PROSPECT
Journal of Cellular Biochemistry 111:1087–1098 (2010)

Journal of Cellular Biochemistry

PRL-3 Phosphatase and Cancer Metastasis

Abdul Qader O. Al-Aidaroos[1,2] and Qi Zeng[1,2,3*]

[1] Institute of Molecular and Cell Biology, A*STAR (Agency for Science, Technology and Research), 61 Biopolis Drive, Proteos, Singapore 138648, Republic of Singapore

[2] NUS Graduate School for Integrative Sciences and Engineering, National University of Singapore, 28 Medical Drive, Singapore 117456, Republic of Singapore

[3] Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, 8 Medical Drive, Singapore 119260, Republic of Singapore

ABSTRACT

The deregulated expression of members of the phosphatase of regenerating liver (PRL) family has been implicated in the metastatic progression of multiple human cancers. Importantly, PRL-1 and PRL-3 both possess the capacity to drive key steps in metastatic progression. Yet, little is known about the regulation and oncogenic mechanisms of this emerging class of dual-specificity phosphatases. This prospect article details the involvement of PRLs in the metastatic cascade, the regulatory mechanisms controlling PRL expression, and recent efforts in the characterization of PRL-modulated pathways and substrates using biochemical and high-throughput approaches. Current advances and future prospects in anti-cancer therapy targeting this family are also discussed. J. Cell. Biochem. 111: 1087–1098, 2010. © 2010 Wiley-Liss, Inc.

KEY WORDS: PRL-3 PHOSPHATASE; CANCER METASTASIS; CANCER CELL SIGNALING

Reversible tyrosine phosphorylation is governed by the balanced action of protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs). PTPs constitutes a large family of enzymes (>100) which play crucial roles in positive and negative regulation of key signaling pathways involved in the control of cell proliferation, adhesion, migration, differentiation, and survival/apoptosis [Alonso et al., 2004]. Aberrant tyrosine phosphorylation resulting from dysregulation of PTP activity has been implicated in the progression of various diseases, including cancer [Ostman et al., 2006].

In recent years, emerging evidence indicates that members of the phosphatase of regenerating liver (PRL) subgroup of PTPs are linked to multiple human cancers [Bessette et al., 2008]. The PRL–PTP family comprises three members, PRL-1, PRL-2, and PRL-3. Based on the conserved amino acid sequences of their catalytic domain, PRLs have been classified as a unique subgroup of VH1-like PTPs with dual-specificity [Alonso et al., 2004]. The first PRL member implicated in cancer metastasis was PRL-3. Using global gene expression profiling of colorectal cancer (CRC) samples, PRL-3 was found to be the only gene expressed at high levels in all of 18 cancer metastases examined but at lower levels in primary tumors and normal epithelium [Saha et al., 2001]. Since then, elevated PRL expression (especially PRL-3) has been shown to be associated with the metastatic potential and poor prognosis of multiple cancers, including CRC, gastric cancer, ovarian cancer, breast cancer, cervical cancer, and lung cancer (reviewed in [Bessette et al., 2008]). In light of this, PRL-3 has been proposed as a potential biomarker for assessing tumor aggressiveness [Bessette et al., 2008]. Interestingly, independent non-biased high-throughput insertional mutagenesis screens [Akagi et al., 2004] (database accessible online at http://RTCGD.ncifcrf.gov) have also identified PRL-3 as a retrovirally tagged cancer gene. Unfortunately, despite significant headway in elucidating the involvement of PRL-3 and PRL-1 in cancer progression, much less attention has been given to the contribution of PRL-2. Nonetheless, PRL-2 was recently found to be upregulated in metastatic and primary breast tumors [Hardy et al., 2010]. Collectively, the reports reviewed here implicate an important, causal role for PRL-3, and possibly other PRLs, in cancer development and metastatic progression.

THE PRL FAMILY

A summarized timeline of key discoveries concerning PRLs is shown in Figure 1. PRL-1, the first PRL member, was identified as an

Abbreviations used: CRC, colorectal cancer; DSP, dual-specificity phosphatase; EMT, epithelial–mesenchymal transition; PRL, phosphatase of regenerating liver; PTEN, phosphatase and tensin homologue deleted on chromosome 10; PTP, protein tyrosine phosphatase; VHR, vaccinia virus phosphatase VH1-related.

*Correspondence to: Dr. Qi Zeng, Institute of Molecular and Cell Biology, A*STAR (Agency for Science, Technology and Research), 61 Biopolis Drive, Proteos, Singapore 138648, Republic of Singapore.
E-mail: mcbzengq@imcb.a-star.edu.sg

Received 1 October 2010; Accepted 4 October 2010 • DOI 10.1002/jcb.22913 • © 2010 Wiley-Liss, Inc.
Published online 4 November 2010 in Wiley Online Library (wileyonlinelibrary.com).

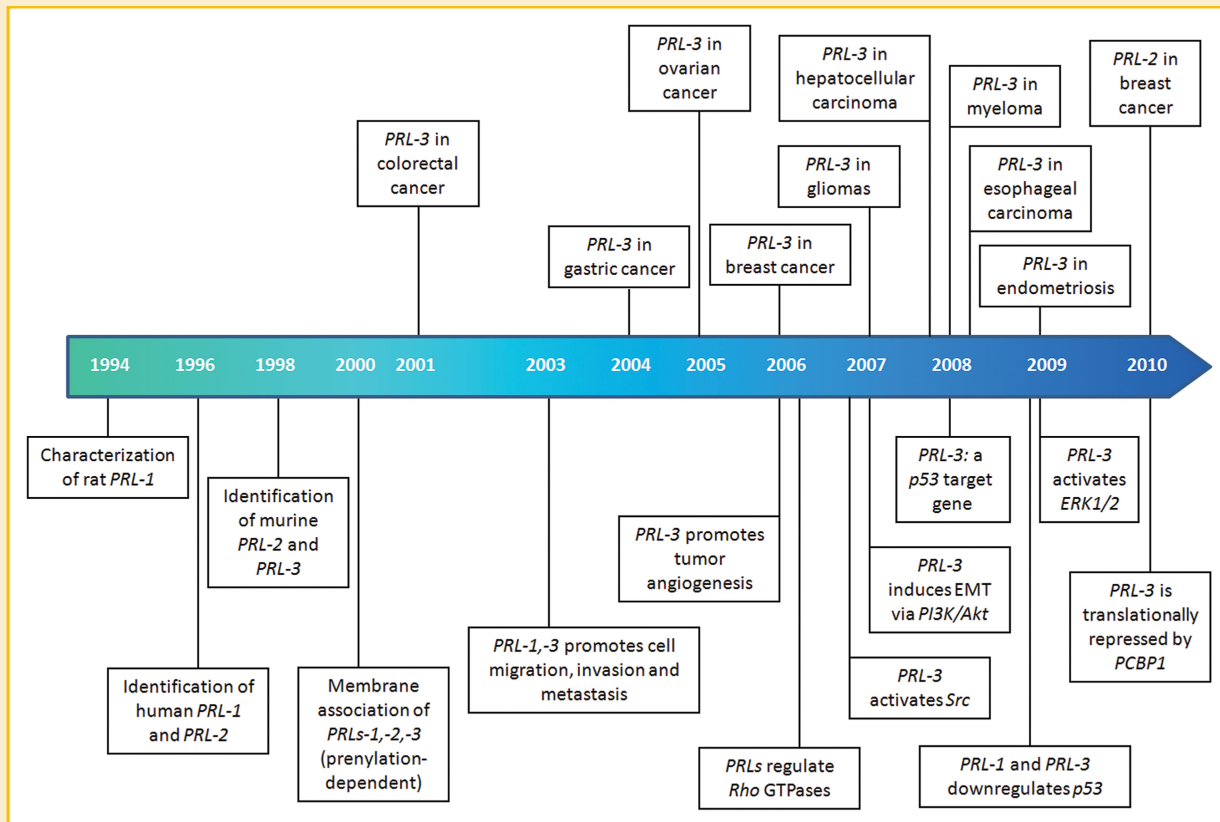

Fig. 1. Summarized timeline of major discoveries in PRL research from 1994 to date. EMT, epithelial–mesenchymal transition.

immediate-early gene upregulated in regenerating rat liver and mitogen-treated cells [Mohn et al., 1991; Diamond et al., 1994], and subsequently PRL-2 and PRL-3 were found in a database screen for PRL-1 homologues in mice [Zeng et al., 1998]. In an independent study, human PRL-1 and PRL-2 were discovered using an in vitro prenylation screen [Cates et al., 1996]. In humans, PRLs are encoded on different chromosomes, with PRL-1, -2, and -3 mapped to chromosomal loci 6q12, 1p35, and 8q24, respectively. PRL family members share significant amino acid identity—highest being 87% between PRL-1 and PRL-2, followed by 79% between PRL-1 and PRL-3, and lowest being 76% between PRL-2 and PRL-3 in humans. Genome database searches reveal that the PRLs are remarkably conserved across different species (Fig. 2), suggesting an important evolutionary role for these phosphatases in development. However, the invertebrates *Caenorhabditis elegans* and *Drosophila melanogaster* each contain only a single PRL member, suggesting that although requirement for PRL activity might exist in multicellular metazoans, it is more complex in mammals with the evolution of three functional members.

