PRL phosphatases as potential molecular targets in cancer

Bret J. Stephens,1 Haiyong Han,3 Vijay Gokhale,2 and Daniel D. Von Hoff3

Departments of 1Molecular and Cellular Biology and 2Pharmacology and Toxicology, University of Arizona, Tucson, Arizona and 3Translational Genomics Research Institute, Phoenix, Arizona

Abstract
The phosphatase of regenerating liver (PRL) family of phosphatases, consisting of PRL-1, PRL-2, and PRL-3, represents an intriguing group of proteins being validated as biomarkers and therapeutic targets in cancer. Individual PRLs are overexpressed in a variety of cancer cell lines and tissues when compared with their normal counterparts. More importantly, several recent studies have shown that PRL-3 is expressed at higher levels and at a greater frequency in colorectal cancer metastases compared with primary colorectal tumors and normal colon tissue. Ectopic expression of PRLs in nontumorigenic cells can influence proliferation and the migratory and invasive properties of cells, while knockdown of endogenous PRL-3 or PRL-1 in cancerous cells using small interfering RNA can abrogate cell motility and ability to metastasize in a mouse model. However, the exact biological function and cellular substrates of the PRLs remain unclear. This review will discuss what is known about the PRLs, what makes the PRLs possible attractive targets for therapeutic intervention, and the possible future directions in PRL biology and inhibitor identification. [Mol Cancer Ther 2005;4(11):1653–61]

Introduction
Phosphorylation and dephosphorylation are major regulatory events affecting the functional activities of diverse proteins that modulate cellular processes, such as transcriptional regulation, apoptosis, cell cycle progression, protein degradation, and protein trafficking (1, 2). The protein tyrosine phosphatase (PTP) superfamily of phosphatases, which are defined by the signature C(X)5R active site motif, encompasses a large group of enzymes that play key roles in the regulation of protein phosphorylation. In recent years, several PTPs have been implicated in a variety of diseases and have emerged as therapeutic targets. These include PTP1B for diabetes and obesity, CD45 for immune dysfunctions, PTP-oc for osteoporosis, CDC25s, PTP-α, and PTP1B for breast and colon carcinomas, and PTP1B and PTP-α for neurodegenerative diseases (2, 3). Using amino acid sequences of published PTPs, Alonso et al. (4) searched several publicly available databases and generated a list of 107 PTP genes contained in the human genome. Based on the amino acid sequences of their catalytic domains, of these 107 genes, 61 can be classified as VH1-like PTPs, many of which have been shown to be dual-specificity phosphatases (which are able to dephosphorylate tyrosine, serine, and threonine residues as well as inositol phospholipids in some cases). Phosphatase of regenerating liver (PRL)-1, PRL-2, and PRL-3 collectively make up a subgroup of VH1-like PTPs (4). Based on amino acid sequence, the most closely related phosphatases outside the subgroup are dual-specificity phosphatases CDC14 (regulates mitotic exit) and PTEN (a tumor suppressor with lipid phosphatase activity), with an amino acid sequence identity of ~20% and 17%, respectively (5).

PRL Genes and Protein Products
The genes encoding PRL-1, PRL-2, and PRL-3 are located on chromosomes 6q12, 1p35, and 8q24.3, respectively. There is a high level of amino acid sequence identity among PRL family members. In humans, PRL-1 and PRL-2 share 86% identity, PRL-1 and PRL-3 share 78%, and PRL-2 and PRL-3 are the least identical at 75% (ref. 6; Fig. 1). There is also a high level of homology among mammalian PRLs. For instance, the amino acid sequences for human and mouse PRL-1 as well as PRL-2 are identical, whereas PRL-3 is 96% identical between the species.

Multiple transcript isoforms of all three PRLs have been identified. However, full-length transcripts, which code for ~20-kDa proteins (i.e., 173 amino acids for both PRL-1 and PRL-3 and 167 amino acids for PRL-2), are predominant in the literature and will be the focus of this review. The short variants, at least for PRL-3, do not seem to fold properly when expressed in vitro, suggesting that the shorter variants might not be translated in vivo (7).

All three PRLs contain a COOH-terminal CAAX motif, where they can be post-translationally farnesylated (see Fig. 1), suggesting that the PRLs might be localized to membrane structures (8). Further, a conserved cluster of charged amino acids is present, including a basic stretch...
of amino acids, at the COOH terminus of the protein. This basic fragment, located next to the prenylation site, could participate in membrane binding through interactions with phospholipids (7, 9). Interestingly, these basic residues might also play a role in nuclear localization as part of a potential bipartite nuclear localization signal (9).

Using phosphorylation prediction software, Zeng et al. (10) predicted that the PRLs might contain several potential phosphorylation sites. Furthermore, pp60 src is able to phosphorylate PRL-1 in vitro (14), suggesting that phosphorylation might play a regulatory role in PRL function (11).

Although the PRLs are closely related, several differences in possible phosphorylation sites exist. For example, PRL-1 and PRL-3 have weak consensus sites for protein kinase C and casein kinase II, whereas PRL-2 does not contain either. PRL-3 also contains multiple potential protein kinase C and casein kinase II phosphorylation sites, whereas PRL-1 only contains one of each (10).

