Overexpression of the Protein Tyrosine Phosphatase PRL-2 Correlates with Breast Tumor Formation and Progression

Serge Hardy, Nau Nau Wong, William J. Muller, Morag Park, and Michel L. Tremblay

Abstract

The PRL-1, PRL-2, and PRL-3 phosphatases are prenylated protein tyrosine phosphatases with oncogenic activity that are proposed to drive tumor metastasis. We found that PRL-2 mRNA is elevated in primary breast tumors relative to matched normal tissue, and also dramatically elevated in metastatic lymph nodes compared with primary tumors. PRL-2 knockdown in metastatic MDA-MB-231 breast cancer cells decreased anchorage-independent growth and cell migration, suggesting that the malignant phenotype of these cells is mediated at least in part through PRL-2 signaling. In different mouse mammary tumor–derived cell lines overexpressing PRL-2, we confirmed its role in anchorage-independent growth and cell migration. Furthermore, injection of PRL-2–overexpressing cells into the mouse mammary fat pad promoted extracellular signal-regulated kinase 1/2 activation and tumor formation. MMTV–PRL-2 transgenic mice engineered to overexpress the enzyme in mammary tissue did not exhibit spontaneous tumorigenesis, but they exhibited an accelerated development of mammary tumors initiated by introduction of an MMTV-ErbB2 transgene. Together, our results argue that PRL-2 plays a role in breast cancer progression. Cancer Res; 70(21): 8959–67. ©2010 AACR.

Introduction

Protein tyrosine phosphatases (PTP) constitute a large family of enzymes that can exert both positive and negative effects on signaling pathways (1). Thus, they play dominant roles in setting the levels of tyrosine phosphorylation in cells and in the regulation of many physiologic processes. Note-worthy, as observed with protein tyrosine kinases, deregulation of PTP activity can also contribute to cancer (2). Onset of various breast cancer subtypes correlates with overexpression of proto-oncogenes such as the epidermal growth factor receptor family member ErbB2 and the cytosolic protein tyrosine kinase src (3, 4). Interestingly, some PTP family members can also promote tumorigenesis (5). For instance, deletion of PTP1B activity in the ErbB2 transgenic mice results in a decrease in mammary tumor latency and resistance to lung metastasis (6, 7). Complementary to these findings, specific overexpression of PTP1B in the mammary gland leads to spontaneous breast cancer development.

The phosphatase of regenerating liver (PRL) phosphatases (PRL-1, PRL-2, and PRL-3) are three closely related small PTPs of ~20 kDa with at least 75% amino acid sequence identity (8). Recent interest in PRL phosphatases relates to their role in cell proliferation, including promotion of cell migration, invasion, and metastasis (9–11). Of the PRL phosphatases, PRL-3 is the most thoroughly investigated and high expression has been shown to correlate with disease progression in several types of cancer, including colorectal, gastric, and ovarian tumors (12–14). Importantly, gene expression profiling of metastatic colorectal liver samples revealed that among 144 upregulated genes detected, PRL-3 was the only gene consistently overexpressed in all 18 of the cancer metastases examined (15). Although the exact biological functions of these phosphatases remain unclear, mechanistically PRL-1 and PRL-3 have been linked to several pathways, including regulation of integrins, c-Src, Akt–phosphatidylinositol 3-kinase (PI3K), and Rho family GTPases (16–19). Interestingly, PRL-1 and PRL-3 have recently been identified as p53-inducible genes involved in cell cycle regulation (20, 21). However, the mechanism of their regulation and the basis of their transforming activity are not yet understood.

The expression of PRL-2 in tumor tissues and metastases has not been extensively investigated. However, few studies indicate that PRL-2 might have a role in tumor progression in colon and pancreatic cancer (22, 23). In addition, small interfering RNA (siRNA)–mediated knockdown of PRL-1 and PRL-2 in combination resulted in a moderate reduction of cellular growth and migration in pancreatic cancer cells as well as in serum-induced Akt phosphorylation (23). Thus, mounting evidence supports the view that PRL-1 and PRL-3 promote tumor progression and metastasis, whereas the lack of information about PRL-2 in these processes requires additional investigation.

