell transformation [51]. In this context, CagA likely mimics host cell Grb2 activity to potentiate cell transformation.

Additional evidence supporting an oncogenic function of SHP2 comes from studies using shRNA to knockdown SHP2 in prostate or lung cancer cells. In both cases, SHP2 was required for tumor growth in xenograft mouse models [52], and decreased growth was attributed to lower activation of ERK and c-myc. Interestingly, phosphoproteomic analysis of tumor tissues from these mice showed both up and down-regulation of tyrosine phosphorylated proteins. For example, the inhibitory tyrosine 530 on c-src was phosphorylated, while Gab2 was dephosphorylated on tyrosines 627 and 659 [52]. This type of analysis underscores the dual role of PTPs in direct dephosphorylation of protein targets (tyrosine kinase substrates) versus the direct regulation of tyrosine kinase activity. To fully appreciate the contribution of PTPs to cancer progression, it will be important to identify the global changes in tyrosine phosphorylation in tumor tissues as a function of PTP expression.

In contrast to the studies highlighted above, there is also evidence that SHP2 may function as a tumor suppressor in some

situations. Conditional knockout of SHP2 in liver hepatocytes caused an increased inflammatory response and activation of STAT3 along with enhanced development of hepatocellular carcinoma in response to challenge with the carcinogen, diethylnitrosamine [53]. Although it is not known if the tumor suppressive effect of SHP2 in liver is downstream of RTK activation, several studies show that SHP2 can differentially regulate RTKs. For example, SHP2 can be a positive regulator of insulin receptor signaling [54,55] however, the insulin receptor and IGF-1R, in particular, can also be suppressed by SHP2 [56,57]. SHP2 can be recruited to IGF1R via IRS-1 (insulin receptor substrate 1) [58,59] or SHPS1 [60] to affect activation of PI3 kinase [56,57]. While most of this evidence is obtained from insulin responsive cells and tissues (muscle, liver, adipose), it will be interesting to investigate the role of SHP2 in IGF-1R signaling in tumor cells.

2.3. PTP-PEST/PTPN12: an emerging tumor suppressor

PTP-PEST (PTPN12) has recently emerged as a putative tumor suppressor. Early studies showed PTP-PEST, a ubiquitously expressed, cytosolic PTP, to be important for cell motility and cytokinesis in fibroblasts [61,62], and knockout of PTP-PEST in mice resulted in embryonic lethal effect at day E9.5 [63]. Incidentally, mice heterozygous for PTP-PEST have no apparent phenotype. Later it was shown that PTP-PEST is involved in apoptosis in response to extrinsic stimuli such as Fas [64]. PTP-PEST has an N-terminal catalytic domain and a C-terminal scaffolding domain containing multiple poly-proline rich sequences [61]. Several groups identified direct targets of PTP-PEST using substrate trapping. These range from cytoplasmic tyrosine kinases (c-abl, PYK2) [65,66], the focal adhesion proteins p130cas and paxillin [67,68], and upstream regulators of Rho GTPases, VAV2 and p190RhoGAP [69]. The effects of PTP-PEST on motility were attributed to its regulation of Rac1 and RhoA through many of these substrates [67,69,70]. An interaction with Grb2 was identified linking PTP-PEST to activated RTKs, EGFR in particular [71].

In more recent studies, the role of PTP-PEST in cancer cells has begun to be examined. Based on cell culture studies, PTP-PEST has been implicated in colon, pancreatic and breast cancers along with lymphoma and sarcoma [72–77]. Although these studies have not shown a direct effect of PTP-PEST on RTK activation, they do indicate that PTP-PEST targets pathways downstream of activated RTKs. In pancreatic cancer cells, PTP-PEST controls activation of c-abl and its association with abl interacting protein, abi to affect invasion [72]. In breast cancer cells, PTP-PEST was shown to regulate EGF signaling [73] and in ras-transformed fibroblasts, PTP-PEST targets FAK to affect anchorage-independent growth and survival [74]. In intestinal epithelial cells, PTP-PEST was shown to affect activation of c-src [75]. Finally, in colon cancer cells, PTP-PEST is required to suppress chemotaxis to HGF [76]. PTP-PEST has also been implicated in non-RTK mediated events in cancer cells. In colon cancer cells, PTP-PEST is required to stabilize and maintain E-cadherin cell-cell junctions through a Rho GTPase-dependent mechanism [76]. Knockdown of PTP-PEST in this system results in loss of cell–cell junctions, enhanced motility, and a mesenchymal phenotype. Collectively, these studies demonstrate that PTP-PEST functions to suppress growth, invasion, and EMT in cancer cells and support an anti-tumor role for PTP-PEST.