All three PRLs contain a conserved cysteine residue (Cys49 in PRL-1 and PRL-3 and Cys46 in PRL-2) that is in close proximity to the catalytic cysteine (Cys104 in PRL-1 and PRL-3 and Cys101 in PRL-2). Structural studies have shown that under oxidizing conditions a disulfide bond can form between the two residues (7, 12). Therefore, the activity of PRLs might be regulated by redox status.

Oxidation as an important regulatory mechanism for the PTP family of enzymes has been reviewed elsewhere (13).

PRL Three-Dimensional Structures and Implications in Their Functions

Using nuclear magnetic resonance, the solution structure for PRL-3 has been solved independently by Kozlov et al. (7) and Kim et al. (5). The resonance assignments of PRL-1 and PRL-2 have also been reported (6, 14). More recently, the crystal structure of PRL-1 has been determined at 2.7-Å resolution (12). Due to the high sequence similarity, it is expected that the PRL-3 and PRL-1 structures would have a large structural similarity. However, significant differences in the P-loop structure exist between the PRL-1 crystal structure and the PRL-3 nuclear magnetic resonance structure published by Kozlov et al. The PRL-3 nuclear magnetic resonance structure P-loop [C(X)R motif] has an open conformation with the side chains of Cys104 and Arg110 orienting away from the pocket. On the other hand, the P-loop in the PRL-1 crystal structure is constricted and the side chains of Cys104 and Arg110 are oriented in toward the pocket (Fig. 2A and B). Both PRL-3 and PRL-1 active site residues are mainly nonpolar in nature, with relatively few polar residues like Cys49 and Asp72 being present.

Along with sequence similarities, the secondary structure elements and overall tertiary structures for the PRLs are typical for dual-specificity phosphatases. Sequence alignments suggest that CDC14 is the closest homologue but PRL-1 and PRL-3 show the closest structural similarity to phosphatases VHR, PTEN, MKP, and KAP (7, 12). However, the P-loop and active site constitution of the PRLs is significantly different from the related phosphatases PTEN (15) and VHR (ref. 16; Fig. 2C and D). The active site P-loop in PTEN is predominantly polar in nature with residues like Asp92, Lys125, Arg159, and Gln171. The VHR phosphatase P-loop is also highly polar in nature (HCREGYSR) due to the presence of Arg125, Glu126, Tyr128, and Ser129. Other polar residues like Asp92, Arg158, and Asn163 also form part of the active site of VHR.

PRL Expression

PRL-1 was first identified as an immediate-early growth response gene in regenerating liver and mitogen-treated 3T3 mouse fibroblasts (11, 17). Database searches for sequences homologous to PRL-1 led to the subsequent identification of PRL-2 and PRL-3 (10).

Normal Tissues

PRL expression in humans is not well characterized, but in adult rodents PRL-1, PRL-2, and PRL-3 are predominantly expressed in skeletal muscle, with PRL-1 also being expressed at high levels in the brain and PRL-3 being expressed at moderate levels in the heart (10, 11). Due to PRL-3 expression in the heart, it is important to note that therapeutics targeting PRL-3 might exhibit cardiotoxicity. PRL-1 and PRL-2 seem to be more ubiquitously expressed than PRL-3, as they are detected at low levels in various tissues (10, 11, 18). In addition to adult tissues, PRL-1 expression has also been...
examined during development, where it is expressed in several digestive epithelial tissues (19). PRL expression in normal tissues seems to correlate most often with terminal differentiation. For example, PRL-1 and PRL-3 expression in the intestine is limited to the terminally differentiated villus but not found in proliferating crypt enterocytes (8, 20). In addition, expression of PRL-3 in heart tissue is primarily found in cardiomyocytes (18). A summary of PRL expression in normal tissues is provided in Table 1.

**Tumor Tissues**

Although PRL expression often correlates with terminal differentiation, PRLs are also found overexpressed in cancerous cells. Saha et al. (21) first described PRL-3 overexpression in colorectal cancer by comparing gene expression levels in colon cancers that had metastasized to the liver with those in primary tumor and normal colon cells. PRL-3 was consistently overexpressed in the cancer metastases, whereas its expression in normal colorectal epithelia was nearly undetectable. In addition, Bardelli et al. (22) observed high PRL-3 mRNA expression in metastatic lesions derived from colorectal cancers regardless of the site of metastasis (liver, lung, brain, or ovary), whereas low levels of PRL-3 were observed in non–colorectal cancer metastases to the lung (pancreas and stomach cancers) or the liver (pancreas, esophagus, and stomach cancers). These results indicated that PRL-3 might be involved in cell type–specific metastatic processes. High PRL-3 expression has also been reported in cancer types other than the colorectal cancers. For example, PRL-3 was found to be highly expressed in the Hodgkin’s lymphoma cell line, L1236, compared with a nonmalignant counterpart (23); Wu et al. (24) showed that PRL-3 was overexpressed in liver carcinoma samples compared with normal liver; and high PRL-3 expression has been detected in invasive breast tumor vasculature (25).

