PRL-3 Expression in Metastatic Cancers

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ABSTRACT

Purpose: Expression of the PRL-3 tyrosine phosphatase is elevated in liver metastases derived from colorectal cancer (CRC). We sought to determine the cellular basis of this elevation and assess the expression of PRL-3 in metastatic lesions derived from cancers of the colon and other tissues.

Experimental Design: We developed modifications of in situ hybridization methods that facilitated the study of paraffin-embedded sections. We also evaluated PRL-3 gene copy numbers using fluorescence in situ hybridization and developed antibodies to assess PRL-3 subcellular localization.

Results: PRL-3 mRNA expression was elevated in nearly all metastatic lesions derived from CRCs, regardless of the site of metastasis (liver, lung, brain, or ovary). Expression was found in neoplastic cells, although tumor endothelium also expressed the gene. In contrast, little or no PRL-3 expression was observed in normal colon, nonmetastatic primary cancers, or metastatic lesions derived from cancers other than those of the colon (pancreas, stomach, or esophagus). Interphase fluorescence in situ hybridization confirmed that gene amplification was not the major cause of PRL-3 overexpression. Immunohistochemical analysis with anti-PRL-3 antibodies showed a cell membrane localization, consistent with the predicted isoprenylation of the protein.

Conclusions: These studies establish an unexpected and unprecedented specificity in metastatic gene expression profiles: PRL-3 is apparently expressed in CRC metastases to any organ but is not expressed in metastases of other cancers to the same organs or in nonmetastatic CRCs. PRL-3 is also expressed in tumor vasculature, regardless of the tumor source. These data raise intriguing questions about the role of protein phosphorylation in angiogenesis and cell-type-specific metastatic processes.

INTRODUCTION

Metastasis represents the phase of tumorigenesis that is responsible for the great majority of cancer deaths. Although much has been learned about the genetic and biochemical basis of the earlier phases of tumorigenesis, the metastatic process has proved far more difficult to investigate. In part, this is because metastasis is, by nature, a process determined by cell-cell interactions and specific microenvironments (1–3). In contrast, advances in understanding the earlier stages of tumorigenesis revolve around cell autonomous processes that determine the rate of cell division and cell death (4). Although cell autonomous processes can be studied in tissue culture and other model systems, cell-cell and cell-microenvironment interactions are much more difficult to model in vitro.

Progress in understanding some aspects of metastasis are notable. For example, many studies have shown that proteases that degrade the basement membrane are likely to be essential for invasion (5). Additionally, cytoskeletal remodeling appears to be required for cancer cell mobility (6). However, the signaling pathways that control the cytoskeletal changes or the expression and activation of proteases and their inhibitors in metastases are largely unknown. This situation is therefore quite unlike the one investigators face when studying cell birth and cell death in which detailed information about the relevant biochemical networks are available.

On the basis of the above considerations, we were intrigued by the finding that a protein tyrosine phosphatase called PRL-3 was expressed at high levels in liver metastases of CRCs (7). Protein tyrosine kinases and phosphatases constitute critical controls of many cellular activities, and PRL-3 thereby promised to provide new insights into the biochemical pathways controlling various aspects of metastasis (8, 9). PRL-3 is a

4 The abbreviations used are: CRC, colorectal cancer; DIG, digoxin; FISH, fluorescent in situ hybridization; CRC, colorectal cancer; HA, hemagglutinin; VEGFR, vascular endothelial growth factor receptor.
member of a small and recently discovered class of protein tyrosine phosphatases (10). The family includes only three members (PRL-1, PRL-2, and PRL-3), and overexpression of PRL-1 or PRL-2 can transform cells in vitro when overexpressed (11, 12). Among normal human adult tissues, PRL-3 (also known as PTP4A3) is expressed predominantly in muscle and heart (13). In the current work, we attempted to address several fundamental questions relating PRL-3 to the metastatic process. One of the most interesting and unexpected results described below concerns the specificity of PRL-3 expression among the various metastatic lesions tested.

MATERIALS AND METHODS

Tumor Tissues. Formalin-fixed, paraffin-embedded tissue samples were retrieved from the surgical pathology files of The Johns Hopkins Hospital.