More definitive evidence of a tumor suppressor function for PTP-PEST is beginning to emerge. One study detected single nucleotide polymorphisms (SNPs) in PTPN12 alleles in a number of cancer tissues including breast, lung and kidney [77]. The SNPs reside in the C-terminal scaffolding region and may affect PTP-PEST's interaction with target molecules. Furthermore, these SNPs have altered catalytic activity. Compelling functional evidence that loss of function of PTP-PEST may contribute to breast cancer progression was recently provided [73]. PTP-PEST was identified in an small hairpin RNA (shRNA) screen as a tumor suppressor in triple negative breast cancers (TNBC), a type of breast cancer that is refractory to targeted therapies. PTPN12 was found to suppress mammary epithelial cell growth and transformation *in vitro*. PTPN12's effects were attributed to direct regulation of EGFR and ErbB2 activation. ShRNA depletion of PTPN12 resulted in hyperphosphorylation of EGFR on Y1148 and ErbB2 on Y1248. Restoring PTPN12 expression diminished phosphorylation specifically at these residues. Substrate trapping confirmed an enhanced interaction between PTPN12 and EGFR or ErbB2. Further, depletion of EGFR or ErbB2 by shRNA or lapatinib (an EGFR and ErbB2 inhibitor) reverted the transformed phenotype. Downstream of EGFR and ErbB2, activation of ERK and S6 kinase was elevated in the absence of PTPN12. Importantly, loss of PTPN12 function, either through SNP inactivation or deletion, strongly correlated with TNBC, both in cell lines and patient tissues [73]. Restoring PTPN12 expression in TNBC cells suppressed primary tumor growth when injected into mouse mammary gland and blocked lung metastasis. Interestingly, suppression of TNBC (PTP-PEST deficient) mammary tumor growth *in vivo* required combination treatment with multiple RTK inhibitors (lapatinib and sunitinib). This finding suggests that loss of PTPN12 may have direct effects on EGFR and ErbB2 signaling and also indirect effects on other RTKs such as PDGFR. As discussed below, this could have significant implications for targeted therapies in breast cancer.

2.3.1. A role for PTPs in acquired resistance to RTK inhibitors

Acquired resistance to RTK-targeted therapies can be caused by any combination of molecular mechanisms including (1) secondary RTK mutations acquired during therapy, (2) cross-talk with other RTK signaling pathways, (3) environmental factors including inflammatory agents and infection, (4) gene amplification and (5) receptor-independent activation of downstream signaling cascades or loss of regulatory mechanisms [11]. The identification of altered PTP activity and activating PTP mutations in cancer, coupled with the general requirement for PTPs as regulators of oncogenic RTK signaling, favors the idea that therapies targeting PTPs could provide new therapeutic options in the clinic. Unfortunately, the development of inhibitors targeting PTP oncogenes such as SHP2 or reagents that counter the loss of PTP tumor suppressors remains elusive. Regardless, an improved understanding of how PTPs contribute to RTK-driven oncogenesis and acquired resistance to RTK targeted therapies, should provide new insights into pathogenesis and will improve the therapeutic use of such inhibitors as they become available. One complicating factor is the high level of conservation between the catalytic domains of PTPs. Accordingly highly specific inhibitors have been difficult to design [10]. A number of strategies to design more selective inhibitors are underway based on detailed structural analysis of PTPs, to expedite the design of specific competitive or allosteric inhibitors [10].

Alternative strategies are needed to identify novel therapeutics to counteract PTP activities in cancers. Insights gained from recent studies in mouse models suggest that, at least in the case of PTP-PEST, loss of function of a PTP *in vivo* may lead to complex signaling alterations in tumors. The requirement for multiple RTK inhibitors to suppress breast tumor growth *in vivo* supports the involvement of PTP-PEST in multiple signaling pathways [73]. Although not tested in cancer models, the same may hold true for additional members of the PTP family. Therefore, future studies using phospho-proteomic approaches to identify the full spectrum of PTP targets in cancers will be of great benefit for developing alternative anti-cancer therapies. Adding to the complexity, mounting evidence implicates epigenetic mechanisms in regulation of PTPs in cancer. MicroRNAs have been the subject of recent intense studies and represent an alternate mechanism for

regulating PTP activity. Interestingly, both PTP-PEST and PTEN are targets of numerous microRNAs [78,79], a finding that could suggest an innovative approach for the development of novel therapeutics to regulate the activity of oncogenic PTPs. The treatment of colon cancer cells with cytotoxic agent 5-fluorouracil led to an upregulation of several microRNAs including mir-200, a microRNA that targets *PTPN12* expression [79]. It is tempting to speculate that tumors may develop resistance to targeted therapies in part by altering their microRNA profile, resulting in the downregulation of (putative) PTP tumor suppressors such as PTP-PEST. Yet, much remains to be learned about the biosynthesis and regulation of microRNAs for normal cell function and under pathological conditions.

Cancer is a complex disease in terms of underlying mechanisms. Given the propensity of tumors to generate resistant RTK-targeted variants due to their inherent genetic instability, acquired drug resistance remains a significant clinical challenge. As such, an increased understanding of the role of additional proteins such as PTPs for amplifying RTK signaling during tumor pathogenesis, could provide novel avenues for future anti-cancer drug discoveries.

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