Wang et al. (9) investigated PRL-1 levels in a panel of 76 different cell lines, including 67 cancer cell lines from various tumor origins and 9 normal/untransformed cell lines. They found that PRL-1 mRNA levels were elevated in several tumor cell lines compared with respective untransformed cell lines. Melanoma cell lines showed the most consistent high levels of expression, where they found that five of six melanoma cell lines expressed high levels of PRL-1. It is interesting, however, that PRL-1 was not found overexpressed in cancerous cell lines derived from brain or colon (9). This result is rather intriguing because of the previously described close correlation between PRL-3 expression and colorectal cancer invasion and metastasis as well as PRL-1 being expressed normally.

![Figure 2](image-url) P-loop $[C(X)_4R$ motif] structure of PRL and other phosphatases. PTEN phosphatase (C), VHR phosphatase (D), and PRL-1 (B) are crystal structures (12, 15, 16); PRL-3 (A) is a nuclear magnetic resonance solution structure (7).
in brain and differentiated colon tissue. This indicates that, although the three PRLs are highly homologous, they might serve distinct functions inside cells. Overexpression of PRL-1 has also been identified in pancreatic cancer cell lines by Han et al. (26). Overexpression of PRL-2 has been observed in the LNCaP prostate cancer cell line and prostate tumor tissue (27). However, increased expression of PRL-1 and PRL-2 in cancer has not been reported as extensively as PRL-3 (see Table 2).

**Regulation of PRL Expression**

Transcriptional regulation of the PRLs is not well understood. As discussed previously, the expression pattern of the PRLs differs among tissues. The 5’ noncoding regions of the PRLs have been described as divergent (10), providing a mechanism for possible differential regulation of transcription.

Two reports have implicated p53 in PRL transcriptional control but in opposing roles with respect to PRL-1 and PRL-3. Daoud et al. (28), using cDNA microarrays, found the presence of p53 correlated with decreased expression of PRL-1 when comparing wild-type p53 HCT 116 colorectal cancer cells and an isogenic p53 knockout counterpart. In contrast, Fontemaggi et al. (29) showed that ectopic expression of both p53 and p73 increased the expression of PRL-3 in H1299 non–small cell lung cancer cells.

Human PRL-1 is in part regulated by early growth response-1 (EGR-1), a growth factor–activated transcription factor (30, 31). Interestingly, in tumors derived from brain tissues, such as gliomas, EGR-1 can act as a tumor suppressor (32); however, it has been reported that EGR-1 expression correlates with gastric cancer progression and metastasis (33).

Table 3 lists some of the proteins and small molecules that have been shown to affect the expression of PRLs.

**PRL-3 overexpression in colorectal cancer can occur through increased gene copy number. Using genomic fluorescence in situ hybridization, Bardelli et al. (22) observed increased copy numbers of PRL-3 in 45% of liver metastases tested but rarely in the nonmetastatic primary lesions. This result combined with their previous work (21) indicated that part of the increased PRL-3 expression was derived from the increased gene copy number of PRL-3 in the cell. The authors pointed out, however, that increased copy number does not account for all the increased expression, because PRL-3 expression is observed in a much higher fraction of metastases than the percentage of samples containing an increased copy number of PRL-3. It is interesting that increased copy numbers of PRL-3 are associated with an amplification of a large part of the telomeric region of chromosome 8q encompassing 12 Mb, which includes the oncogene c-Myc. Buffart et al. (34) reported that increased copy numbers of several oncogenes, including PRL-3, located on 8q24.12-q24.23 correlate with metastatic disease in colorectal cancer.

**Biological Functions of PRLs**

The biological functions of the PRLs remain largely unclear. Although the PRLs are very closely related, the expression pattern of the PRLs differs among tissues, indicating that PRLs could be divergent in their functions. As discussed previously, all three PRLs are highly conserved among mammals, underscoring the idea that all three PRLs might have distinct and important regulatory roles. However, the particularly strong sequence conservation around the catalytic site suggests similar substrate specificity in PRLs (7). Therefore, if the PRLs have distinct functions, it may be due to differences in protein interactions and differences in regulation. Alternatively, the PRLs might all function...
Similarly and be regulated by the same factors but are under the control of separate promoters as a means of tissue/cell–specific regulation. The latter seems improbable, because several of the protein interactions described to date seem to be PRL type specific.

Many earlier articles describing PRLs focused on their involvement in proliferation and ability to transform nontumorigenic immortalized cell lines. Ectopic expression of PRL-1, PRL-2, and PRL-3 can cause increased levels of cellular proliferation as well as transform otherwise nontumorigenic cell lines, whereas transfection with catalytically inactive mutants of the PRLs have minimal effect (11, 35, 36). However, at least in the tumorigenic colorectal adenocarcinoma DLD-1 cell line, transient down-regulation of PRL-3 or PRL-1 by small interfering RNA seems to have no effect on proliferation (37).