In Situ Hybridization. DIG-labeled antisense RNA probes were generated by PCR amplification of multiple PCR products (500–800 bp in length) corresponding to the entire PRL-3 transcript. Various sense PRL-3 probes served as negative controls. T7 promoter sequences were incorporated into the antisense primers as described previously (14). In vitro transcription was performed with DIG RNA labeling reagents and T7 RNA polymerase according to the manufacturer’s instructions (Roche, Indianapolis, IN). Six-μm-thick sections obtained from formalin-fixed, paraffin-embedded tissue blocks were deparaffinized, treated with pepsin, blocked with 1% blocking reagent (DIG Nucleic Acid Detection kit; Roche) containing purified nonspecific rabbit immunoglobulins (Dako). For signal amplification, a horseradish peroxidase rabbit anti-DIG antibody (Dako) was used to catalyze the signal amplification of the corresponding genomic locus using the following set of primers: PRL3-3F, CCAGGTTTTTG- GACAGGACT; PRL3-3R, CTGGTACAACCCCTCAATGTG; PRL3-4F, TTCAAGGCTTGTCTTGCT; PRL3-4R, GAT- CATTCCCGGCGTGA; PRL3-5F, TTCTTCAGGGCAG- GAATTG; PRL3-5R, TCTCTGGTCTCGAGGAGT; PRL3-6F, CCACAGAGTGGGTGTGCTCA; PRL3-6R, AAC- GGTGTCACATTCTCC; PRL3-7F, GAGGGAAGCCAT- GAGGA; PRL3-7R, ATGTGAAATTCTCTGGGTGAC; PRL3-8F, GGTGTCCCACCTACATTCA; PRL3-8R, CTTT- GTCTGTCACCATCAGTT; PRL3-9F, GCCACCGAGCT- GAAATG; PRL3-9R, CTTGGGATAGGGCAGTAG; PRL3-10F, TCTCCCCCTGATAGGGTCC; PRL3-10R, AGT- CCTGACTGCGGTGTGG; PRL3-11F, GCCACACGCGC- TGGAGAC; PRL3-11R, CATAGGCTTGAGGCTGCC; PRL3-12F, GCCACCCGCTGCAGTT; PRL3-12R, GAGTGTAAGCTGGGCGTGG; PRL3-13F, CTTCTGTCGCTCCAGTCT; PRL3-13R, CTGTTGACCGTGAGGCTGC; PRL3-14F, GGAGTACATGTTCCACACCGT; PRL3-14R, GTGGTACCAGCTACTCAGGA.

Quantification of PRL-3 expression by real-time PCR. Total RNA was isolated using RNeasy (Quiagen, Valencia, CA) and mRNA was selected using Superase RNase (Promega, Madison, WI). Single-stranded cDNA was generated using Superscript II Reverse Transcriptase Life Technologies, Inc.) following the manufacturer’s directions. Mock template preparations were prepared in parallel without the addition of reverse transcriptase. Quantitative PCR was performed with an iCycler (Bio-Rad, Hercules, CA) using Sybr Green I dye (Molecular Probes, Eugene, OR), and threshold cycle numbers were obtained using iCycler software version 2.3. Primer sets were described previously (7). Conditions for amplification were: one cycle of 95°C, 2 min followed by 35 cycles of 95°C, 15 s, 58°C, 15 s, and 72°C, 15 s.

Expression Constructs and Immunofluorescence. HA-tagged PRL-1, PRL-2, and PRL-3 expression vectors were constructed by PCR amplification of the corresponding cDNA. PCR products were cloned into pHAHA (15). Expression constructs were transfected into HCT-116 CRC cells seeded on glass chamber slides (Nalgene Nunc; Lab-Tek, Christchurch, New Zealand). The expression of fusion proteins was detected by fluorescence microscopy and immunoblotting with a monoclonal anti PRL-3 antibody (MA no. 14) as described previously (15). Nuclei were counterstained with 1 μg/ml 4’,6-diamidino-2-phenylindole.