Herein, we have identified PRL-2 as a PTP that is overexpressed during breast cancer development. In multiple...
breast cancer cellular models, we have confirmed the role of PRL-2 in cell migration and in the transformation process. In addition, PRL-2 promoted tumor formation in vivo in a xenograft model and in breast cancer–prone MMTV-ErbB2 transgenic mice. Together, these results provide the first evidence that PRL-2 plays a role in breast cancer progression.

Materials and Methods

Cell culture

The human breast cancer cell line (MDA-MB-231) and the mouse mammary cell lines (NMuMG and EpH4) were authenticated by the supplier (American Type Culture Collection) based on viability, recovery, growth, morphology, and isoenzymology. All cell lines were maintained in DMEM supplemented with 10% fetal bovine serum (FBS) and 50 μg/mL gentamicin (Life Technologies) at 37°C in a 5% CO₂ humid atmosphere.

Laser capture microdissection, RNA extraction, and real-time PCR

All procedures have been previously described (24). For quantification by real-time PCR of PRL-2 mRNA, cDNA synthesis was performed using SuperScript II (Invitrogen) using random hexamer according to the manufacturer’s instructions. Quantitative real-time PCR was performed on LightCycler (Roche) using the Quantitect SYBR Green PCR kit (Qiagen, Inc.) according to the manufacturer’s instructions. The primers used were as follows: 5′-GGACGCTGTTCCAT-CAGTAT-3′ (forward) and 5′-GGAGGCTAATTGTTC-CATGTGGC-3′ (reverse). Conditions for amplification were 35 cycles of 95°C for 15 seconds, 60°C for 15 seconds, and 72°C for 10 seconds with the acquisition of the fluorescence at 72°C. Threshold cycle numbers were determined using the second derivative maximum obtained with the LightCycler software version 3.5, and PRL-2 expression levels are calculated with 10⁻¹⁵ internal control gene.

Immunoblots

Cells were rinsed on ice with PBS and lysed [1% NP40, 0.5% sodium deoxycholate, 150 mmol/L NaCl, 50 mmol/L Tris (pH 7.4), 0.1% SDS, 50 mmol/L NaF, 1 mmol/L Na₃VO₄, Complete protease inhibitors (Roche)]. Equal amounts of denatured protein were separated by SDS-PAGE and transferred to polyvinylidene difluoride membranes (Immobilon-P, Millipore). Antibodies specific for phospho–extracellular signal-regulated kinase 1/2 (ERK1/2; Thr202/Tyr204), phospho-Akt (Ser473), ERK1/2, Akt, calnexin (Cell Signaling), actin, and Flag-M2 (Sigma) were used according to the manufacturer’s instructions. Blots were stripped using Re-Blot Plus Strong (Chemicon International) and reprobed according to the instructions from the manufacturer.

Production of PRL-2 stable cell lines

DB7, TM15, Met-1, EpH4, and NMuMG cells were stably infected by retroviral infection using pMSCVpuro vector (Clontech) containing the human PRL-2 gene. Ecotropic Phoenix packaging cells were transfected with the appropriate retroviral construct using Lipofectamine 2000 (Invitrogen). Culture supernatants were collected 36 to 48 hours after transfection and filtered. Target cells were infected with the filtered viral supernatants in the presence of 4 μg/mL Polybrene for 48 hours, after which the medium was changed. Following infection, cells were selected with 4 μg/mL puromycin for 2 weeks, and the resistant population was used for cellular assays.

Colony formation in soft agar

Anchorage-independent growth was determined by assaying colony formation in soft agar as described (25), with minor modifications. Briefly, single-cell suspensions were prepared from monolayer cultures by treatment with trypsin-EDTA. Cells were suspended in serum-free DMEM containing 10% FCS and 0.33% Seaplaque low-melting temperature agarose, and 5,000 cells were plated in a well of a six-well plate over a layer of solidified DMEM/10% FCS/0.6% agarose. The cells were fed every 2 days by adding DMEM/10% FCS. Colonies were counted and photographed at ×10 magnification after 2 weeks. The size of each colony was evaluated by counting the number of cells per colony.