The characterized domains of PRLs are highlighted in Figure 2. PRLs have a conserved core PTP domain with the signature $C(X)_5R$ active site motif. Importantly, PRLs are the only PTPs known to bear the membrane-targeting CAAX prenylation motif at their COOH-terminus, and have been characterized as farnesylated proteins in vitro and in vivo [Cates et al., 1996; Zeng et al., 2000]. The CAAX motif is proceeded by a conserved polybasic region; this provides a cluster of positive charges which, for PRL-1, have been shown to also play a crucial role in lipid binding, presumably via electrostatic interactions [Sun et al., 2007]. Although predominantly reported to be localized to the cytomembrane and cytosol in multiple cancer cell lines, human colon cancer and endometriosis tissues [Wang et al., 2007b; Ruan et al., 2009], nuclear localization of PRL-1 and PRL-3 has also been reported [Diamond et al., 1994; Fagerli et al., 2008]. This contrasting result was partially explained by recent work in myeloma cells, where PRL-3 was found to shuttle between the nucleus and cytoplasm during S-G2M and G0/G1 phases, respectively [Fagerli et al., 2008]. Although the C-terminal polybasic sequence might serve as a nuclear localization signal, recent evidence invalidates this hypothesis [Pascaru et al., 2009], suggesting that nuclear import might occur via passive diffusion or alternative active processes. PRL-1 has also been found to cycle between the endoplasmic reticulum and the centrosome in mitotic cells in a farnesylation-independent manner [Wang et al., 2002]. Interestingly, deletion of the C-terminus prenylation motif of PRLs promotes their nuclear accumulation [Zeng et al., 2000], suggesting that reversible prenylation could regulate PRL nucleo-cytoplasmic distribution.

Structural studies have revealed that the PRLs share similar secondary structures and overall fold. Unlike other dual-specificity phosphatases (DSPs), PRLs posses unusually shallow and wide active site clefts surrounded by few protruding loops [Kozlov et al., 2004]. Such a layout might allow PRLs to accommodate both the short and

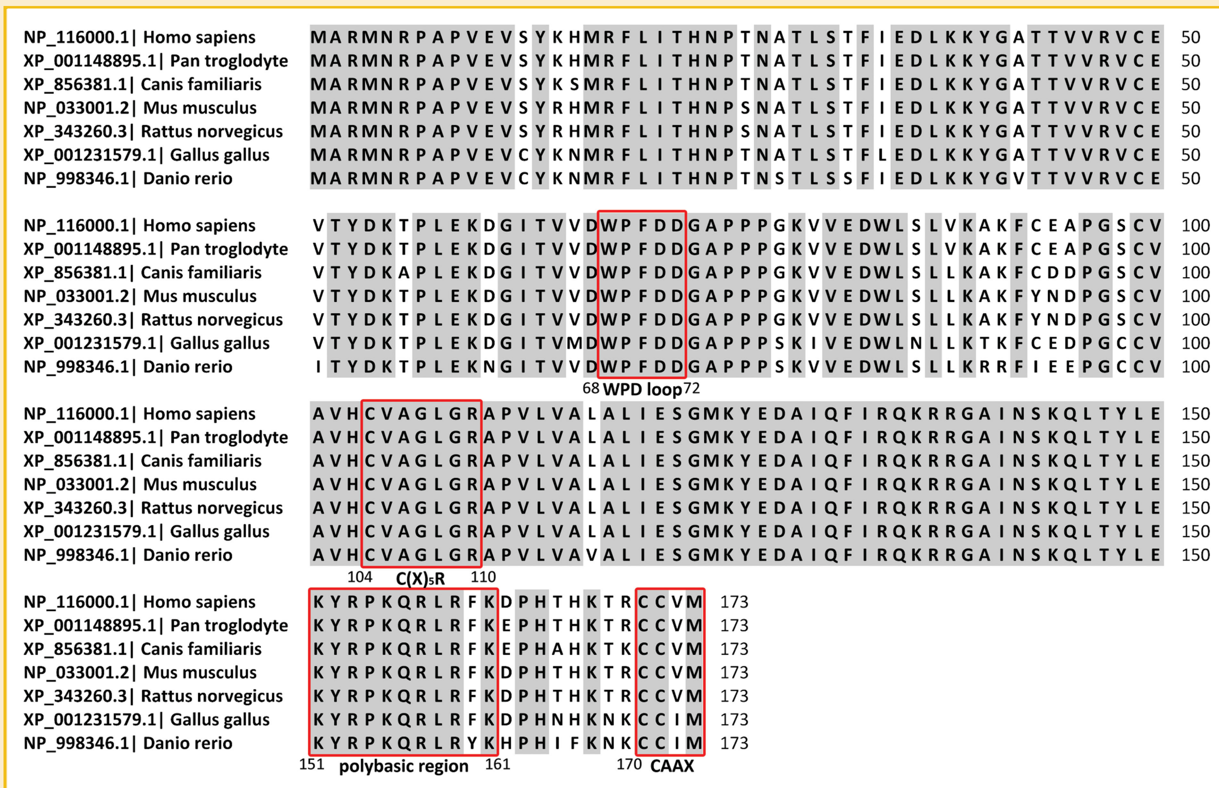

Fig. 2. Amino acid sequence alignment of known and predicted PRL-3 homologues across different species using Clustal W. For NCBI accession numbers, an "NP_" prefix indicates a known protein whereas an "XP_" prefix indicates a predicted protein based on genetic analysis. Key functional motifs of PRLs are boxed and labeled. Amino acids common to all species are shaded.

long sidechains of phospho-Ser/phospho-Thr or phospho-Tyr residues, respectively. Although this active site layout could also accommodate phosphoinositol headgroups, a lipid phosphatase activity of PRLs has not been described to date. Collectively, these features establish PRLs as a unique subclass of PTPs with a diverse and potentially redundant substrate repertoire.

PRL members have distinct expression profiles. PRL-1, unlike the almost ubiquitously expressed PRL-2, has a somewhat more restricted pattern of expression, with an overall lower expression level than PRL-2 in the same tissue or cell types [Zhao et al., 1996; Dumaual et al., 2006]. Unlike PRL-1 and PRL-2, prominent PRL-3 mRNA expression has been described in the heart, skeletal muscle, and pancreas, although it is found at lower levels in other organs [Diamond et al., 1994; Zeng et al., 1998; Matter et al., 2001; Bardelli et al., 2003; Stephens et al., 2005]. Importantly, PRL-3 protein has been detected in fetal heart, developing blood vessels, and developing erythrocytes, but not in their mature counterparts [Guo et al., 2006]. This observation, taken together with other reports describing the expression and function of PRL-3 in endothelial cells [Parker et al., 2004; Rouleau et al., 2006], suggest important roles for PRL-3 during the early development of the cardiovascular system and angiogenesis. Since PRL-3 is not expressed in adult heart, it can be considered as a potential therapeutic target for future clinical trial.

PRLs: DRIVERS OF THE METASTATIC CASCADE

Metastasis encompasses the stepwise process in which malignant cells spread from the primary tumor or origin to colonize distant organs. As a pre-requisite to metastasis, cells are first transformed by acquisition of tumor-initiating alterations that promote unlimited proliferation, apoptosis resistance, genomic instability, attraction of a blood supply, cell motility, and the maintenance of progenitor-like phenotypes [Hanahan and Weinberg, 2000]. Subsequently, transformed cells acquire additional alterations or mutations conferring the ability to penetrate blood or lymph vessels, survive in the circulation, infiltrate distant organs, and eventually colonize the new microenvironments they encounter. The influence of PRLs, particularly PRL-3, in the key steps of the metastatic process—cellular proliferation, invasion, motility, and survival—will be reviewed here.

PRL SIGNALING IN CELL PROLIFERATION

Progression towards metastasis typically starts with the oncogenic transformation of cells, giving them an unlimited proliferative advantage. PRL-1 was first implicated in cell proliferation with its discovery as an immediate-early gene expressed throughout the

course of hepatic regeneration [Mohn et al., 1991]. Subsequently, overexpression of either PRL-1 or PRL-2 was shown to promote proliferation in epithelial cells, concomitant with induction of transformed phenotypes [Diamond et al., 1994; Cates et al., 1996]. Similarly, PRL-3 has been shown to promote proliferation in various cell lines [Matter et al., 2001; Werner et al., 2003; Ming et al., 2009].

p53 is a transcription factor that activates and represses various target genes which participate in cell-cycle arrest, including the cyclin-dependent kinase (CDK) inhibitor p21 [Vousden and Lu, 2002]. Concomitant with a role in cell cycle progression, PRL-1 or PRL-2 overexpression in cancer cells resulted in downregulation of p21Cip/Waf1 expression [Werner et al., 2003]. Subsequently, PRL-1 and PRL-3 were found to enhance ubiquitination and proteosome-mediated degradation of p53 itself, an event mediated by a synergistic increase in PIRH2 transcription and MDM2 phosphorylation, both negative regulators of p53 stability [Min et al., 2009, 2010]. Since PRL-1 and PRL-3 promoted p53 downregulation via similar mechanisms, it raises the notion that common substrate(s) are likely to be shared amongst the PRL members. It is worth noting that PRL-1 has also been shown to regulate the expression of other transcription factors involved in cell proliferation, including serum response factor (SRF) [Fiordalisi et al., 2006] and early growth response protein 1 (Egr-1) [Min et al., 2009]. However, the role these regulators play in PRL-1-induced proliferation requires further study.

The phosphatidylinositol-3-kinase/protein kinase B (PI3K/AKT) signaling pathway is an important driver of cell proliferation and survival [Cully et al., 2006]. PRL-3 potently promotes PI3K–AKT activity [Wang et al., 2007a; Basak et al., 2008]. PRL-3 most likely lies upstream of PI3K, as treatment with the PI3K inhibitor LY294002 abrogated PRL-3-mediated AKT activation [Wang et al., 2007a]. Additionally, PRL-3 overexpression could down-regulate the expression of phosphatase and tensin homologue deleted on chromosome 10 (PTEN) in DLD-1 colorectal carcinoma cells [Wang et al., 2007a]. Since PTEN is the most important negative regulator of the PI3K/AKT pathway, PTEN downregulation could be an important means of PRL-3-driven PI3K/AKT signaling, although PI3K activation by other mechanisms might exist.