Recent findings have focused on the role that PRLs play in metastasis. Zeng et al. (38) showed that Chinese hamster ovary cells stably expressing PRL-1 or PRL-3 exhibited enhanced motility and invasiveness. In addition, PRL-3 or PRL-1 expressing Chinese hamster ovary cells (but not control cells) induced metastatic tumor formation in mice (38). Overexpression of PRL-1 or PRL-2 in D27 hamster pancreatic ductal epithelial cells resulted in loss of contact

| Tissue | Observation | Method | Ref. |
|--------|-------------|--------|------|
| PRL-1  | Melanoma    |        |      |
| Cell lines | 5 of 6 showed elevated expression | Quantitative PCR | (9) |
| Pancreatic adenocarcinoma | High expression relative to normal | cDNA microarray | (26) |
| PRL-2  | Prostate    |        |      |
| Cell lines | Overexpression in LNCaP, PC-3, and DU-145 cells | Reverse transcription-PCR | (27) |
| Patient tumors | 2 of 3 samples showed overexpression | Reverse transcription-PCR | (27) |
| PRL-3  | Breast      |        |      |
| Invasive tumor vasculature | Expression in vasculature but not in tumor | Serial analysis of gene expression | (25) |
| Colon  | Cancer metastases | Expressed at high levels in 18 of 18 samples | Serial analysis of gene expression | (21) |
| Normal epithelia | Not expressed | In situ hybridization | (22) |
| Nonmetastatic carcinoma | Not expressed | In situ hybridization | (22) |
| Carcinoma metastases | Expressed in 86–100% of samples (depending on site) | In situ hybridization | (22) |
| Primary tumors | Expressed at high levels in 44.6% of samples | In situ hybridization | (37) |
| Primary tumors with metastases | Expressed at high levels in 51.8–88.9% of samples (varies by site) | In situ hybridization | (37) |
| Carcinoma metastases | Expressed at high levels in 47.5–100% of samples | In situ hybridization | (37) |
| Normal epithelia | Expressed in 7.1% of samples | Immunohistochemistry | (50) |
| Primary tumors | Expressed in 23.9% of samples | Immunohistochemistry | (50) |
| Carcinoma metastases | Expressed in 53.7–66.7% of samples | Immunohistochemistry | (50) |
| Gastric | Cell lines | Expressed in 87.5% of cell lines tested | Reverse transcription-PCR | (51) |
| Primary carcinoma without nodal metastasis | Expressed in 50% of samples | Reverse transcription-PCR | (51) |
| Primary carcinoma with nodal metastasis | Expressed in 81.5% of samples | Reverse transcription-PCR | (51) |
| Liver | Carcinoma samples | Overexpressed in all 27 samples tested | Northern | (24) |
inhibition, and when injected into nude mice, there was tumor formation in all mice tested but no tumor formation in the controls (35). Moreover, Wu et al. (24) showed that PRL-3 could convert a tumor cell line with low metastatic potential into highly metastatic cells both in vitro and in vivo. B16 melanoma cells stably transfected with a PRL-3 expression vector showed a significant increase in adhesion to fibronectin and laminin compared with the mock vector transfectants, whereas the adhesion of the cells to type I collagen decreased (24).

Several findings suggest that PRLs are involved in cell cycle control. Cell cycle analysis of HeLa cells expressing a phosphatase dead mutant of PRL-1 indicates that the PRL-1 phosphatase activity is required for progression of cells through mitosis (9). In addition, expression of a farnesylation site mutant of PRL-1 (C170S), which was found enriched in the nucleus throughout the cell cycle, resulted in mitotic defects without compromising spindle checkpoint function (9). Werner et al. (36) found that PRL-1 or PRL-2 transfected cells had markedly higher rates of DNA synthesis than vector-transfected control cells. Cell cycle analysis confirmed that PRL-1- or PRL-2-overexpressing cells had at least 3-fold more cells in S phase than control cells with a concomitant decrease in the proportion of cells in G1. Significantly lower levels of p21 were observed in both PRL-1- and PRL-2-overexpressing cell lines compared with the control cells. Therefore, the authors postulate that PRL-1 and PRL-2 might regulate the cell cycle by modulating p21 levels. Interestingly, it was also shown that PRL-1 (but not PRL-2) overexpression resulted in higher cyclin A levels (36). Unlike some proteins involved in cell cycle regulation, PRL-1 mRNA as well as protein levels do not seem to fluctuate during the cell cycle (9).

Determining the intracellular localization of a protein can sometimes provide clues as to the possible biological function(s) of the protein. Although PRL-1 was first described as being localized to the nucleus (11), most subsequent studies (which have been focused on PRL expression in cancer) place PRL proteins at membrane structures, such as the plasma membrane, early endosomes, and endoplasmic reticulum (8, 9, 22). Using normal tissue samples from several digestive organs, Kong et al. (19) observed that PRL-1 expression was mostly nuclear. Interestingly, several tissues, which displayed a nuclear localization pattern for PRL-1 in mature tissue, showed evidence of some cytoplasmic expression during tissue development. It was also observed that the expression of PRL-1 became more consistently nuclear, as well as more cell type restricted, as development progressed (19). Based on these findings, it is an intriguing possibility that PRL proteins localized to the nucleus might function in terminal differentiation and/or that localization to membranes might allow PRLs to be involved in cell growth and metastasis. Alternatively, PRL protein localization might simply be dependent on the level of differentiation of a cell. However, it remains unclear if, in general, PRL protein localization correlates with the level of differentiation of a cell.