Migration assay

Migration was measured using a 24-well Boyden chamber (Neuro Probe, Inc.). DMEM with 0.5% or 10% FBS was placed into the base wells separated from the top wells by polycarbonate filters (8-μm pore size). Cells were harvested by trypsinization, washed once with serum-free DMEM containing 0.5 μg/mL soybean trypsin inhibitor (Sigma), and washed twice with serum-free DMEM. Cells were resuspended in serum-free DMEM and added to the upper chamber at 30,000 per well. Cells were incubated for 6 hours at 37°C, and the number of migrated cells was determined by CyQuant DNA-binding fluorescence (Molecular Probes, Invitrogen). Relative fluorescence was detected at 485-nm excitation and 527-nm emission by a plate reading fluorometer (Fluoroskan Accent FL, ThermoLabsystems).

Lentivirus production and shRNAmir-mediated knockdown of PRL-2

The pcDNA_6.2-GW/EmGFP-miR plasmid (Invitrogen) was used for the cloning of two different miR155-based PRL-2 short hairpin RNA (shRNA), and the production of lentivirus was prepared using the BLOCK-iT Lentiviral Pol II miR RNAi Expression System (Invitrogen) according to the manufacturer’s instructions. MDA-MB-231 breast cancer cells were infected with the filtered viral supernatants in the presence of 4 μg/mL Polybrene for 48 hours, after which the medium was changed. Following infection, cells were selected with 10 μg/mL blasticidin for 2 weeks and the resistant population was used for cellular assays.

Mouse experimental procedure

The PRL-2 WT and C/S DNA fragment was cloned into a plasmid cassette containing the MMV LTR and SV40 splicing and polyadenylation sites (26). We generated MMTV...
PRL-2 WT and PRL-2 C/S transgenic mice by microinjecting the linearized expression construct into the pronucleus of FVB/N mouse embryos as described previously (6) MMTV-ErbB2 (FVB/N) and MMTV–PRL-2 (FVB/N) mice were interbred to generate females of the required genotypes. For xenograft assay, mice (FVB/N; 6 weeks old) were injected in the #4 mammary fat pad with DB-7 mammary cancer cells stably expressing Flag–PRL-2 or Flag–PRL-2 C/S as described previously (27). Mice were kept on a 12-hour light/12-hour dark cycle and allowed free access to food and water. Animals were monitored daily for physical well-being and examined twice a week for tumor occurrence. All procedures were carried out according to the Canadian Council on Animal Care ethical regulations and approved by the McGill University Research and Ethics Animal Committee.

Results

PRL-2 is implicated in the growth and the migration of mouse mammary cancer cells

To identify new candidates that contribute to cancer pathogenesis, we integrated the use of laser capture microdissection (LCM) and T7-based RNA amplification with DNA microarrays. LCM provides means to analyze the specific cancer cells and avoids contamination by surrounding stromal and inflammatory cells (24). In addition, RNA amplification and expression analysis at the subnanogram input level is both feasible and accurate (28). This approach allowed the subsequent PTP4A2 (PRL-2) expression pattern to be obtained within a cellular-based, rather than a tissue-based, resolution. Using these techniques, the expression of PTP4A2 was shown to be elevated in laser capture microdissected primary breast tumor epithelial cells when compared with matched normal tissue from 16 of 19 patients analyzed (Supplementary Fig. S1). To further investigate the role of PRL-2 in mammary tumorigenesis, we first established cells retrovirally infected with pmscv vector (empty), pmscv–Flag–PRL-2 (PRL-2 WT), and pmscv–Flag–PRL-2 C101S (PRL-2 C101S). TM15 and DB7 cells are, respectively, NeuNT (activated ErbB2) knock-in and MMTV-PyMT tumor-derived cell lines that form tumors when injected into the mammary fat pad of FVB/N mice (27, 29). Although control original TM15 and DB7 cells proliferated at relatively the same rate as PRL-2 WT- and PRL-2 C101S–infected cells in bidimensional