Despite most reports associating PRL expression to enhanced cell proliferation, there are reports noting otherwise [Qian et al., 2007; Fagerli et al., 2008; Zhou et al., 2009]. For instance, PRL-3 overexpression in mouse embryonic fibroblasts was found to induce cell-cycle arrest in late G1 [Basak et al., 2008]. This p53-independent phenomenon occurred through a pathway involving PI3K–AKT1/2 mediated upregulation of p21 expression and was dependent on cyclin-dependent kinase 2 (CDK2) activity. Surprisingly, ablation of PRL-3 in MEFs *also* elicited a potent arrest response, a phenomenon dependent on p53 [Basak et al., 2008]. The authors noted different observations in cancer cells: in RKO colon carcinoma cells, no arrest was seen upon PRL-3 overexpression, whereas in U2OS osteosarcoma cells, PRL-3 overexpression dramatically enhanced G1/S cell cycle progression [Basak et al., 2008]. Collectively, these observations suggest that cell-cycle regulation is tightly regulated by the basal levels of PRL-3 expression, and depends on the activity of CDK2 or other downstream component(s) which might be lost or modified in some genetically unstable cancer cells. Interestingly, CDK2 has been identified as an interacting partner of PRL-3 [Ewing et al., 2007]. The biochemical significance of this, however, requires further study.

PRL SIGNALING IN CELL INVASION AND MOTILITY

To enter the circulation and infiltrate distant organs, transformed cancer cells must acquire additional abilities to invade the surrounding tissues. Mechanisms conferring such invasiveness onto cancer cells include cellular motility and basement membrane degradation. In addition, the aberrant expression of developmental transcription factors might trigger epithelial–mesenchymal transition (EMT), which is associated with cellular plasticity and invasion [Yang and Weinberg, 2008]. Here we review the contribution of PRL-1 and PRL-3 to these mechanisms supporting the causal role for PRLs as metastasis-promoting genes.

Focal adhesions are dynamic structures regulated by complex signaling pathways emanating from the clustering and interactions of cell-surface integrins with a variety of extracellular matrix (ECM) components [Huveneers and Danen, 2009]. Integrin engagement regulates the activity of members of the Rho family of small GTPases–key regulators of actin cytoskeletal dynamics associated with cell motility and invasion [Sahai and Marshall, 2002]. Members of the Src family of tyrosine kinases (SFKs) also localize in cell-matrix adhesions; by partnering with focal adhesion kinase (FAK), FAK-Src complexes regulate guanine-exchange factors (GEFs) and GTPase-activating proteins (GAPs) that act on Rho-GTPases. Thus, integrin signaling through FAK-Src complexes can regulate the localization and activity of these GTPases to coordinate membrane protrusion, focal adhesion turnover and cell motility [Huveneers and Danen, 2009]. Both PRL-1 and PRL-3 have been implicated in integrin/Src signaling. In A549 lung cancer cells, ablation of PRL-1 resulted in increased adherence and decreased invasive activity, a phenotype attributed to downregulation of c-Src and p130Cas expression in these cells [Achiwa and Lazo, 2007]. Notably, tyrosine phosphorylation of FAK (Tyr397) and an intracellular integrin adaptor protein, paxillin, were rapidly and constantly elevated in PRL-1 depleted cells plated on the integrin ligand, fibronectin. In these cells there was also decreased activity of the Rho-family members Rac1 and Cdc42, both positive regulators of actin-mediated protrusion and cell motility [Achiwa and Lazo, 2007]. In another study using SW480 colorectal carcinoma cells, PRL-1 and PRL-3 overexpression was found to increase RhoA and RhoC activity by 4–7-fold, a phenomenon which was essentially abrogated by inhibition of the Rho kinase (ROCK), a key Rho effector [Fiordalisi et al., 2006]. However, although no change in Cdc42 activity was found, Rac activity was reduced by both PRL members. On the other hand, both RhoA and Rac1 activity was found to be reduced in PRL-3-overexpressing Chinese hamster ovary (CHO) and DLD-1 colorectal carcinoma cells [Wang et al., 2007a]. These seemingly disparate results on Rho family members might be explained by the dynamic focal adhesion turnover during different stages of cell adhesion and spreading, which involves spatiotemporal oscillations in the activities of RhoA, Rac1, and Cdc42 [Huveneers and Danen, 2009].

Like PRL-1, PRL-3 has also been linked to regulation of various focal adhesion components, including integrin, Src, and paxillin. Integrin beta-1 has been reported to be necessary for PRL-3 mediated activation of ERK1/2 in LoVo colorectal carcinoma cells [Peng et al., 2009]. Significantly, PRL-3 induced cell motility and invasion in vitro and metastasis in vivo was abrogated upon ablation of integrin beta-1 expression. In another study using human embroyonic kidney cells (HEK293), PRL-3 overexpression promoted Src activation by downregulating c-Src tyrosine kinase (Csk) expression and consequently its inhibitory phosphorylation on Tyr527 of Src [Liang et al., 2007]. With decreased Src inhibition, the authors found activation of several downstream targets of Src-modulated oncogenic pathways including ERK1/2, STAT3, and p130Cas [Liang et al., 2007]. Later, the same group elucidated the mechanism behind PRL-3-induced downregulation of Csk to be due to translational suppression via eukaryotic initiation factor 2 (eIF2) [Liang et al., 2008]. In HEK293 cells, PRL-3 upregulated eIF2 Ser51 phosphorylation, thus leading to a suppression of global protein synthesis including that of Csk, thereby reducing the amount of available Csk to phosphorylate and inhibit Src activity [Liang et al., 2008]. These two independent studies highlight a potentially important role of ERK1/2 in PRL-3 signaling.

In CHO and DLD-1 cells, PRL-3 overexpression caused pronounced decrease in expression and phosphorylation of other focal adhesion components including adaptors paxillin and vinculin [Wang et al., 2007a]. Vinculin promotes focal adhesion assembly and cell spreading by promoting assembly of stress fibres [Ezzell et al., 1997]. Concomitant with the downregulation of vinculin, less filamentous actin and reduced stress fiber formation were observed in PRL-3 overexpressing cells [Wang et al., 2007a]. These results imply that PRL-3 either (1) reduced the number of focal adhesions and/or (2) increased focal adhesion turnover in these cells, thereby inhibiting stress fiber formation and promoting cell migration [Thiery and Sleeman, 2006].

Besides focal adhesions, PRL-3 can also modulate other membrane proteins involved in cell–cell and cell–matrix interactions. Ezrin, an ERM family member, has been reported as a PRL-3 substrate [Forte et al., 2008]. Ezrin links cell surface growth factor receptors to the actin cytoskeleton and plays important roles in tumor–endothelium interactions, cell migrations, cell adhesion, tumor progression and metastasis [Martin et al., 2003]. PRL-3 overexpression in HCT116 cells promoted ezrin dephosphorylation on residue Thr567, whereas its ablation increased ezrin Thr567 phosphorylation in vivo. Interestingly, Thr567 phosphorylation of ezrin is known to switch it from a "closed" conformation to an active, "open" conformation, thereby bridging F-actin to the cell membrane and recruiting positive (GEFs) and negative Rho regulators (RhoGDI) [McClatchey, 2003]. It is thus conceivable that loss of ezrin Thr567 phosphorylation could inhibit recruitment of these regulators and indirectly contribute to the PRL-3 mediated deregulation of Rho family activity and increased cell motility observed in previous reports [Fiordalisi et al., 2006; Achiwa and Lazo, 2007; Wang et al., 2007a]. The cytoskeletal intermediate filament protein keratin 8 has also been suggested as another candidate target of PRL-3 [Mizuuchi et al., 2009]. By comparing the global phosphorylation profiles of SW480 colorectal carcinoma cells upon overexpression of wild-type or catalytically dead mutant (C104S) of PRL-3, keratin 8 was identified as a protein specifically dephosphorylated at Ser71 and Ser431 by elevated PRL-3 expression. Treatment with a PRL-3 specific inhibitor corroborated this result in vivo, and immunehistochemistry (IHC) analysis of a CRC patient sample revealed an inverse relationship between elevated PRL-3 expression and keratin 8 phosphorylation at the invasive front and liver metastasis, suggesting a clinical relevance of this finding [Mizuuchi et al., 2009]. However, the role of keratin 8 in PRL-3 driven motility and metastatic potential was not further examined.

Invasion is a key process of cancer cell metastasis. It involves the secretion of proteolytic enzymes, including matrix metalloproteinases (MMPs), to degrade the ECM and basement membranes, thereby permitting the movement of tumor cells into the surrounding mesenchyme, as well as the recruitment of endothelial cells during angiogenesis [Chang and Werb, 2001]. MMP activity can be regulated by integrin/Src and ERK signaling pathways [Kuo et al., 2006]. In agreement with this, PRL-3 was found to promote cell invasion by increasing MMP2 activity and decreasing the expression of the MMP inhibitor, TIMP2 [Peng et al., 2009]. Besides proteolysis of the ECM, cells also undergo a phenotypic change characteristic of EMT, which is associated with cellular plasticity, invasion, and motility [Yang and Weinberg, 2008]. In DLD-1 colorectal carcinoma cells, PRL-3 overexpression led to down-regulation of the epithelial markers E-cadherin, plakoglobin, and integrin beta-3, whilst upregulating expression of the mesenchymal markers fibronectin and Snail [Wang et al., 2007a], hallmarks of EMT. PRL-3 induced EMT could be abrogated by overexpression of catalytically inactive PRL-3 or inhibition of PI3K, suggesting that (1) the PI3K/AKT pathway was crucial to this process and (2) PRL-3 acts upstream of PI3K/AKT signaling. Thus, by synergistically increasing MMP activity and promoting EMT, PRL-3 exploits multiple pathways to effectively promote invasion and cancer progression, potentially right from the early stages of tumor development.