### Table 3. Regulators of PRL expression

| PRL expression regulators | Observed in                                      | Ref. |
|---------------------------|--------------------------------------------------|------|
| **PRL-1**                 |                                                  |      |
| Genes                     |                                                  |      |
| *Early growth response-1* | Rat liver and rat H35 cells                      | (31) |
| *Interleukin-15*          | T Lymphocytes (human)                            | (52) |
| *Pax3* (mouse)            | Medulloblastoma derived DAOY cell line           | (53) |
| p53                       | HCT 116 colon carcinoma cell line                | (28) |
| Other                     | Human glial cells                                | (54) |
| Tetrodotoxin              | Polymorphonuclear leukocytes (human)             | (55) |
| Camptothecin              | Rat Sertoli cells                                | (56) |
| Follicle-stimulating hormone | Erythroleukemic SKT6 cells                    | (57) |
| Erythropoietin            |                                                  |      |
| **PRL-2**                 |                                                  |      |
| Genes                     |                                                  |      |
| *Progesterone receptor A* | T47D breast cancer cell line                    | (58) |
| *Progesterone receptor B* | T47D breast cancer cell line                    | (58) |
| Other                     | Polymorphonuclear leukocytes (human)             | (55) |
| Camptothecin              | Polymorphonuclear leukocytes (human)             | (55) |
| Anti-Fas antibody         |                                                  |      |
| **PRL-3**                 |                                                  |      |
| Genes                     |                                                  |      |
| p53                       | H1299 non–small cell lung cancer                 | (29) |
| p73                       | H1299 non–small cell lung cancer                 | (29) |
| Other                     | Mouse skeletal muscle                            | (59) |
| Denervation               | CSIAN S3.G2 fibroblast                           | (60) |
| H2O2                      |                                                  |      |

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Recently, Jeong et al. (12) presented evidence suggesting that PRL-1 might form trimers in cells. Because farnesylation alone is often insufficient for the translocation of the protein to the plasma membrane, it is possible that three farnesyl groups in the trimeric PRL-1 may provide strong adhering forces to the membrane, stabilizing the membrane association. It would be interesting to see if PRL-1 proteins carrying mutations at the trimeric interface show any reduction in their ability to facilitate invasion and proliferation when ectopically expressed. PRL localization might also be dependent on the cell cycle. In nonmitotic HeLa cells, PRL-1 localizes to the endoplasmic reticulum, whereas in mitotic cells PRL-1 is associated with the centrosomes and the spindle apparatus (9). A similar localization pattern has also been observed with PRL-3. Wu et al. (24) detected enhanced green fluorescent protein–tagged PRL-3 in COS-7 cells primarily in membrane compartments, but in cells progressing through mitosis, PRL-3 was also found at the metaphase plate. This localization pattern during mitosis suggests that PRLs could be involved in cell cycle regulation.

The identification of proteins that interact with the PRLs will be important in the understanding of the functions of PRLs. PRL-1 is able to interact with and dephosphorylate activating transcription factor-5 (ATF-5; also called ATF-7 and ATFx) in vitro (39). ATF-5 transcription seems to be tightly regulated during cell cycle progression, with expression peaking in G1-S and decreasing to undetectable levels in G2-M (40). ATF-5 is a regulator of cyclic AMP–induced expression by binding to CRE elements and is expressed in association with differentiation in the Caco-2 cell model of intestinal differentiation (39). In contrast, down-regulation of ATF-5 is required for the progression of neural progenitor cells to neurons (41). ATF-5 has also been described as an antiapoptotic protein in murine FL5.12 cells (40). However, ectopic expression of PRL-1 or PRL-2 does not seem to affect apoptosis at least in the hamster pancreatic D27 cell line (36).

The β-subunit of a prenyltransferase, geranylgeranyltransferase, was found to specifically interact with PRL-2 in mammalian cells. This interaction was found to be dependent on the prenylation status of PRL-2. A unique region of PRL-2 that is not present in PRL-1 or PRL-3 might be dependent on the prenylation status of PRL-2. Interaction of PRL-2 with α-tubulin in in vitro binding assays (9). It has been pointed out that PRL-2 is missing three amino acids before the CAAX box, which may account for their differences in interaction with tubulins (9).

Targeting PRLs in Cancer

Several findings have helped to preliminarily validate PRLs as therapeutic targets. Pathak et al. (43) showed that the anti-leishmaniasis drug pentamidine inhibited all three recombinant PRLs in vitro and caused tumor shrinkage in a melanoma mouse xenograft model. Because pentamidine inhibited the phosphatase activity of all three PRLs as well as other phosphatases like PTP1B and MKP1, it is not clear if the inhibition of tumor growth was caused by the inhibition of a specific PRL, a combination of the PRLs, or another phosphatase. Moreover, it should be noted that pentamidine is a known DNA minor groove binder and has also been shown to disrupt hERG protein processing and hence lower functional hERG protein levels (44–46).