Figure 1. PRL-2 promotes anchorage-independent growth and migration of mouse mammary cancer cells. A, TM15, DB-7, and Eph4 cells stably expressing Flag–PRL-2 or Flag–PRL-2 C/S were tested for their potential to form colonies in soft agar. B, number of colonies was quantified. Columns, mean of three independent experiments; bars, SE. *, P < 0.05 versus control empty vector. C, expression of Flag–PRL-2 transgenes in the various cell lines was measured by SDS-PAGE and immunoblotted with a Flag antibody. D, TM15, DB-7, and Eph4 cells stably expressing Flag–PRL-2 or Flag–PRL-2 C/S were tested for migration using the Boyden chamber assay. Columns, mean of three independent experiments; bars, SE. *, P < 0.05 versus control empty vector.
culture conditions (data not shown), we hypothesized that overexpression of PRL-2 may be advantageous under more stringent growth conditions, such as growth in soft agar. Whereas TM15 and DB7 empty control cells formed very few colonies in agar, PRL-2 expression induced about a 2.5-fold increase in colony formation (Fig. 1A and B). However, using Eph4 cells, a nontransformed mammary epithelial cell line, we did not observe any change on soft agar growth when PRL-2 was overexpressed, suggesting that PRL-2 will exert its oncogenic cell growth potential only in fully transformed cancer cells. It is noteworthy that the same phenomenon was observed in the MMTV-PyMT highly metastatic tumor-derived cell line Met-1 (27) and in the nontransformed mammary epithelial cell line NMuMG (Supplementary Fig. S2).

Because overexpression of PRL-1 and PRL-3 has been shown to promote cell migration in many types of cancer cells (30), we next evaluated the migratory capacity of PRL-2–overexpressing cells using Boyden chamber assay. Serum-stimulated cell migration was increased ~2-fold in TM15 and DB7 mammary cancer cells with high levels of PRL-2 when compared with the control situation and the catalytically inactive PRL-2 mutant–expressing cells (Fig. 1D). However, because the metastatic cell line Met-1 already has a high migratory potential (27), we did not observe enhanced cell migration in PRL-2–overexpressing cells. Taken together, these results show that PRL-2 promotes cellular transformation and contributes to the migration of mammary cancer cells.

**PRL-2 increases tumor growth in a syngeneic orthotopic xenograft mouse model**

Orthotopic xenograft models can represent a more clinically relevant tumor model with respect to the primary site of the tumor. Moreover, implanting tumor cells into the original anatomic sites more closely mimics host microenvironments. DB7 cells expressing pmscv vector (empty), pmscv–Flag–PRL-2 (PRL-2 WT), and pmscv–Flag–PRL-2 C101S (PRL-2 C/S) were injected in the mammary fat pad of immune intact, syngeneic FVB/N female mice. After 5 weeks, tumors were isolated and weighed. We observed a
50% increase in tumor size and weight generated from DB7–PRL-2 WT–injected mice compared with the DB7 empty control and the DB7 catalytically inactive PRL-2 mutant (Fig. 2A). Immunoblot analysis of the lysates from the DB7 tumors showed that enhanced tumor growth correlated with increased phosphorylation of ERK1/2 in PRL-2 overexpressing tumors (Fig. 2B). On the other hand, phosphorylation of Akt was not affected by high levels of PRL-2. Taken together, these results suggest that PRL-2 promotes tumor growth and correlates with increased ERK1/2 activity in vivo.

PRL-2 is implicated in the migration and growth of human breast cancer cells

When we looked at the expression of PRL-2 in different breast cancer cell lines, we noticed that PRL-2 is elevated in all human breast cancer cell lines tested when compared with the immortalized breast epithelial cell line MCF10A (Fig. 3A). Interestingly, the metastatic breast cancer cell line MDA-MB-231 has the highest PRL-2 expression level. Because PRL-1 and PRL-3 have been shown to mediate cell migration of cancer cells, we examined whether the alteration of PRL-2 expression in MDA-MB-231 breast cancer cells influenced their motility. To determine if PRL-2 is implicated in serum-induced migration of MDA-MB-231 cells, we first attempted to reduce the expression level of PRL-2 in MDA-MB-231 cells using siRNA. Interestingly, the increase in migration following serum stimulation was significantly reduced by 40% in cells expressing the PRL-2– or ERK2-specific siRNA (Fig. 3B), which also corresponded to a decrease of PRL-2 mRNA by 80% in siRNA-transfected cells as
determined by real-time PCR (Fig. 3D). Using ERK2-specific siRNA as a positive control in this experiment, we observed a 90% decrease of its proteins when analyzed by Western blotting (Fig. 3C). To test PRL-2 potency to alter colony formation in soft agar, MDA-MB-231 breast cancer cells were stably transduced with lentiviral particles expressing emGFP plus two PRL-2–directed shRNAs and tested for their potential to form colonies in soft agar (Fig. 4A). PRL-2 mRNA expression was reduced by ~70% in cells expressing the shRNA targeting PRL-2 as determined by semiquantitative PCR when compared with the scrambled control (Fig. 4B). Interestingly, we observed a decrease on the size and the number of colonies in the population expressing the two shRNAs targeting PRL-2 gene when compared with the control (Fig. 4C). Altogether, these results indicate that migration and growth of MDA-MB-231 breast cancer cells is mediated at least in part through PRL-2 signaling.