Antibodies. Rabbit polyclonal antibodies were raised against a PRL-3 COOH-terminal peptide (DPTHKTRC-CVM). Immunoreactive sera were affinity purified with the antigenic peptide. Monoclonal antibodies were raised against His and glutathione S-transferase-tagged recombinant PRL-3 produced in bacteria and purified using glutathione and nickel resin respectively (Ivratech, Inc., Kyiv, Ukraine). Specificity of the anti-PRL-3 antibodies was evaluated by ELISA assays and Western immunoblotting using recombinant PRL-1, PRL-2, and PRL-3 produced in CRC cells.

FISH Analysis. The c-myc and the chromosome 8 centromere probe (both labeled with Spectrum Orange) were obtained from Vysis (Downers, IL). PRL-3 probe was prepared by PCR amplification of the recombinant genomic locus using the following set of primers: PRL3-3F, CCAGGTTTTTG-GACAGGACT; PRL3-3R, CTGGTACAACCCCTCAATGTG; PRL3-4F, TTCAAGGCTTGTCTTGCT; PRL3-4R, GAT-CATTCCCGGCGTGA; PRL3-5F, TTCTTCAGGGCAG-GAAATG; PRL3-5R, TCTCTGGTCTCGAGGAGT; PRL3-6F, CCACAGAGTGGGTGTGCTCA; PRL3-6R, AAC-GGTGTCACATTCTCC; PRL3-7F, GAGGGAAGCCATGAGGA; PRL3-7R, ATGTGAAATTCTCTGGGTGAC; PRL3-8F, GGTGTCCCACCTACATTCA; PRL3-8R, CTTTGTCTGTCACCATCAGTT; PRL3-9F, GCCACCGAGCTGGAAATG; PRL3-9R, CTTGGGATAGGGCAGTAG; PRL3-10F, TCTCCCCCTGATAGGGTCC; PRL3-10R, AGT-CCTGACTGCGGTGTGG; PRL3-11F, GCCACACGCGCTGGAGAC; PRL3-11R, CATAGGCTTGAGGCTGCC; PRL3-12F, GCCACCCGCTGCAGTT; PRL3-12R, GAGTGTAAGCTGGGCGTGG; PRL3-13F, CTTCTGTCGCTCCAGTCT; PRL3-13R, CTGTTGACCGTGAGGCTGC; PRL3-14F, GGAGTACATGTTCCACACCGT; PRL3-14R, GTGGTACCAGCTACTCAGGA.

Cycling conditions using Platinum Taq (Invitrogen) were: 94°C for 2 min, followed by 35 cycles of 94°C for 10 s, 58°C for 60 s, and 68°C for 30 s. PCR products were purified using the QiaQuick PCR purification kit (Qiagen, Valencia, CA) and labeled with biotin dUTP using nick translation as described previously (16). Tissue sections were deparaffinized with xylene for 60 min three times and then washed in 100% ethanol for 10 min. Sections were dried completely and then incubated with 1 μ NaSCN at 80°C for 15 min. Slides were then washed with water and treated with the protease solution (Vysis 32-801210)
for 10 min at 45°C–50°C. After digestion, the sections were
rinsed 1–3 min in water and fixed with 10% buffered formalin
for 10 min at room temperature. Sections were washed with
70% ethanol and then aged in 70% ethanol for 24 h at −20°C.
FISH hybridization and detection were performed as previously
described (16) with the following modifications. Aged slides
were washed three times with water and immersed for 5 min in
denaturing solution: 7 parts deionized formamide (American
Bioanalytical, Natick, MA) three parts 2× SSC, pH 7.5) for 15
min at 73°C. At the end of the hybridization, slides were
immersed in washing solution: 0.1% IGEPAL CA-630 (Sigma)
and 2× SSC, final pH 7.5 for 2 min at 73°C.

Cell Culture. The human colon cancer cell lines and
their derivatives were cultured in McCoy’s 5A medium supple-
mented with 10% FCS and penicillin/streptomycin (Invitrogen
Corp.).

Immunoprecipitation and Immunoblotting. Cells
were lysed with EB buffer [100 mM Tris (pH 7.4), 150 mM
NaCl, 5 mM EDTA, 10% glycerol, 1% Triton X-100, and 1 mM
sodium orthovanadate], and immunoprecipitation was per-
formed with the indicated antibodies. Protein extracts were
resolved via SDS-PAGE and transferred to polyvinylidene difluoride membranes by high-intensity wet blotting. Filters were
probed with the indicated antibodies, and specific binding was
detected by enhanced chemiluminescence system (Amersham).