Specific down-regulation of PRL-3 or PRL-1 by small interfering RNA has been reported to abrogate motility (in vitro) and hepatic colonization (in vivo) of the tumorigenic colorectal adenocarcinoma DLD-1 cell line (37). Further experiments using small interfering RNA and other technologies to specifically knockdown gene expression will be needed to more extensively validate individual PRLs as drug targets.

Due to the close relationship between the PRLs and PTEN and SHP-2, identifying an inhibitor with a high degree of specificity to the PRLs would be important. Although PTEN and SHP-2 are already mutated or deleted in several cancers, a drug with low specificity might have unintended consequences and weaken the tumor suppressive abilities of a normal cell by inhibiting closely related tumor suppressor proteins.

Because of the high level of primary sequence identity between the PRL family members, identifying inhibitors specific to a certain PRL might be challenging. However, because all the PRLs seem to be involved in growth and transformation, an agent affecting all three might be desirable.

Recent structural determinations have given much insight and identified several features that could be exploited in identifying selective inhibitors for the PRLs. The catalytic clefts of the PRLs seem to be shallow, which normally suggests a broad range of specificity (7, 12). However, the PRLs exhibit a wide active site pocket entrance with a relatively flat active site surface, which may be exploited in designing specific drugs toward PRL phosphatases (7, 12). In addition, the hydrophobic character of the P-loop might indicate a preference for more hydrophobic substrates than those of other phosphatases (7, 12).

If PRLs must be localized to membrane structures to function in tumor progression, then inhibition of PRL activity might also be carried out using farnesytransferase inhibitors. Treatments with farnesyltransferase inhibitors have been shown to cause nuclear accumulation of PRLs (8), and proteins other than Ras are thought to be important targets of farnesyltransferase inhibitors (47).

Conclusion

The PRL family of phosphatases represents a group of enzymes for further validation as metastasis biomarkers and as possible therapeutic targets. High PRL-3 expression correlates with metastasis in multiple tumor types, and
several recent reports suggest that PRLs may play key causal roles in promoting tumor cell motility and invasion. Although current research has been focused on the role PRL-3 plays in colorectal cancer invasion and metastasis, all three PRLs can be found overexpressed in certain cancer types and seem to be able to regulate cellular growth and metastatic ability. The exact biological functions and the physiologic substrates of the PRLs are not fully understood. However, identifying pathways that PRLs regulate would be important in understanding metastases. In addition, recently resolved structures of the PRL proteins should aid in identifying specific inhibitors.