Coexpression of PRL-2 and ErbB2 results in faster mammary tumor formation

We observed previously (Fig. 1) that overexpression of PRL-2 in the activated ErbB2 knock-in tumor-derived cell line TM15 increased the size and the number of colonies in soft agar assay. To explore whether PRL-2 signaling plays a role in mammary tumor progression in the ErbB2 mouse model, we derived transgenic mice that coexpress both activated ErbB2 and PRL-2 in the mammary epithelium. To achieve this, transgenic mice carrying an oncogenic version of ErbB2 under the MMTV promoter (ErbB2) were interbred with mice expressing Flag–PRL-2 protein from the same mammary-targeted promoter (PRL-2) or the catalytically inactive mutant (PRL-2 C/S). Previous studies have shown that this ErbB2 model (NDL2 strain) develops multifocal mammary tumors (31), whereas our MMTV–PRL-2 mice failed to develop mammary tumors and showed no phenotypic alteration (data not shown). We observed that the double transgenic mice (ErbB2/PRL-2) show a short but significantly reduced latency of mammary tumor formation, with 50% of the animals showing tumor formation at 132 days ($T_{50}$) as opposed to 144 days for the ErbB2 and ErbB2/PRL-2 C/S mice (Fig. 5A). The expression of ErbB2 and the Flag–PRL-2 proteins was confirmed by Western blot analysis on lysates from the mammary tumors of the transgenic mice (Supplementary Fig. S3A and B). Furthermore, as observed previously in the xenograft assay, immunoblot analysis of the lysates from tumors at the initiation stage showed an increase in the phosphorylation of ERK1/2 in PRL-2–overexpressing tumors (Fig. 5B and C). However, phosphorylation of Akt was not affected by overexpression of PRL-2 at this stage. Thus, in an in vivo mouse model, the results indicate that expression of PRL-2 can accelerate the induction of ErbB2 mammary tumors.

The PRL-2 gene is overexpressed in breast cancer and lymph node metastases

Because overexpression of the two other PRLs is associated with progression and poor prognosis of several human cancers, we desired to evaluate the expression of PRL-2 in human breast cancer specimens. Thus, to determine the gene expression profiles of PRL-2 (PTP4A2) during breast cancer progression in human cancer tissues, we used quantitative PCR on laser capture microdissected epithelial
cancer materials at different stages of disease progression. Importantly, we observed an increase of PRL-2 expression in primary breast tumor cells using real-time quantitative PCR analysis compared with normal tissue (Fig. 6). In addition, this phosphatase was shown to be dramatically elevated in breast cancer metastatic lymph nodes when compared with the primary tumor, suggesting that expression of the PRL-2 gene is increased during breast cancer progression. These findings also support our murine studies in showing that PRL-2 contributes to the development of breast cancer.

**Discussion**

PTPs, such as PRL-1 and PRL-3, have been proposed to possess oncogenic properties and to play a role in tumor development (30). However, the role of PRL-2 in cancer has been poorly studied. Here, we have identified PRL-2 as a potential therapeutic candidate that promotes breast cancer progression. Moreover, in multiple cellular and in vivo models, we have confirmed the role of PRL-2 in cell migration and in the transformation process. In addition, for the first time, we provide data derived from a transgenic animal model of a member of the PRL phosphatase family.