Concomitant with increasing tumor invasiveness, PRL-3 also promotes tumor angiogenesis. We found that, in nude mice, subcutaneous injection of PRL-3 overexpressing CHO cells led to increased recruitment of host endothelial cells within the tumor mass [Guo et al., 2006]. Likewise, in an in vitro angiogenesis model system, both CHO and DLD-1 cells overexpressing PRL-3, but not its catalytically dead mutant (C104S), could dramatically enhance endothelial vascular formation. This phenomenon was partially attributed to PRL-3 mediated downregulation of the angiogenesis inhibitor, interleukin-4 [Guo et al., 2006]. In contrast to interleukin-4, the pro-angiogenic cytokines vascular endothelial growth factor (VEGF) and its isoform VEGF-C have been reported to be upregulated in PRL-3-positive non-small cell lung cancers (NSCLC) [Ming et al., 2009]. IHC analysis of PRL-3 expression in NSCLC revealed a significant association between PRL-3 expression and VEGF and VEGF-C expression, micro vessel density and lymphatic vessel density. In addition, treatment of A549 lung cancer cells with an anti-PRL-3 antibody resulted in a decrease in VEGF and VEGF-C expression, concomitant with a decrease in invasive and migratory abilities [Ming et al., 2009]. Thus, by regulating

secretion of pro- and antiangiogenic factors, PRL-3 facilitates subsequent metastatic dissemination by promoting efficient endothelial cell attraction and tumor angiogenesis.

PRL SIGNALING IN CELL SURVIVAL

In addition to forming a locally aggressive tumor, entering the circulation and then exiting it to infiltrate distant organs, distant organ infiltration and colonization are general steps that primary tumor cells must accomplish to successfully metastasize. PRL-3 may facilitate these final steps of tumor metastasis by promoting extravasation, micro-metastasic tumor formation, and ultimately cell survival by inhibiting apoptosis. We first demonstrated the causative role of PRLs in metastasis using an experimental metastatic assay [Zeng et al., 2003]. In this assay, cells are injected directly into the bloodstream of mice via the tail-vein, thereby bypassing the requirement for the early steps of metastasis, including intravasation. Intriguingly, all mice injected with CHO cells overexpressing either PRL-1 or PRL-3 had extensive metastasis to the lungs, whereas none was observed for control CHO cells overexpressing β-gal [Zeng et al., 2003]. We subsequently found that this effect was dependent on the catalytic activity of PRL-3 [Guo et al., 2004]. Currently, the biochemical mechanism behind the ability of a single gene (PRL-1 or PRL-3) to confer the potent metastatic capacity seen in the tail-vein assay is still poorly defined. Specifically, the contribution of Src and/or PI3K–AKT signaling to this process constitutes a research area deserving more attention.

After intravasation, tumor cells face harsh conditions in the circulation, leading to many malignant cells perishing at this stage [Al-Mehdi et al., 2000]. Interestingly, we found that after tail-vein injection into nude mice, CHO cells overexpressing PRL-3 could form solid tumor emboli within blood vessels and survive in this intravascular location [Guo et al., 2004]. This observation alludes to a dominant pro-survival effect of PRL-3 for circulating tumor cells, in addition to promoting tumor extravasation. Furthermore, in line with promoting survival and reducing cell death, both PRL-1 and PRL-3 have been reported to promote resistance to p53-induced apoptosis by targeting this potent tumor-suppressor for degradation [Min et al., 2009, 2010]. Thus, by simultaneously promoting extravasation and increasing cell survival, PRL-1 and PRL-3 employ a potent dual-pronged approach to drive tumor dispersion in late metastasis.

PRL INTERACTING PROTEINS

Despite advancements in elucidating the roles of PRLs in cancer progression and their signaling pathways, a major challenge in studying the detailed signaling mechanism of PRLs is the lack of physiologically relevant substrates identified to date—a problem largely due to the transient nature of the phosphatase–substrate interaction. Nonetheless, several PRL interacting proteins have been reported (Table I). To date, the only direct PRL substrate known is the transcription factor ATF-7 (now known as ATF-5 or ATF-X) by PRL-1 [Peters et al., 2001]. ATF-7 belongs to the basic leucine zipper protein family of transcription factors, and is involved in regulating expression of genes involved in proliferation and survival [Persengiev and Green, 2003]. However, despite PRL-1's ability to dephosphorylate ATF-7 on tyrosine residues in vitro, the function of tyrosine phosphorylation in regulating ATF-7 activity is still unknown.

As mentioned above, the ERM component, ezrin, has been reported as a PRL-3 substrate [Forte et al., 2008]. PRL-3 overexpression or ablation inversely affected ezrin phosphorylation on Thr567 in vivo, and immunopurified PRL-3 possessed apparent phosphatase activity towards Thr657 of immunopurified ezrin in vitro. In an alternative approach involving comparative proteomic analysis of differentially phosphorylated proteins in HCT116 cells with and without PRL-3 overexpression, besides ezrin, the same group identified elongation factor 2 (eEF-2) as another phosphorylated protein regulated by PRL-3 [Orsatti et al., 2009]. It is worth pointing out that in an independent differential phospho-proteomic approach conducted in SW480 colorectal carcinoma cells, no influence of PRL-3 on ezrin phosphorylation was found [Mizuuchi et al., 2009]. In this latter study, the authors found the intermediate filament component, keratin 8, to be a physiological PRL-3 target. PRL-3 interacted with and promoted keratin 8 Ser73 and Ser431 dephosphorylation in vivo. It is worth highlighting here that for both ezrin and keratin 8, PRL-3 induced differential phosphorylation on non-tyrosine residues. As a DSP, these observations allude to

TABLE I. Identified PRL Binding Partners and Putative Substrates

	Interacting protein	Outcome of interaction	Validation method(s)	Refs.
PRL-1	ATF-7	ATF-7 dephosphorylation in vitro	Y2H, IP	Peters et al. [2001]
	PRL-1	Trimer formation in vitro and in vivo	IP	Jeong et al. [2005], Sun et al. [2007]
PRL-2	βGGT-II	Binding competition with αGGT-II in vivo	Y2H, IP	Si et al. [2001]
PRL-3[a]	CDH22	N.d.	Y2H, PD, IP	Liu et al. [2009]
	Ezrin	Ezrin dephosphorylation in vitro (indirect) and in vivo	—	Forte et al. [2008], Orsatti et al. [2009]
	Elongation factor 2	N.d.	IP	Orsatti et al. [2009]
	Keratin 8	KRT8 dephosphorylation in vivo	IP	Mizuuchi et al. [2009]
	Integrin-α1	N.d.	Y2H, PD	Peng et al. [2006]
	PRL-3	Oligomer formation in vitro	IP	Sun et al. [2007], Pascaru et al. [2009]

[a]In a separate unbiased high-throughput study employing ESI-MS/MS, 36 novel PRL-3 interactors (not listed here) were identified [Ewing et al., 2007].
Y2H, yeast 2-hybrid assay; IP, immunoprecipitation; PD, GST pull-down. N.d., not determined.

the wide repertoire of potential PRL target—future efforts could employ "substrate-trapping" mutants to facilitate identification of genuine PRL substrates [Flint et al., 1997].

Besides functioning as enzymes, PRLs could act as competitors or scaffolds in signaling protein complexes, thereby modulating downstream signaling pathways. For instance, a closely-related phosphatase, PTEN, forms crucial non-substrate interactions with a variety of molecules, including MSP58 [Okumura et al., 2005]. The beta subunit of the prenyltransferase geranylgeranyltransferase II (βGGT-II) has been identified as a PRL-2 interacting protein, although PRL-2 itself is not geranylgeranylated [Si et al., 2001]. PRL-2 competes with the alpha subunit of GGT-II (αGGT-II) for binding to βGGT-II in vivo, thereby regulating GGT-II activity. Significantly, GGT-II is a Rab GGT—prenylation of Rab GTPase is essential for its function in the vesicle transport pathway [Calero et al., 2003]. This competitive interaction suggests that PRL-2 might regulate intracellular protein trafficking though functional GGT-II assembly. Similarly, using the yeast-2-hybrid approach, PRL-3 has been shown to interact with integrin-α1 [Peng et al., 2006] and cadherin-22 [Liu et al., 2009]; however, PRL-3-dependent regulation of either interacting proteins' phosphorylation in mammalian cells has not been studied. Both these integral membrane proteins are known to be important in cell–ECM and cell–cell interactions. Interestingly, PRL-3 overexpression in COS-7 cells caused a decrease in tyrosine phosphorylation of integrin beta-1, a heterodimeric binding partner of integrin alpha-1 [Peng et al., 2006]. While it is tempting to hypothesize that integrin alpha-1 could act as a scaffold to recruit PRL-3 for tyrosine dephosphorylation of integrin beta-1, no direct interaction between PRL-3 and integrin beta-1 has been detected in vitro [Peng et al., 2009]. In addition, PRL-3 could co-immunoprecipitate with integrin beta-1 in human colon cancer LoVo cells, which lack integrin alpha-1 [Peng et al., 2009]. These results suggest that the integrin beta-1 interaction is indirect, and may be facilitated by PRL-3 recruitment via integrin alpha-1 or alternative heterodimeric partners.

REGULATION OF PRL EXPRESSION AND FUNCTION

With the establishment of key PRL functions in metastatic progression, the mechanisms underlying regulation of PRL expression and function have attracted both basic science and pharmaceutical researchers. Since an excess of PRL phosphatase activity is clearly a key alteration contributing to the acquisition of metastatic properties in tumor cells, much focus has been placed on exploring and understanding the regulation of PRLs, particularly PRL-3 (summarized in Fig. 3).

GENE AMPLIFICATION

PRL overexpression can occur through gene amplification in genetically unstable cancer cells, as shown for PRL-3, which is located on 8q24.3 and found to be amplified in 25% (3/12) of liver metastasis of CRCs [Saha et al., 2001]. Indeed, a statistically significant difference in PRL-3 gene copy number between liver metastasis and primary lesions of CRC was reported [Bardelli et al., 2003]. Increased gene copy numbers of PRL-3 have also been found in primary CRCs with liver metastasis compared to those without liver metastasis [Buffart et al., 2005], as well as in several myeloma cell lines [Fagerli et al., 2008]. c-myc, a well-characterized oncogene, is located on 8q24.12–q24.23, a locus just upstream of PRL-3. Interestingly, c-myc is similarly amplified and overexpressed in metastasizing CRCs [Buffart et al., 2005]. Due to the physical proximity of these two gene loci, it is not yet clear if PRL-3 gene amplification is specifically selected in synthesis with c-myc amplification, or an indirect consequence of c-myc locus amplification.