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References

1. Hunter T. Signaling—2000 and beyond. Cell 2000;100:113–27.
2. Lyon MA, Ducruet AP, Wipf P, Lazo JS. Dual-specificity phosphatases as targets for antineoplastic agents. Nat Rev Drug Discov 2002;1:961–76.
3. van Huijtduijn RH, Bombrun A, Swinnen D, Selecting protein tyrosine phosphatases as drug targets. Drug Discov Today 2002;7:1013–9.
4. Alonso A, Sasin J, Bottini N, et al. Protein tyrosine phosphatases in the human genome. Cell 2004;117:699–711.
5. Kim KA, Song JS, Jee J, et al. Structure of human PRL-3, the phosphatase associated with cancer metastasis. FEBS Lett 2004;565:181–7.
6. Zhou H, Gallina M, Mao H, et al. 1H, 13C, and 15N resonance assignments and secondary structure of the human protein tyrosine phosphatase PRL-2. J Biomol NMR 2003;25:397–8.
7. Kozlov G, Cheng J, Ziemek E, Banville D, Gehring K, Ekiel I. Structural insights into molecular function of the metastasis-associated phosphatase PRL-3. J Biol Chem 2004;279:11882–9.
8. Zeng Q, Si X, Horstmann H, et al. Prenylation-dependent association of protein-tyrosine phosphatases PRL-1, -2, and -3 with the plasma membrane and the early endosome. J Biol Chem 2000;275:21444–52.
9. Wang J, Kirby CE, Herbst R. The tyrosine phosphatase PRL-1 localizes to the endoplasmic reticulum and the mitotic spindle and is required for normal mitosis. J Biol Chem 2002;277:46569–68.
10. Zeng Q, Hong W, Tan YH. Mouse PRL-2 and PRL-3, two potentially prenylated protein tyrosine phosphatases homologous to PRL-1. Biochem Biophys Res Commun 1998;244:421–7.
11. Diamond RH, Cressman DE, Laz TM, Abrams CS, Taub R. P-R1, a unique nuclear protein tyrosine phosphatase, affects cell growth. Mol Cell Biol 1994;14:3752–62.
12. Jeong DG, Kim SJ, Kim JH, et al. Trimeric structure of PRL-1 phosphatase reveals an active enzyme conformation and regulation mechanisms. J Mol Biol 2005;345:401–13.
13. den Hertog J, Groen A, van der Wijk T. Redox regulation of protein-tyrosine phosphatases. Arch Biochem Biophys 2005;434:11–5.
14. Laurence JS, Hallenga K, Stauffercher CV. 1H, 13C, 15N resonance assignments of the human protein tyrosine phosphatase PRL-1. J Biol NMR 2004;29:417–8.
15. Lee JO, Yang H, Georgescu MM, et al. Crystal structure of the PTL tumor suppressor: implications for its phosphoinositide phosphatase activity and membrane association. Cell 1999;99:323–34.
16. Yuvaliyma J, Denu JM, Dixon JE, Saper MA. Crystal structure of the dual specificity protein phosphatase VHR. Science 1996;272:1328–31.
17. Mohn KL, Laz TM, Hsu JC, Melby AE, Bravo R, Taub R. 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 1991;11:381–90.
18. Matter WF, Estridge T, Zhang C, et al. Role of PRL-3, a human muscle-specific tyrosine phosphatase, in angiotensin-II signaling. Biochem Biophys Res Commun 2001;283:1061–8.
19. Kong W, Swain GP, Li S, Diamond RH. PRL-1 PT-Pase expression is developmentally regulated with tissue-specific patterns in epithelial tissues. Am J Physiol Gastrointest Liver Physiol 2000;279:G613–21.
20. Diamond RH, Peters C, Jung SP, et al. Expression of PRL-1 nuclear PT-Pase is associated with proliferation in liver but with differentiation in intestine. Am J Physiol 1998;271:G121–9.
21. Saha S, Bardelli A, Buckhaults P, et al. A phosphatase associated with metastasis of colorectal cancer. Science 2001;294:1343–6.
22. Bardelli A, Saha S, Sager JA, et al. PRL-3 expression in metastatic cancers. Clin Cancer Res 2003;9:5607–15.
23. Schwerin I, Brauninger A, Distler V, et al. Profiling of Hodgkin’s lymphoma cell line L1236 and germinal center B cells: identification of Hodgkin’s lymphoma-specific genes. Mol Med 2003;9:85–95.
24. Wu X, Zeng H, Zhang X, et al. Phosphatase of regenerating liver-3 promotes motility and metastasis of mouse melanoma cells. Am J Pathol 2004;164:2039–54.
25. Parker BS, Argani P, Cook BP, et al. Alterations in vascular gene expression in invasive breast carcinoma. Cancer Res 2004;64:7785–66.
26. Han H, Bearss DJ, Browne LW, Calaluce R, Nagle RB, Von Hoff DD. Identification of differentially expressed genes in pancreatic cancer cells using cDNA microarray. Cancer Res 2002;62:2890–6.
27. Wang Q, Holmes DI, Powell SM, Lu QL, Waxman J. Analysis of stromal-epithelial interactions in prostate cancer identifies PTPCAAX2 as a potential oncogene. Cancer Lett 2002;175:63–9.
28. Daoud SS, Munson PJ, Reinhold W, et al. Impact of p53 knockout and tumor treatment on gene expression profiles in human colon carcinoma cells: a pharmacogenomic study. Cancer Res 2003;63:2782–93.
29. Fontemb教吉 G, Kela I, Amariglio N, et al. Identification of direct p73 target genes combining DNA microarray and chromatin immunoprecipitation analyses. J Biol Chem 2002;277:43359–68.
30. Peng Y, Genin A, Spinning NB, Diamond RH, Taub R. The gene encoding human nuclear protein tyrosine phosphatase, PRL-1. Cloning, chromosomal localization, and identification of an intron enhancer. J Biol Chem 1998;273:17286–95.
31. Zeng Q, Dong JM, Guo K, et al. PRL-3 and PRL-1 promote cell growth, differentiation, and invasion in human colorectal cancer: a predictive molecular marker of metachronous liver and lung metastases. Clin Cancer Res 2004;10:7318–28.