The present study shows that expression of PRL-2 in breast cancer cells leads to a complex modulation of important aspects of the transformed phenotype. We observed that overexpression of PRL-2 was advantageous under stringent growth conditions, such as growth in soft agar, and that both of our in vivo models indicate that PRL-2 expression contributes to breast cancer cell growth. Earlier studies describing PRLs focused on their involvement in proliferation and ability to transform nonmalignant immortalized cell lines. Ectopic expression of PRL-1 and PRL-3 can cause increased levels of cellular proliferation as well as transform nonmalignant cell lines (10, 11, 32), whereas transfection with catalytically inactive mutants of the PRLs has a minimal effect as we also noticed in our study. Werner and colleagues (33) found that PRL-1– or PRL-2–transfected cells had a higher rate of DNA synthesis. Indeed, cell cycle analysis confirmed that PRL-1– or PRL-2–overexpressing cells had more cells in S phase with a concomitant decrease in the proportion of cells in G1 phase. On the other hand, in the tumorigenic colorectal adenocarcinoma DLD-1 cell line, downregulation of PRL-3 or PRL-1 by siRNA decreased cell migration but had no effect on cell proliferation (34). We also observed that cells proliferated at relatively the same rate in PRL-2–infected cells in bidimensional culture conditions but showed increased anchorage-independent growth. It is possible that the difference observed could reflect differences in the cell types or the methodologies used. Nonetheless, those studies in addition to our results support an important role for PRL-2 in tumor cell transformation and not only in motility.

To date, the presence or absence of lymph node metastasis is the most valuable single prognostic attribute in breast cancer patients. Analysis of PRL expression in seven neoplastic breast carcinomas and seven normal breast tissue samples by semiquantitative reverse transcription-PCR (RT-PCR) found a very small increase in the PRL-3 transcript in the tumors, but no significant changes in PRL-1 and PRL-2 mRNA levels (35). Upregulated PRL-3 was also validated in larger sets of tumors (36), but no further studies of PRL-1 and PRL-2 expression in this type of cancer have yet been reported. Here, we showed that PRL-2 expression levels increase during breast cancer progression. By quantitative RT-PCR on LCM tumors, the PRL-2 mRNA expression was significantly elevated in neoplastic compared with normal breast tissue, and we have found a significantly more frequent expression of PRL-2 in lymph node metastases compared with primary tumors. This increased expression during breast cancer progression suggests that PRL-2 could be a novel marker for breast cancer metastasis. The development of PRL-specific antibodies and specifically anti–PRL-2 reagents will be essential to validate by immunohistochemistry the clinical relevance of PRL-2 as a breast cancer metastasis marker.

The migration capacity of breast cancer cells is a critical determinant of their metastatic potential to migrate into lymph nodes. The present work also provides, for the first time, evidence that expression of PRL-2 increases the migration of breast cancer cells because PRL-2–expressing cells display significantly enhanced migratory ability in cell-based assays. However, whether this change is accompanied by enhanced metastasis of PRL-2–overexpressing cells in vivo remains to be further investigated. Because the ErbB2 model (NDL2 strain) already develops aggressive metastatic lesions to the lung, we did not observe more metastases in the ErbB2/PRL-2 mice (data not shown). This is also consistent with the observation that the metastatic mammary cancer cell Met-1 did not migrate more when PRL-2 was overexpressed. However, when we looked at the expression levels of PRL-2 in the metastatic human breast cancer cell line MDA-MB-231, we noticed the highest expression among all the cell lines tested, and knocking down PRL-2 expression

---

**Figure 6.** mRNA levels of PRL-2 gene (PTP4A2) in laser capture microdissected human breast cancer tumor samples. Nonmalignant, malignant breast, and lymph node metastatic tissue samples (each: n = 7) were selected for analysis using real-time quantitative RT-PCR. Columns, mean of three independent measurements performed in duplicates. *, P < 0.05 versus normal; **, P < 0.05 versus tumor.
resulted in a decrease in cell migration. Thus, in addition to the contribution of PRL-2 in the transformation process of breast cancer cells, our data support that this PTP could also participate in tumor dissemination and might be an early event in the progression of a primary tumor to a more aggressive phenotype.

The exact biological functions and the physiologic substrates of PRL-2 are not fully understood. However, identifying pathways that PRL-2 regulates would be important in understanding its role in tumor progression. Most studies have focused on the role that PRL-1 and PRL-3 play in cancer cell lines. Recently, it was reported that PRL-1 and PRL-3 promoted motility and invasion in colon cancer cells by stimulating Rho activity (19). In addition, they have been linked to several pathways, including regulation of integrins, c-Src, and Akt-P19K (16–18, 37). Our study showed that PRL-2 increased ERK1/2 activity in vivo, but not Akt activity, suggesting that this pathway might be involved in PRL-2--induced tumor growth, and in turn suggesting a more complex function of this intriguing family of phosphatase.