RESULTS

In Situ Hybridization to Assess PRL-3 Expression. To
identify the cell types responsible for PRL-3 expression, we
developed a modified in situ hybridization technique that al-
lowed us to study archival samples with nonradioactive probes.
As described in detail in the “Materials and Methods” section,
we hybridized an adjacent section of each tumor with a probe for VEGFR2, known to be expressed in endothelial cells, as a control. Examples of the results obtained in CRC metastases to the liver are shown in Fig. 1. Prominent hybridization to the neoplastic epithelial cells was observed in nearly all cases (10 of 11 studied). The differences in cellular localizations of the mRNA for PRL-3 versus VEGFR2 were striking, with the former largely decorating the neoplastic component and the latter decorating the stromal (endothelial) component (Fig. 1B, E, H, and M). However, PRL-3 signals were also observed in a subset of the endothelial cells of some of the tumors (Fig. 1, H and I). This localization to tumor endothelial cells is consistent with previous analyses of global patterns of gene expression in such cells (14).

To determine whether PRL-3 was expressed in metastases outside the liver, we analyzed 7 lung lesions, 4 brain lesions, and 3 ovarian lesions, each from a different patient. In 13 of the 14 cases, prominent hybridization to the neoplastic cells of the metastases was observed (examples in Fig. 2, I and N). In the rare case that did not exhibit PRL-3 expression in the neoplastic cells, PRL-3 hybridization was still observed in the endothelial cells within the metastasis (data not shown). We also studied mesenteric lymph node metastases and found that these lesions generally expressed PRL-3 (Fig. 1I).

Six primary, nonmetastatic CRCs and 8 colorectal adenomas were evaluated with the same technique. No expression of PRL-3 was observed in the epithelial cells within these samples, and only weak hybridization, if any, was observed in the endothelial compartment (Table 1).

Finally, we examined 10 liver or lung metastases derived from esophageal, stomach, or pancreatic cancers. None of the neoplastic epithelial cells within these metastatic lesions expressed PRL-3 at detectable levels, although PRL-3 expression was sometimes observed in endothelial cells of these lesions (data not shown). The fraction of CRC metastases expressing PRL-3 (92%) was significantly different from that of non-CRC metastases (P < 0.00001, χ² test).

**Evaluation of the PRL-3 Genomic Locus.** To assess the status of the PRL-3 genomic locus in situ, we identified a bacterial artificial chromosome from chromosome 8q24.3 con-
taining the entire gene and used it for FISH analyses of liver metastases derived from CRC. No true gene amplification was detected among 20 such cases. This is consistent with previous data showing that gene amplification is not a major cause of PRL-3 overexpression (7). However, we found that 9 of 20 cases had significantly increased copy numbers of PRL-3 (i.e., >4 copies/cell; examples in Fig. 3, A–C). In all of these cases, the increases were associated with extra copies of chromosome 8q but not the entire chromosome 8. This was demonstrated through hybridization with a probe for chromosome 8 centromeric sequences (Fig. 3B). Through hybridization of adjacent slides to a bacterial artificial chromosome containing the c-myc gene, we found that a relatively large segment of chromosome 8q24, of size at least 12 Mb, was involved in all these cases (example in Fig. 3C). This situation is different from that expected to result from gene amplification, wherein only a relatively small region, <2 Mb, is present at increased copy number.

To determine whether increased copies of the PRL-3 gene were present in primary CRCs, we analyzed 19 Duke’s B and C cancers with the same probes. Only 2 of these 19 cancers had extra PRL-3 (or chromosome 8q) sequences, as determined by the ratio of PRL-3 signals to centromere 8 signals. The difference in PRL-3 gene copy number between metastatic and primary lesions was statistically significant ($P < 0.02$).