However, gene amplification alone seems unlikely to account for the overexpression of PRLs seen at high frequency in multiple advanced tumors (reviewed in [Bessette et al., 2008]). In myeloma cell lines, no strict correlation was found between PRL-3 gene copy numbers and mRNA expression level [Fagerli et al., 2008]. The converse holds true as well; not all cases with an amplification of the PRL-3 gene display mRNA overexpression [Saha et al., 2001; Polato et al., 2005]. For instance, amongst myeloma cell lines, IH-1 with 3–5 copies of chromosome 8 had high levels of PRL-3, whereas CAG with 4 copies of chromosome 8 had very low levels of PRL-3 [Fagerli et al., 2008]. These observations implicate that PRL-3 expression must be tightly regulated at transcriptional and/or post-transcriptional levels.

mRNA EXPRESSION

The first hint at the post-genomic regulation of PRLs came from the early finding that PRL-1 was specifically upregulated during the course of hepatic regeneration [Mohn et al., 1991]. Subsequent reports on induction of PRL mRNA expression in cells upon direct mitogenic stimulation [Rouleau et al., 2006; Fagerli et al., 2008] and exposure to conditioned medium from carcinoma-associated fibroblasts [Molleví et al., 2009] suggests that extracellular stimuli transduced via growth factor signaling networks are involved in PRL regulation. Egr-1, a growth factor-activated transcription factor, is one such factor implicated in the transcriptional upregulation of PRL-1 [Peng et al., 1999]. The PRL-1 intron enhanced complex (PIEC), a developmentally-regulated factor, regulates PRL-1 expression by direct binding to the first intron of PRL-1 [Peng et al., 1998]. p53 is a transcription factor that activates and represses various target genes proposed to participate in cell-cycle arrest or apoptotic responses [Vousden and Lu, 2002]. Surprisingly, both PRL-3 and PRL-1 have been reported to have p53-binding elements in their first intron and have been verified as p53-inducible target genes [Basak et al., 2008; Min et al., 2009, 2010]. The discordance between being direct p53-upregulated targets yet causal factors in metastasis progression might be explained by their feedback loop on p53 expression: both PRL-3 and PRL-1 have been shown to downregulate p53 via its increased ubiquitination and proteosomal degradation [Min et al., 2009, 2010]. However, as p53 activity is frequently disrupted early in the progression of many tumor types [Hollstein et al., 1991], the elevated PRL-3/-1 levels observed in metastatic cells require perturbations of this negative feedback loop or alternative transcriptional activation to allow PRL accumulation independent from p53 activity. Bioinformatics prediction tools (CONSITE, TRED) suggest that PRL-3 has several putative promoter binding sites including that for n-MYC, STAT3, and NF-kB.

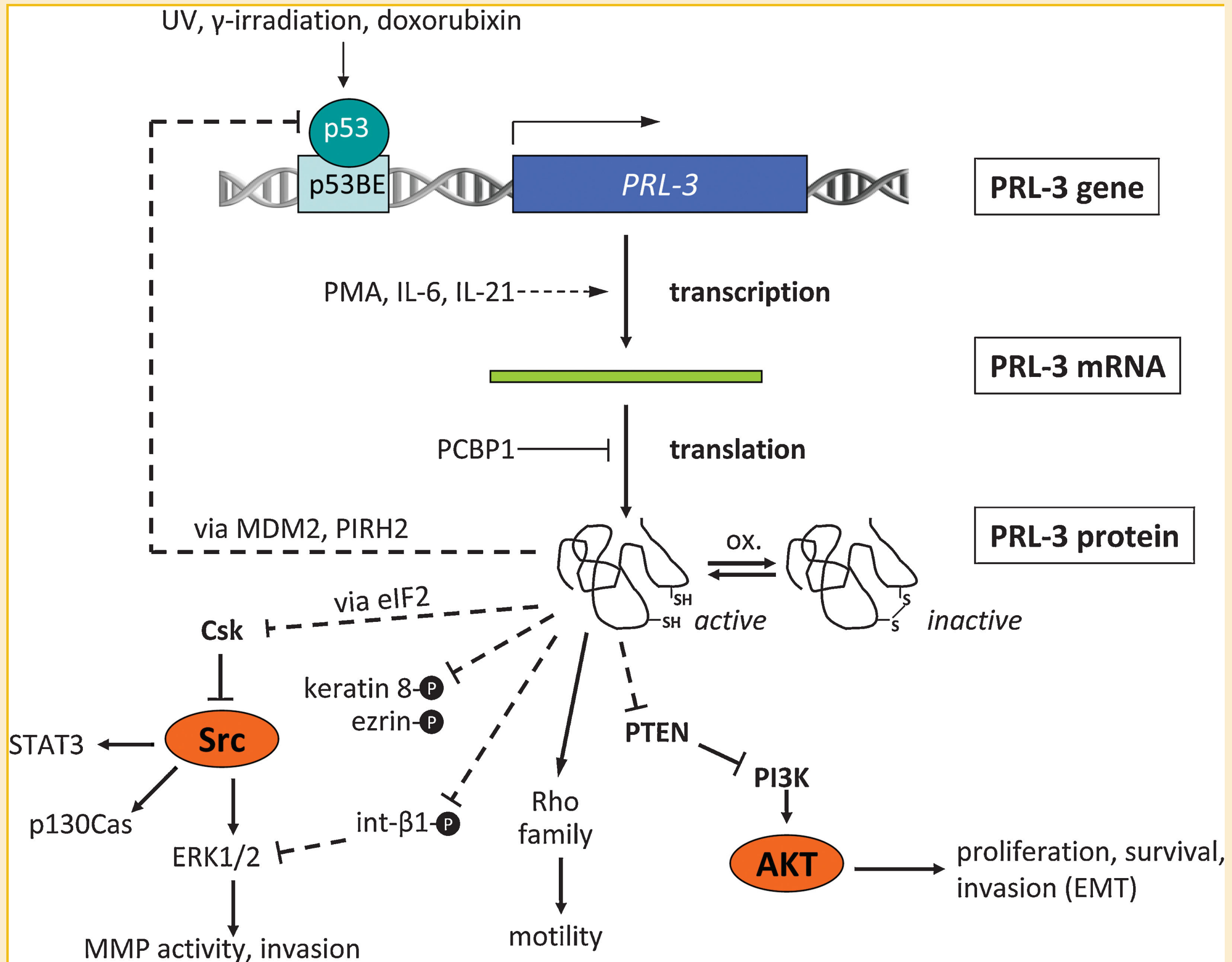

Fig. 3. Proposed regulatory network and signaling pathways downstream of PRL-3 culminating in metastatic progression. See text for details.

Although the functional relevance of these transcription factors on PRL-3 expression have not directly been reported, a recent study showed that stimulation of myeloma cells with interleukin-6, a cytokine which promotes STAT3 transcriptional activity [Heinrich et al., 2003], could potently induce a 2.5–3.5-fold increase in PRL-3 mRNA levels [Fagerli et al., 2008].

PROTEIN TRANSLATION

Beyond transcriptional regulation of genes, translation is an important relay between transcript levels and protein expression. We recently found that PRL-3 expression could be negatively regulated at the translational level by direct interaction between poly(C)-binding protein 1 (PCBP1) and triple GCCCAG motifs within the 5′-untraslated region (5′-UTR) of PRL-3 mRNA [Wang et al., 2010]. PCBP1 is a member of the hnRNP family of RNA- and/or DNA-binding proteins, with described roles in the regulation of RNA transcription, pre-mRNA processing, maturation, and mRNA export [Choi et al., 2009]. Overexpression or ablation of PCPBP1 resulted in suppression or increase in PRL-3 protein levels, respectively, without any change in PRL-3 transcript levels. The mechanism behind this regulation was the PCBP1-induced retardation of PRL-3 mRNA incorporation into polyribosomes, thereby negatively regulating PRL-3 protein translation [Wang et al., 2010]. Significantly, immunoblotting and immunohistochemical analysis revealed an inverse correlation between PRL-3 and PCBP1 in multiple tumor types, thereby establishing a clinical relevance of this finding.

OXIDATION, OLIGOMERIZATION AND PHOSPHORLYATION

The architecture of the active site of PTPs renders them sensitive to reversible oxidation, which has emerged to be an important regulatory mechanism of PTP activity [Tonks, 2005]. Due to participation of the catalytic cysteine in disulfide bond formation, oxidized PTPs lose catalytic function and require reduction by cellular reducing agents to regain activity. Structural analysis has revealed conserved cysteine residues in PRL-1 and PRL-3 (Cys49) which lie in close proximity with the catalytic cysteine (Cys104) which, under oxidizing conditions, can form a disulfide bond [Kozlov et al., 2004; Sun et al., 2005]. Biochemical analysis of PRL-1 found it to have a reduction potential of approximately −365 mV at pH 7.5 [Skinner et al., 2009], a value much lower than the reduction potential in most cellular compartments. This suggests

that PRL-1 is largely kept inactive by catalytic cysteine oxidation intracellularly, and might require activation by some post-translational modification or binding partner. Interestingly, mutation of Cys170 of PRL-1, which is also the cysteine residue targeted for farnesylation in the prenylation motif (CAAX), makes it less prone to inactivation. This implies that in vivo, farnesylation could serve to regulate both localization and enzymatic activity by promoting reduction of catalytic cysteine. This might provide basis for previous reports describing the requirement of prenylation for the biological activities of PRL-1 [Fiordalisi et al., 2006; Sun et al., 2007; Skinner et al., 2009] and PRL-3 [Song et al., 2009; Fiordalisi et al., 2006], in addition to proper localization for enzyme–substrate interaction. Oxidative stress may also play a part in PRL expression and regulation. PRL-1 expression was found to be upregulated in retinal cells induced by oxidative stress upon light exposure [Yu et al., 2007], and after transient forebrain ischemia in the rat cerebral cortex [Takano et al., 1996]. These observations suggest that PRLs might have additional roles in the cellular response to oxidative stress—an intriguing possibility which might unravel novel pro-tumorigenic roles for this family in solid cancers, which routinely experience hypoxic conditions in vivo.