32. Kato H, Semba S, Miskad UA, Seo Y, Kasuga M, Yokozaki H. High expression of early growth response-1 as a metastasis-regulatory factor in gastric cancer. Anticancer Res 2002;22:9367–70.
33. Buffert TE, Coffa J, Hermens MA, et al. DNA copy number changes at 9p21 in colorectal cancer: a predictive molecular marker of metachronous colorectal and breast carcinoma. J Mol Med 2004;82:4513–20.
34. Calogero A, Arcella A, De Gregorio G, et al. The early growth response gene EGR-1 behaves as a suppressor gene that is downregulated independent of ARF/Mdm2 but not p53 alterations in human gliomas. Clin Cancer Res 2001;7:2788–96.
35. Kobayashi D, Yarnada M, Kamagata C, et al. Overexpression of early growth response-1 as a metastasis-regulatory factor in gastric cancer. Anticancer Res 2002;22:9367–70.
36. Peters CS, Liang X, Li S, et al. Identification of direct p73 target genes combining DNA microarray and chromatin immunoprecipitation analyses. J Biol Chem 2002;277:43359–68.
37. Pesce A, Giavedoni A, Forni G, et al. Analysis of gene expression in colorectal cancer. Cancer Res 2000;60:926–30.
38. Peters CS, Liang X, Li S, et al. ATFx: a novel role for a member of the ATF/CREB family of mammalian bZIP transcription factors. Genes Dev 2002;16:1806–14.
39. Fontemaggi G, Kela I, Amariglio N, et al. Identification of direct p73 target genes combining DNA microarray and chromatin immunoprecipitation analyses. J Biol Chem 2002;277:43359–68.
40. Persengiev SP, Devireddy LR, Green MR. Inhibition of apoptosis by ATFa: a novel role for a member of the ATF/CREB family of mammalian bZIP transcription factors. Genes Dev 2002;16:1806–14.
41. Angelastro JM, Ignatova TN, Kukkov K, et al. Regulated expression of ATFa is required for the progression of neural progenitor cells to neurons. J Neurosci 2003;23:4950–600.
42. Si X, Zeng Q, Ng CH, Hong W, Pallen CJ. Interaction of farnesylated PRL-2, a protein-tyrosine phosphatase, with the j-subunit of geranylgeranylaniltransferase II. J Biol Chem 2001;276:32875–82.
43. Pathak MK, Dhanaw D, Lindner DJ, Borden EC, Farver C, Yi T. Pentamidine is an inhibitor of PRL phosphatases with anticancer activity. Mol Cancer Ther 2002;1:1255–64.
44. Fox KR, Sansom CE, Stevens MF. Footprinting studies on the sequence-selective binding of pentamidine to DNA. FEBS Lett 1990;286:150–4.
45. Cordes JS, Sun Z, Lloyd DB, et al. Pentamidine reduces hERG expression to prolong the QT interval. Br J Pharmacol 2005;145:15–23.
46. Kuryshev YA, Ficker E, Wang L, et al. Pentamidine-induced long QT syndrome and block of hERG trafficking. J Pharmacol Exp Ther 2005;312:316–23.
47. Sebti SM, Der CJ. Opinion: searching for the elusive targets of farnesyltransferase inhibitors. Nat Rev Cancer 2003;3:945–51.
48. Takano S, Fukuyama H, Fukumoto M, et al. 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 1996;40:105–15.
49. Yarovinsky TO, Rickman DW, Diamond RH, Taub R, Hageman GS, Bowes Rickman C. Expression of the protein tyrosine phosphatase, phosphatase of regenerating liver 1, in the outer segments of primate cone photoreceptors. Brain Res Mol Brain Res 2000;77:95–103.
50. Peng L, Ning J, Meng L, Shou C. The association of the expression level of protein tyrosine phosphatase PRL-3 protein with liver metastasis and prognosis of patients with colorectal cancer. J Cancer Res Clin Oncol 2004;130:521–6.
51. Miskad UA, Semb S, Kato H, Yokozaki H. Expression of PRL-3 phosphatase in human gastric carcinomas: close correlation with invasion and metastasis. Pathobiology 2004;71:176–84.
52. Liu K, Catalfamo M, Li Y, Henkart PA, Weng NP. IL-15 mimics T cell receptor crosslinking in the induction of cellular proliferation, gene expression, and cytotoxicity in CD8+ memory T cells. Proc Natl Acad Sci U S A 2002;99:6192–7.
53. Mayani CS, George D, Freilich L, et al. Microarray analysis detects novel Pax3 downstream target genes. J Biol Chem 2001;276:49299–308.
54. Raghavendra Prasad HS, Qi Z, Srivivasan KN, Gopalakrishnakone P. Potential effects of tetrodotoxin exposure to human glial cells postulated using microarray approach. Toxicol 2004;44:597–608.
55. Kobayashi SD, Voyich JM, Braughton KR, DeLeo FR. Down-regulation of proinflammatory capacity during apoptosis in human polymorphonuclear leukocytes. J Immunol 2003;170:3357–68.
56. McLean DJ, Friel PJ, Pouchnik D, Griswold MD. Oligonucleotide microarray analysis of gene expression in follicle-stimulating hormone-treated rat Sertoli cells. Mol Endocrinol 2002;16:2780–92.
57. Gregory RC, Lord KA, Panek LB, Gaines P, Dillon SB, Wojchowski DM. Subtraction cloning and initial characterization of novel epo-immediate response genes. Cytokine 2000;12:845–57.
58. Richer JK, Jacobsen BM, Manning NG, Abel MG, Wolf DM, Horwitz KB. Differential gene regulation by the two progesterone receptor isoforms in human breast cancer cells. J Biol Chem 2002;277:5208–18.
59. Magnusson C, Svensson A, Christerson U, Tagerud S. Denervation-induced alterations in gene expression in mouse skeletal muscle. Eur J Neurosci 2005;21:577–80.
60. Kyung KJ, May A, Brosh RM, Jr., et al. The transcriptional response after oxidative stress is defective in Cockayne syndrome group B cells. Oncogene 2003;22:1135–49.