**Expression of PRL-3 in Cancer Cell Lines.** Additional studies of the role of PRL-3 in neoplasia would be facilitated if cancer cell lines expressing this gene were available. We therefore analyzed colorectal tissue samples as well as cell lines

**Table 1** Summary of PRL-3 expression in colorectal tumor samples

<table>
<thead>
<tr>
<th>Stage/Histology</th>
<th>No. of samples</th>
<th>PRL-3-positive epithelial cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR normal colon</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Nonmetastatic CRC</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>CRC metastases to lymph node</td>
<td>4</td>
<td>4 (100%)</td>
</tr>
<tr>
<td>CRC metastases to the liver</td>
<td>11</td>
<td>10 (91%)</td>
</tr>
<tr>
<td>CRC metastases to the lung</td>
<td>7</td>
<td>6 (86%)</td>
</tr>
<tr>
<td>CRC metastases to the brain</td>
<td>4</td>
<td>4 (100%)</td>
</tr>
<tr>
<td>CRC metastases to the ovary</td>
<td>3</td>
<td>3 (100%)</td>
</tr>
<tr>
<td>Non-CRC metastases to the liver</td>
<td>8</td>
<td>0</td>
</tr>
<tr>
<td>Pancreas</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Esophagus</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Non-CRC metastases to the lung</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Stomach</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td>1</td>
<td></td>
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</tbody>
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(passaged in vitro or as xenografts in nude mice) to determine whether any expressed high levels of PRL-3. We identified two cancer cell lines, V9P and V9M, respectively, derived from a CRC and a liver metastasis of the same patient, that exhibited relatively high levels of PRL-3 expression (Fig. 4, A and B). Five other lines that expressed somewhat lower levels of PRL-3 were also identified; each of these was derived from a CRC that had metastasized to lymph nodes or to the liver (Fig. 4B and data not shown). In contrast, PRL-3 expression, as assessed by Northern blotting, was very low or absent in normal colonic epithelium.

Subcellular Localization of PRL-3. Previous studies have shown that PRL-3 protein appeared to be either membrane associated or nuclear (10). However, these studies were complicated by the extensive similarity between PRL-1, PRL-2, and PRL-3 and the consequent difficulty in establishing the specificity of the antibodies used. To address the localization of PRL-3 in metastatic cancer cells, we first developed a new set of antibodies to the PRL-3 protein. Polyclonal antibodies were generated through immunization of rabbits with a PRL-3 COOH-terminal peptide that was partially divergent among the three PRL family members. Monoclonal antibodies were generated through immunization of mice with the entire PRL-3 protein produced as glutathione S-transferase or His(6)-fusion proteins in bacteria.

To test the specificity of these antibodies, we also generated mammalian expression vectors for each of the PRL-3 family members and used them to transfect HCT-116 cells. The PRL-3 expression vectors were HA-tagged at their NH$_2$ terminus to ensure equal levels of proteins in the transfected cell extracts (Fig. 5A). Western blotting performed with the various antibodies described above showed that the polyclonal antibody displayed specific reactivity with PRL-3 but not with PRL-1 or PRL-2 (Fig. 5A). Similarly, of 13 different monoclonal antibodies reactive with PRL-3, 1 (mAb MA no. 14) reacted with PRL-3 and PRL-2 and did not react with PRL-1. We first used these antibodies to determine whether PRL-3 protein was expressed at the expected levels in extracts of the CRC cell lines described above. We found that the levels of PRL-3 protein were consistent with the levels of PRL-3 mRNA, with the highest levels of protein found in cell line V9P (Fig. 5B).

We then used the anti PRL-3 antibodies to study the intracellular distribution of PRL-3 in CRC cells. Staining of PRL-3-transfected cells with the MA no. 14 showed that PRL-3 expression was largely confined to the cytoplasmic membrane, consistent with the predicted prenylation (Fig. 6). Similar experiments performed with an antibody to E-cadherin confirmed membrane expression of PRL-3 (data not shown). No staining was evident in cells with undetectable levels of PRL-3 mRNA or protein, as assessed by Northern and Western blots, respectively.

DISCUSSION

Several observations made in this study are important for understanding the potential role of PRL-3 in metastasis. PRL-3 was previously shown to be expressed in cells immunomagnetically purified from cancers using the BerEP4 antibody (7). However, it was impossible to be certain that the expression of PRL-3 was derived from the neoplastic cells in these fractions; it was conceivable, for example, that other nonneoplastic cells express the cell surface protein recognized by the BerEP4 antibody used for positive selection or were tightly bound to neoplastic cells that did express this protein. The in situ hybridization results presented here confirm the elevated expression of PRL-3 in metastases and prove that the great majority of this expression is derived from the neoplastic epithelial cells within the metastatic lesions. A relatively minor portion of the expression within metastases was derived from the tumor vasculature. The signal within the vasculature was localized both to the endothelial cells and to the surrounding smooth muscle cells of the larger vessels. Interestingly, very little PRL-3 expression was observed in the vasculature of nonmetastatic CRC or normal tissues. These in situ observations are consistent with serial analysis of gene expression data, indicating that PRL-3 is expressed in neoplastic but not normal endothelial cells, as well as in metastatic cancer cells (14).