Oligomerization of PRLs may also regulate their activity. Previous studies have illustrated the propensity of PRL-1 [Jeong et al., 2005; Sun et al., 2005] and PRL-3 [Sun et al., 2007; Pascaru et al., 2009] to form oligomers both in vitro and in vivo. Regulated dimerization has been implicated as a mechanism for the negative regulation of receptor-like PTPs [Ostman et al., 2006]. Intriguingly, PRL-1 trimerization, which requires C-terminal prenylation, was found to be essential for its biological function in vivo [Sun et al., 2007]. Similarly, C-terminus prenylation of PRL-3 promoted oligomer formation; however, an inhibition in catalytic activity was observed for oligomerized PRL-3 in vitro [Pascaru et al., 2009]. These contradicting results are likely due to the different model systems employed—within the intracellular milieu, there likely exist additional regulators which serve to control PRL function by direct interaction or post-translational modifications. Direct kinetic measurements of phosphatase activity in vitro cannot take these events into consideration, and likely accounts for the difference in results.

Another common mechanism for regulating protein activity is phosphorylation. Mono-or multi-phosphorylation of a protein can modulate its intrinsic biological activity, subcellular location, half-life, and/or docking with other proteins [Cohen, 2000]. Computational prediction using the ScanProsite program suggested that PRLs possess both conserved and unique putative phosphorylation sites between the three highly homologous members [Zeng et al., 1998]. For instance, all three PRLs have consensus phosphorylation sites for casein kinase II, yet only PRL-1 and PRL-3 have consensus protein kinase C phosphorylation sites. Human PRL-3 has been shown to be directly phosphorylated by pp60Src in vitro [Matter et al., 2001], but the functional relevance of this finding in vivo is unknown. Given the potent capacity of post-translational regulation in regulating protein function, the regulation of PRLs activity and stability via phosphorylation, ubiquitination, glycosylation, and other mechanisms deserve attention. Importantly, increased PRL activity due to post-translational regulation might have significance in tumors without overt PRL overexpression.

PRLS AS THERAPEUTIC TARGETS

Despite substantial advances in cancer therapy, metastatic disease remains the primary cause of morbidity and mortality in cancer. With the recognition of the causal role of PRLs in the multiple stages of cancer metastasis and the characterization of their molecular determinants, PRLs are becoming increasingly attractive as drug targets for metastatic intervention. Several anti-PRL drugs have been reported in recent years [Pathak et al., 2002; Ahn et al., 2006; Daouti et al., 2008; Guo et al., 2008; Park et al., 2008; Wang et al., 2009], and they can be generally classified into (1) small molecule inhibitors or (2) antibody therapy.

Pentamidine is an anti-protozoan compound shown to have somewhat selective inhibition of several PTPs including MKP-1, PTP1B, and the PRLs [Pathak et al., 2002]. Interestingly, pentamidine's inhibitory effect on phosphatase activity was prolonged despite washing away the compound after preincubation. It could also inactivate ectopically expressed PRL-2 in cancer cells. Despite the tumor suppressive effects of pentamidine on growth of WM9 human melanoma cells in nude mice, this observation could not be attributed to PRL inhibition solely, as other endogenous PTPs could be suppressed as well. A more promising small molecule PRL inhibitor recently identified is the thienopyridone, 7-amino-2-phenyl-5H-thieno[3,2-c]pyridin-4-one. This drug was shown to selectively inhibit all three PRL members, but not 11 other tyrosine and DSPs of different classes, in vitro [Daouti et al., 2008]. Inhibition of PRLs by thienopyridone resulted in the inhibition of tumor cell anchorage-independent growth and the induction of anoikis (a type of apoptosis). Thienopyridone treatment also inhibited mitogen-induced endothelial cell migration. These effects were shown to occur through a novel p53-independent mechanism involving the inhibitory cleavage of p130Cas [Daouti et al., 2008].

Modern high-throughput screening methods have unveiled new classes of PRL-3 small molecule inhibitors, including rhodamine [Ahn et al., 2006]. In this study, two unique rhodamine derivates were found to be more efficient than pentamidine in blocking invasiveness of B16F10 cells in vitro. In an alternative study employing structural-based virtual docking of PRL-3, 12 novel and structurally diverse compounds were identified to bind and inhibit PRL-3 [Park et al., 2008]. These compounds had binding activity to both active and peripheral sites of PRL-3. However, like pentamidine, the value and specificity of these newly identified small molecule PRL-3 inhibitors will require further characterization before preclinical testing and drug development.

A novel method of PRL inhibition is the use of antibody therapy against these intracellular phosphatases. Using the well experimental tail-vein metastasis assay, we found that mouse monoclonal (mAb) and rabbit polyclonal antibodies to PRL-1 and PRL-3 could specifically and dramatically reduce PRL-1- and PRL-3-expressing metastatic lung tumors in nude mice [Guo et al., 2008]. Importantly, PRL-1 mAb specifically blocks PRL-1 but not PRL-3 metastatic tumors; while PRL-3 mAb specifically blocks PRL-3 but not PRL-1 metastatic tumors. This specificity is impressive, considering the high amino acid sequence identity between murine PRL-1 and PRL-3. PRL-3 mAb specifically inhibited cells expressing endogenous PRL-3 (A2780, HCT116), but not those cells having low

or no PRL-3 expression (DLD-1, CT26). This event is not unique; others have presented evidence of intracellular PRL-3 inhibition by PRL-3 antibodies added directly to the culture medium as well [Ming et al., 2009]. Interestingly, we found that serum starvation could promote antibody uptake into cells [Guo et al., 2008]. The reason for this is unknown, but the enhanced uptake due to serum (or mitogen) deprivation might have additional benefit by efficiently and selectively targeting tumor cells in nutrient-deprived niches. We have successfully repeated this intracellular antibody therapy approach in vivo using clinically-relevant chimeric antibodies to PRL-1 and PRL-3 as well (Tang et al., manuscript in preparation). Ultimately, in view of the differences in the expression profiles between PRLs (particularly PRL-2), specific antibody targeting of individual PRL members constitutes an important consideration in minimizing unwanted side effects in anti-PRL cancer therapy.

CONCLUSION

As clinicians move towards personalized cancer medicine, there is an urgent need to understand and identify key factors involved in the biology of metastasis. In light of the evidence discussed here, we propose that PRL-3 is a key metastasis-initiating gene deregulated early in the metastatic process, driving metastasis progression from primary to distant sites. With pronounced overexpression in multiple metastatic tumor types and direct implications in cellular transformation and key metastatic events, PRLs, particularly PRL-1 and PRL-3, may represent ideal candidates for personalized cancer therapy. It should be noted that in a comparison between several different commercially available PRL-3 antibodies and those we generated [Li et al., 2005], most of these antibodies failed to detect endogenous PRL-3 protein satisfactorily in immunoblots under conditions that our antibodies were successful. We also found that several commercial PRL-antibodies lacked PRL isoform-specific binding (unpublished data). In light of this, we feel that the scientific community needs to stringently validate the specificity and usefulness of such antibodies before embarking on studies using them. Future work should be directed towards understanding the biochemical regulation and mechanisms of action of members of this important family.

REFERENCES

Achiwa H, Lazo JS. 2007. PRL-1 tyrosine phosphatase regulates c-Src levels, adherence, and invasion in human lung cancer cells. Cancer Res 67:643–650.

Ahn JH, Kim SJ, Park WS, Cho SY, Ha JD, Kim SS, Kang SK, Jeong DG, Jung SK, Lee SH, Kim HM, Park SK, Lee KH, Lee CW, Ryu SE. 2006. Synthesis and biological evaluation of rhodanine derivatives as PRL-3 inhibitors. Bioorg Med Chem Lett 16:2996–2999.

Akagi K, Suzuki T, Stephens RM, Jenkins NA, Copeland NG. 2004. RTCGD: retroviral tagged cancer gene database. Nucleic Acids Res 32:D523–D527.

Al-Mehdi AB, Tozawa K, Fisher AB, Shientag L, Lee A, Muschel RJ. 2000. Intravascular origin of metastasis from the proliferation of endothelium-attached tumor cells: a new model for metastasis. Nat Med 6:100–102.

Alonso A, Sasin J, Bottini N, Friedberg I, Friedberg I, Osterman A, Godzik A, Hunter T, Dixon J, Mustelin T. 2004. Protein tyrosine phosphatases in the human genome. Cell 117:699–711.

Bardelli A, Saha S, Sager JA, Romans KE, Xin B, Markowitz SD, Lengauer C, Velculescu VE, Kinzler KW, Vogelstein B. 2003. PRL-3 expression in metastatic cancers. Clin Cancer Res 9:5607–5615.

Basak S, Jacobs SBR, Krieg AJ, Pathak N, Zeng Q, Kaldis P, Giaccia AJ, Attardi LD. 2008. The metastasis-associated gene Prl-3 is a p53 target involved in cell-cycle regulation. Mol Cell 30:303–314.

Bessette DC, Qiu D, Pallen CJ. 2008. PRL PTPs: mediators and markers of cancer progression. Cancer Metastasis Rev 27:231–252.