No protein substrate has yet been identified for PRL-2, but the β-subunit of the Rab geranylgeranyltransferase II (RabGGT II) was found to specifically interact with PRL-2 (38). In addition, we also validated this interaction in co-overexpression experiments using these two proteins in HeLa cells (Supplementary Fig. S4). RabGGT II is well known to prenylate Rab proteins, and emerging evidence implicates the Rab proteins in multiple human diseases including breast cancer (39). Members of the Rab families have been shown to regulate cell motility by controlling integrin internalization and recycling back to the plasma membrane (40). Interestingly, PRL-2 may reside in the later recycling subcompartment of the early endosome, where some Rab family members are located (41). In addition, PRL-3 was recently shown to promote cell migration via an integrin β1–ERK1/2 pathway (42). Our present study shows that PRL-2 overexpression promotes cellular properties associated with breast cancer progression likely via the ERK1/2 pathway, and because PRL-2 may function as a regulator of RabGGT II activity, this phosphatase might also have a role related to Rab-regulated recycling pathways that contribute to cell transformation and migration. Whether PRL-2 is implicated in Rab signaling is currently under investigation.

Taken together, we document for the first time that PRL-2 is overexpressed during breast cancer progression. In multiple cellular models, we confirmed the role of PRL-2 in cell migration and in the transformation process. Notably, we showed for the first time that PRL-2 promoted tumor formation in vivo in a xenograft model and in breast cancer–prone transgenic mice. To date, most studies have focused on the role that PRL-1 and PRL-3 play in cancer development. Hence, our results, in addition to the observation that the levels of PRLs are increased in several types of cancer, indicate that overexpression of PRL-2 might facilitate breast tumor cells to invade into lymphatics, and therefore could contribute to the development of local lymph node and distant metastases. Further studies are now justified to characterize the molecular profiles and potential outcome that are linked to PRL-2--expressing human breast cancers.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

Quebec Breast Cancer Foundation and Canadian Breast Cancer Research Alliance (M.L. Tremblay). This work was also supported by an operating grant to M. Park from the Canadian Institutes of Health Research. S. Hardy was supported by a postdoctoral fellowship from the Canadian Institute of Health Research. M.L. Tremblay is a Jeune et Jean-Louis Lévesque chair in Cancer Research and a Chercheur National of the Fonds de la recherche en santé du Québec. M. Park holds the Diane and Sal Guerrera Chair in Cancer Genetics.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received 06/10/2010; revised 09/09/2010; accepted 09/10/2010; published OnlineFirst 09/14/2010.

References

14. Ren T, Jiang B, Xing X, et al. Prognostic significance of phosphatase...


38. Si X, Zeng Q, Ng CH, Hong W, Pallen CJ. Interaction of farnesylated PRL-2, a protein-tyrosine phosphatase, with the β-subunit of geranylgeranyltransferase II. J Biol Chem 2001;276:32875–82.


42. Peng L, Xing X, Li W, et al. PRL-3 promotes the motility, invasion, and metastasis of LoVo colon cancer cells through PRL-3 integrin β1-ERK1/2 and -MMP2 signaling. Mol Cancer 2009;8:110.
Overexpression of the Protein Tyrosine Phosphatase PRL-2 Correlates with Breast Tumor Formation and Progression

Serge Hardy, Nau Nau Wong, William J. Muller, et al.

Cancer Res 2010;70:8959-8967. Published OnlineFirst September 14, 2010.

Updated version Access the most recent version of this article at: doi:10.1158/0008-5472.CAN-10-2041

Supplementary Material Access the most recent supplemental material at: http://cancerres.aacrjournals.org/content/suppl/2010/09/14/0008-5472.CAN-10-2041.DC1

Cited articles This article cites 42 articles, 17 of which you can access for free at: http://cancerres.aacrjournals.org/content/70/21/8959.full.html#ref-list-1

Citing articles This article has been cited by 5 HighWire-hosted articles. Access the articles at: /content/70/21/8959.full.html#related-urls

E-mail alerts Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.