The genomic FISH results show that increased copy
numbers of PRL-3 were present in 45% of the liver metastatic lesions but rarely in nonmetastatic primary lesions. This result is consistent with the idea that part of the increased PRL-3 expression is derived from the increased number of PRL-3 genes in the cell. However, this cannot be the whole story because elevated PRL-3 expression is observed in a much higher fraction (95%) of metastases than is increased PRL-3 gene copy numbers. Nevertheless, it is possible that the increased copy number of PRL-3 accounts for a portion of the increased expression in a subset of tumors.

We previously identified a small amplicon containing the PRL-3 gene in a fraction of metastases [3 of 18 metastases analyzed, unpublished data and (7)]. In these cases, there was >10-fold amplification of the gene (>20 copies/cell). Interestingly, we could not identify such amplifications through FISH. Although increased copy numbers of PRL-3 were observed in 45% of the cancers, these were always associated with increases of a large part of the telomeric region of chromosome 8q, encompassing ~12 Mb, including c-myc and PRL-3, rather than localized to the PRL-3 gene itself. It is statistically possible that the cases we analyzed did not contain high level amplifications because these events are not common. However, we were able to perform FISH on one metastasis in which quantitative PCR results, as well as Southern blot hybridizations, demonstrated PRL-3 amplification, and only two signals for PRL-3/nucleus were observed in this case. One explanation for these results is that the small amplicon in these tumor cells, containing only 40–50 Kb of sequence, was arranged in tandem form on chromosome 8q24.3 and therefore might not be resolvable as separate signals. Regardless, the new data are consistent with our previous data and definitively show that classic gene amplification is not the general cause of PRL-3 overexpression in CRC metastases.

Our Northern blotting results on cancer cell lines were consistent with previous quantitative PCR data showing that PRL-3 was expressed in metastatic cancer cells but not in earlier stages of tumorigenesis. However, quantitative PCR experiments showed that the level of PRL-3 expression in the metastatic cancer cell lines was still significantly less than that generally observed in metastatic cancer cells in vivo (data not shown). This agrees with previous data showing that the gene expression patterns in cancers in their natural setting can be considerably different from those of cell lines grown in artificial environments (17).

In situ hybridization also revealed that PRL-3 was expressed in nearly all CRC metastases analyzed, whether these lesions were located in the liver or other tissues (Figs. 1 and 2). In contrast, liver metastases derived from cancers which...
did not originate in the colon or rectum did not express PRL-3 at detectable levels. These results have two important implications. First, they demonstrate that the process of metastasis is associated with cell-type-specific gene expression. The earlier phases of tumorigenesis have been associated with cell-specific patterns of gene expression or mutation (18–20). However, this point has not been previously established for the late stages of tumorigenesis. It is important to note that PRL-3 is not expressed in normal colorectal epithelium or in nonmetastatic CRCs so that the expression of PRL-3 in metastases does not simply reflect the expression of the tissue of origin. Second, our results show that PRL-3 expression is not the result of residence of the metastatic cells in the liver. One might have imagined that the liver microenvironment induces the specific expression of PRL-3 in the same way as regenerating liver induces the expression of PRL-1 in hepatocytes (11, 21). However, the new in situ data demonstrating PRL-3 expression in metastases to the lung, brain, and ovary exclude this possibility.

One possibility is that PRL-3 expression is required for the earlier phases of the metastatic process such as those required for invasion into the lymphatics or vasculature. The cell lines, expression vectors, and antibodies described here should considerably facilitate future studies to address this and related questions.

Note added in proof: Zeng et al. have recently reported that PRL-3 expression promotes cell migration, invasion, and metastasis in experimental systems (22).

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References

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