Buffart TE, Coffa J, Hermsen MAJA, Carvalho B, van der Sijp JRM, Ylstra B, Pals G, Schouten JP, Meijer GA. 2005. DNA copy number changes at 8q 11–24 in metastasized colorectal cancer. Cell Oncol 27:57–65.

Calero M, Chen CZ, Zhu W, Winand N, Havas KA, Gilbert PM, Burd CG, Collins RN. 2003. Dual prenylation is required for Rab protein localization and function. Mol Biol Cell 14:1852–1867.

Cates CA, Michael RL, Stayrook KR, Harvey KA, Burke YD, Randall SK, Crowell PL, Crowell DN. 1996. Prenylation of oncogenic human PTP(CAAX) protein tyrosine phosphatases. Cancer Lett 110:49–55.

Chang C, Werb Z. 2001. The many faces of metalloproteases: cell growth, invasion, angiogenesis and metastasis. Trends Cell Biol 11:S37–S43.

Choi HS, Hwang CK, Song KY, Law PY, Wei LN, Loh HH. 2009. Poly(C)-binding proteins as transcriptional regulators of gene expression. Biochem Biophys Res Commun 380:431–436.

Cohen P. 2000. The regulation of protein function by multisite phosphorylation – a 25 year update. Trends Biochem Sci 25:596–601.

Cully M, You H, Levine AJ, Mak TW. 2006. Beyond PTEN mutations: the PI3K pathway as an integrator of multiple inputs during tumorigenesis. Nat Rev Cancer 6:184–192.

Daouti S, Li WH, Qian H, Huang KS, Holmgren J, Levin W, Reik L, McGady DL, Gillespie P, Perrotta A, Bian H, Reidhaar-Olson JF, Bliss SA, Olivier AR, Sergi JA. 2008. A selective phosphatase of regenerating liver phosphatase inhibitor suppresses tumor cell anchorage-independent growth by a novel mechanism involving p130Cas cleavage. Cancer Res 68:1162–1169.

Diamond RH, Cressman DE, Laz TM, Abrams CS, Taub R. 1994. PRL-1, a unique nuclear protein tyrosine phosphatase, affects cell growth. Mol Cell Biol 14:3752–3762.

Dumaual CM, Sandusky GE, Crowell PL, Randall SK. 2006. Cellular localization of PRL-1 and PRL-2 gene expression in normal adult human tissues. J Histochem Cytochem 54:1401–1412.

Ewing RM, Chu P, Elisma F, Li H, Taylor P, Climie S, McBroom-Cerajewski L, Robinson MD, O'Connor L, Li M, Taylor R, Dharsee M, Ho Y, Heilbut A, Moore L. 2007. Large-scale mapping of human protein-protein interactions by mass spectrometry. Mol Syst Biol 3:89.

Ezzell RM, Goldmann WH, Wang N, Parashurama N, Parasharama N, Ingber DE. 1997. Vinculin promotes cell spreading by mechanically coupling integrins to the cytoskeleton. Exp Cell Res 231:14–26.

Fagerli UM, Holt RU, Holien T, Vaatsveen TK, Zhan F, Egeberg KW, Barlogie B, Waage A, Aarset H, Dai HY, Shaughnessy JD, Sundan A, Børset M. 2008. Overexpression and involvement in migration by the metastasis-associated phosphatase PRL-3 in human myeloma cells. Blood 111:806–815.

Fiordalisi JJ, Keller PJ, Cox AD. 2006. PRL tyrosine phosphatases regulate rho family GTPases to promote invasion and motility. Cancer Res 66:3153–3161.

Flint AJ, Tiganis T, Barford D, Tonks NK. 1997. Development of "substrate-trapping" mutants to identify physiological substrates of protein tyrosine phosphatases. Proc Natl Acad Sci USA 94:1680–1685.

Forte E, Orsatti L, Talamo F, Barbato G, De Francesco R, Tomei L. 2008. Ezrin is a specific and direct target of protein tyrosine phosphatase PRL-3. Biochim Biophys Acta 1783:334–344.

Guo K, Li J, Tang JP, Koh V, Gan BQ, Zeng Q. 2004. Catalytic domain of PRL-3 plays an essential role in tumor metastasis: formation of PRL-3 tumors inside the blood vessels. Cancer Biol Ther 3:945–951.

Guo K, Li J, Wang H, Osato M, Tang JP, Quah SY, Gan BQ, Zeng Q. 2006. PRL-3 initiates tumor angiogenesis by recruiting endothelial cells in vitro and in vivo. Cancer Res 66:9625–9635.

Guo K, Tang JP, Tan CPB, Wang H, Zeng Q. 2008. Monoclonal antibodies target intracellular PRL phosphatases to inhibit cancer metastases in mice. Cancer Biol Ther 7:750–757.

Hardy S, Wong NN, Muller WJ, Park M, Tremblay ML. 2010. Overexpression of the protein tyrosine phosphatase PRL-2 correlates with breast tumor formation and progression. Cancer Res. Published Online First as DOI: 10.1158/0008-5472.CAN-10-2041.

Hanahan D, Weinberg RA. 2000. The hallmarks of cancer. Cell 100:57–70.

Heinrich PC, Behrmann I, Haan S, Hermanns HM, Müller-Newen G, Schaper F. 2003. Principles of interleukin (IL)-6-type cytokine signalling and its regulation. Biochem J 374:1–20.

Hollstein M, Sidransky D, Vogelstein B, Harris CC. 1991. p53 mutations in human cancers. Science 253:49–53.

Huveneers S, Danen EHJ 2009 Adhesion signaling-crosstalk between integrins, Src and Rho. J Cell Sci 122:1059–1069.

Jeong DG, Kim SJ, Kim JH, Son JH, Park MR, Lim SM, Yoon TS, Ryu SE. 2005. Trimeric structure of PRL-1 phosphatase reveals an active enzyme conformation and regulation mechanisms. J Mol Biol 345:401–413.

Kozlov G, Cheng J, Ziomek E, Banville D, Gehring K, Ekiel I. 2004. Structural insights into molecular function of the metastasis-associated phosphatase PRL-3. J Biol Chem 279:11882–11889.

Kuo L, Chang HC, Leu TH, Maa MC, Hung WC. 2006. Src oncogene activates MMP-2 expression via the ERK/Sp1 pathway. J Cell Physiol 207:729–734.

Li J, Guo K, Koh VWC, Tang JP, Gan BQ, Shi H, Li HX, Zeng Q. 2005. Generation of PRL-3- and PRL-1-specific antibodies as potential diagnostic markers for cancer metastases. Clin Cancer Res 11:2195–2204.

Liang F, Liang J, Wang WQ, Sun JP, Udho E, Zhang ZY. 2007. PRL3 promotes cell invasion and proliferation by down-regulation of Csk leading to Src activation. J Biol Chem 282:5413–5419.

Liang F, Luo Y, Dong Y, Walls CD, Liang J, Jiang HY, Sanford JR, Wek RC, Zhang ZY. 2008. Translational control of C-terminal Src kinase (Csk) expression by PRL3 phosphatase. J Biol Chem 283:10339–10346.

Liu Y, Zhou J, Chen J, Gao W, Le Y, Ding Y, Li J. 2009. PRL-3 promotes epithelial mesenchymal transition by regulating cadherin directly. Cancer Biol Ther 8:1352–1359.

Martin TA, Harrison G, Mans RE, Jiang WG. 2003. The role of the CD44/ezrin complex in cancer metastasis. Crit Rev Oncol Hematol 46:165–186.

Matter WF, Estridge T, Zhang C, Belagaje R, Stancato L, Dixon J, Johnson B, Bloem L, Pickard T, Donaghue M, Acton S, Jeyaseelan R, Kadambi V, Vlahos CJ. 2001. Role of PRL-3, a human muscle-specific tyrosine phosphatase, in angiotensin-II signaling. Biochem Biophys Res Commun 283:1061–1068.

McClatchey AI. 2003. Merlin and ERM proteins: unappreciated roles in cancer development? Nat Rev Cancer 3:877–883.

Min SH, Kim DM, Heo YS, Kim YI, Kim HM, Kim J, Han YM, Kim IC, Yoo OJ. 2009. New p53 target, phosphatase of regenerating liver 1 (PRL-1) down-regulates p53. Oncogene 28:545–554.

Min SH, Kim DM, Heo YS, Kim HM, Kim IC, Yoo OJ. 2010. Downregulation of p53 by phosphatase of regenerating liver 3 is mediated by MDM2 and PIRH2. Life Sci 86:66–72.

Ming J, Liu N, Gu Y, Qiu X, Wang EH. 2009. PRL-3 facilitates angiogenesis and metastasis by increasing ERK phosphorylation and up-regulating the levels and activities of Rho-A/C in lung cancer. Pathology 41:118–126.

Mizuuchi E, Semba S, Kodama Y, Yokozaki H. 2009. Down-modulation of keratin 8 phosphorylation levels by PRL-3 contributes to colorectal carcinoma progression. Int J Cancer 124:1802–1810.

Mohn KL, Laz TM, Hsu JC, Melby AE, Bravo R, Taub R. 1991. The immediate-early growth response in regenerating liver and insulin-stimulated H-35 cells: comparison with serum-stimulated 3T3 cells and identification of 41 novel immediate-early genes. Mol Cell Biol 11:381–390.

Molleví DG, Aytes A, Berdiel M, Padullés L, Martínez-Iniesta M, Sanjuan X, Salazar R, Villanueva A. 2009. PRL-3 over expression in epithelial cells is induced by surrounding stromal fibroblasts. Mol Cancer 8:46.

Okumura K, Zhao M, Depinho RA, Furnari FB, Cavenee WK. 2005. Cellular transformation by the MSP58 oncogene is inhibited by its physical interaction with the PTEN tumor suppressor. Proc Natl Acad Sci USA 102:2703–2706.

Orsatti L, Forte E, Tomei L, Caterino M, Pessi A, Talamo F. 2009. 2-D Difference in gel electrophoresis combined with Pro-Q Diamond staining: a successful approach for the identification of kinase/phosphatase targets. Electrophoresis 30:2469–2476.

Ostman A, Hellberg C, Böhmer FD. 2006. Protein–tyrosine phosphatases and cancer. Nat Rev Cancer 6:307–320.

Park H, Jung SK, Jeong DG, Ryu SE, Kim SJ. 2008. Discovery of novel PRL-3 inhibitors based on the structure-based virtual screening. Bioorg Med Chem Lett 18:2250–2255.

Parker BS, Argani P, Cook BP, Liangfeng H, Chartrand SD, Zhang M, Saha S, Bardelli A, Jiang Y, St Martin TB, Nacht M, Teicher BA, Klinger KW, Sukumar S, Madden SL. 2004. Alterations in vascular gene expression in invasive breast carcinoma. Cancer Res 64:7857–7866.

Pascaru M, Tanase C, Vacaru AM, Boeti P, Neagu E, Popescu I, Szedlacsek SE. 2009. Analysis of molecular determinants of PRL-3. J Cell Mol Med 13:3141–3150.

Pathak MK, Dhawan D, Lindner DJ, Borden EC, Farver C, Yi T. 2002. Pentamidine is an inhibitor of PRL phosphatases with anticancer activity. Mol Cancer Ther 1:1255–1264.

Peng L, Jin G, Wang L, Guo J, Meng L, Shou C. 2006. Identification of integrin alpha1 as an interacting protein of protein tyrosine phosphatase PRL-3. Biochem Biophys Res Commun 342:179–183.

Peng L, Xing X, Li W, Qu L, Meng L, Lian S, Jiang B, Wu J, Shou C. 2009. PRL-3 promotes the motility, invasion, and metastasis of LoVo colon cancer cells through PRL-3-integrin beta1-ERK1/2 and-MMP2 signaling. Mol Cancer 8:110.

Peng Y, Du K, Ramirez S, Diamond RH, Taub R. 1999. Mitogenic up-regulation of the PRL-1 protein–tyrosine phosphatase gene by Egr-1. Egr-1 activation is an early event in liver regeneration. J Biol Chem 274:4513–4520.

Peng Y, Genin A, Spinner NB, Diamond RH, Taub R. 1998. The gene encoding human nuclear protein tyrosine phosphatase, PRL-1. Cloning, chromosomal localization, and identification of an intron enhancer. J Biol Chem 273:17286–17295.

Persengiev SP, Green MR. 2003. The role of ATF/CREB family members in cell growth, survival and apoptosis. Apoptosis 8:225–228.

Peters CS, Liang X, Li S, Kannan S, Peng Y, Taub R, Diamond RH. 2001. ATF-7, a novel bZIP protein, interacts with the PRL-1 protein–tyrosine phosphatase. J Biol Chem 276:13718–13726.

Polato F, Codegoni A, Fruscio R, Perego P, Mangioni C, Saha S, Bardelli A, Broggini M. 2005. PRL-3 phosphatase is implicated in ovarian cancer growth. Clin Cancer Res 11:6835–6839.

Qian F, Li YP, Sheng X, Zhang ZC, Song R, Dong W, Cao SX, Hua ZC, Xu Q. 2007. PRL-3 siRNA inhibits the metastasis of B16-BL6 mouse melanoma cells in vitro and in vivo. Mol Med 13:151–159.

Rouleau C, Roy A, St Martin T, Dufault MR, Boutin P, Liu D, Zhang M, Puorro-Radzwill K, Rulli L, Reczek D, Bagley R, Byrne A, Weber W, Roberts B, Klinger K. 2006. Protein tyrosine phosphatase PRL-3 in malignant cells and endothelial cells: expression and function. Mol Cancer Ther 5:219–229.

Ruan F, Lin J, Wu RJ, Xu KH, Zhang XM, Zhou CY, Huang XF. 2009. Phosphatase of regenerating liver-3: a novel and promising marker in human endometriosis. Fertil Steril. in Press, DOI: 10.1016/j.fertnstert.2009.10.065

Saha S, Bardelli A, Buckhaults P, Velculescu VE, Rago C, St Croix B, Romans KE, Choti MA, Lengauer C, Kinzler KW, Vogelstein B. 2001. A phosphatase associated with metastasis of colorectal cancer. Science 294:1343–1346.

Sahai E, Marshall CJ. 2002. RHO-GTPases and cancer. Nat Rev Cancer 2:133–142.

Si X, Zeng Q, Ng CH, Hong W, Pallen CJ. 2001. Interaction of farnesylated PRL-2, a protein–tyrosine phosphatase, with the beta-subunit of geranylgeranyltransferase II. J Biol Chem 276:32875–32882.

Skinner AL, Vartia AA, Williams TD, Laurence JS. 2009. Enzyme activity of phosphatase of regenerating liver is controlled by the redox environment and its C-terminal residues. Biochemistry 48:4262–4272.

Song R, Qian F, Li YP, Sheng X, Cao SX, Xu Q. 2009. Phosphatase of regenerating liver-3 localizes to cyto-membrane and is required for B16F1 melanoma cell metastasis in vitro and in vivo. PLoS ONE 4:e4450.

Stephens BJ, Han H, Gokhale V, Von Hoff DD. 2005. PRL phosphatases as potential molecular targets in cancer. Mol Cancer Ther 4:1653–1661.

Sun JP, Luo Y, Yu X, Wang WQ, Zhou B, Liang F, Zhang ZY. 2007. Phosphatase activity, trimerization, and the C-terminal polybasic region are all required for PRL1-mediated cell growth and migration. J Biol Chem 282:29043–29051.

Sun JP, Wang WQ, Yang H, Liu S, Liang F, Fedorov AA, Almo SC, Zhang ZY. 2005. Structure and biochemical properties of PRL-1, a phosphatase implicated in cell growth, differentiation, and tumor invasion. Biochemistry 44:12009–12021.

Takano S, Fukuyama H, Fukumoto M, Kimura J, Xue JH, Ohashi H, Fujita J. 1996. PRL-1, a protein tyrosine phosphatase, is expressed in neurons and oligodendrocytes in the brain and induced in the cerebral cortex following transient forebrain ischemia. Brain Res Mol Brain Res 40:105–115.

Thiery JP, Sleeman JP. 2006. Complex networks orchestrate epithelial–mesenchymal transitions. Nat Rev Mol Cell Biol 7:131–142.

Tonks NK. 2005. Redox redux: revisiting PTPs and the control of cell signaling. Cell 121:667–670.

Vousden KH, Lu X. 2002. Live or let die: the cell's response to p53. Nat Rev Cancer 2:594–604.

Wang H, Quah SY, Dong JM, Manser E, Tang JP, Zeng Q. 2007a. PRL-3 down-regulates PTEN expression and signals through PI3K to promote epithelial–mesenchymal transition. Cancer Res 67:2922–2926.

Wang H, Vardy LA, Tan CP, Loo JM, Guo K, Li J, Lim SG, Zhou J, Chng WJ, Ng SB, Li HX, Zeng Q. 2010. PCBP1 suppresses the translation of metastasis-associated PRL-3 phosphatase. Cancer Cell 18:52–62.

Wang J, Kirby CE, Herbst R. 2002. The tyrosine phosphatase PRL-1 localizes to the endoplasmic reticulum and the mitotic spindle and is required for normal mitosis. J Biol Chem 277:46659–46668.

Wang L, Shen Y, Song R, Sun Y, Xu J, Xu Q. 2009. An anticancer effect of curcumin mediated by down-regulating phosphatase of regenerating liver-3 expression on highly metastatic melanoma cells. Mol Pharmacol 76:1238–1245.

Wang Y, Li ZF, He J, Li YL, Zhu GB, Zhang LH, Li YL. 2007b. Expression of the human phosphatases of regenerating liver (PRLs) in colonic adenocarcinoma and its correlation with lymph node metastasis. Int J Colorectal Dis 22:1179–1184.

Werner SR, Lee PA, DeCamp MW, Crowell DN, Randall SK, Crowell PL. 2003. Enhanced cell cycle progression and down regulation of p21(Cip1/Waf1) by PRL tyrosine phosphatases. Cancer Lett 202:201–211.

Yang J, Weinberg RA. 2008. Epithelial–mesenchymal transition: at the crossroads of development and tumor metastasis. Dev Cell 14:818–829.

Yu L, Kelly U, Ebright JN, Malek G, Saloupis P, Rickman DW, McKay BS, Arshavsky VY, Bowes Rickman C. 2007. Oxidative stress-induced expression and modulation of Phosphatase of Regenerating Liver-1 (PRL-1) in mammalian retina. Biochim Biophys Acta 1773:1473–1482.

Zeng Q, Dong JM, Guo K, Li J, Tan HX, Koh V, Pallen CJ, Manser E, Hong W. 2003. PRL-3 and PRL-1 promote cell migration invasion, and metastasis. Cancer Res 63:2716–2722.

Zeng Q, Hong W, Tan YH. 1998. Mouse PRL-2 and PRL-3, two potentially prenylated protein tyrosine phosphatases homologous to PRL-1. Biochem Biophys Res Commun 244:421–427.

Zeng Q, Si X, Horstmann H, Xu Y, Hong W, Pallen CJ. 2000. Prenylation-dependent association of protein-tyrosine phosphatases PRL-1, -2, and -3 with the plasma membrane and the early endosome. J Biol Chem 275:21444–21452.

Zhao Z, Lee CC, Monckton DG, Yazdani A, Coolbaugh MI, Li X, Bailey J, Shen Y, Caskey CT. 1996. Characterization and genomic mapping of genes and pseudogenes of a new human protein tyrosine phosphatase. Genomics 35:172–181.

Zhou J, Wang S, Lu J, Li J, Ding Y. 2009. Over-expression of phosphatase of regenerating liver-3 correlates with tumor progression and poor prognosis in nasopharyngeal carcinoma. Int J Cancer 124:1879–1886.