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PCBP1 Suppresses the Translation of Metastasis-Associated PRL-3 Phosphatase

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SUMMARY

Overexpression of phosphatase of regenerating liver (PRL)-3 is associated with the progression of diverse human cancers. We show that the overexpression of PRL-3 protein is not directly associated with its transcript levels, indicating the existence of an underlying posttranscriptional regulation. The 5′ untranslanted region (UTR) of PRL-3 mRNA possesses triple GCCCAG motifs capable of suppressing mRNA translation through interaction with PolyC-RNA-binding protein 1 (PCBP1), which retards PRL-3 mRNA transcript incorporation into polyribosomes. Overexpression of PCBP1 inhibits PRL-3 expression and inactivates AKT, whereas knockdown of PCBP1 causes upregulation of PRL-3 protein levels, activation of AKT, and promotion of tumorigenesis. An inverse correlation between protein levels of PRL-3 and PCBP1 in human primary cancers supports the clinical relevance.

INTRODUCTION

Cancer metastasis is the major cause of cancer mortality. Despite this, metastasis remains the most poorly understood aspect of human malignancy (Hanahan and Weinberg, 2000). Phosphorylation and dephosphorylation are major regulatory events affecting the functional activities of diverse proteins that modulate cellular processes leading to cancer progression. Although considerable attention has centered on protein kinases in cancer development, the role of protein phosphatases in cancer remains an underexplored area.

The first phosphatase induced in regenerating liver (PRL-1) was identified in 1994 (Diamond et al., 1994). Subsequently, we identified two more PRL-family members, PRL-2 and PRL-3 (Zeng et al., 1998). Among the 107 human protein tyrosine phosphatases (PTPs), PRL-1, PRL-2 and PRL-3 represent an oncogenic subgroup of the PTP-family with a unique C-terminal prenylation motif (Alonso et al., 2004). The three PRL-members represent an intriguing group of proteins that have emerged as potential biomarkers for assessing tumor aggressiveness and useful therapeutic targets in a variety of cancers (Bessette et al., 2008; Stephens et al., 2005).

Global gene expression profiling of metastatic colorectal cancer (CRC) in comparison with primary cancers and normal colorectal epithelium revealed that PRL-3 was the only gene consistently overexpressed in all 18 metastatic colorectal

Significance

Metastasis is the most malevolent aspect of cancer. Upregulation of PRL-3 causes metastases, but the mechanism regulating PRL-3 overexpression is poorly understood. Our identification of PCBP1 as an upstream regulator of PRL-3 translation reveals a molecular mechanism responsible for the overexpression of PRL-3 in cancer. The inverse correlation between PCBP1 and PRL-3 protein levels in several human cancers suggests that PCBP1-mediated PRL-3 regulation is of physiological and clinical relevance. The axis of PCBP1 – PRL-3 – AKT may be a pathway in controlling cancer progression. The finding of PCBP1 as a tumor suppressor is highly significant as a similar mechanism may be regulating other cancer genes.

cancers (Saha et al., 2001). The mRNA expression of PRL-3 was elevated in nearly all metastatic lesions derived from CRCs, regardless of the site of metastasis (Bardelli et al., 2003). Since then, PRL-3 has attracted the most attention among the three members. PRL-3, also known as PTP4A3, is a retroviral tagged cancer gene (http://rtcgd.abcc.ncifcrf.gov/mm9/index.html). PRL-3 expression is implicated in ovarian cancer growth (Polato et al., 2005) and might also play a significant role in invasion and metastasis of gastric carcinoma (Miskad et al., 2004). Overexpression of PRL-3 correlates negatively with the prognosis of liver cancers (Peng et al., 2004). By northern hybridization analysis, PRL-3 mRNA was shown to be upregulated in almost all 27 human liver carcinoma samples examined as compared to normal liver samples (Wu et al., 2004). The level of PRL-3 protein in primary colorectal tumors has prognostic significance in predicting the development of liver and lung metastases (Kato et al., 2004). PRL-3 expression was higher in bone marrow (BM) plasma cells from multiple myeloma patients with newly diagnosed monoclonal gammopathies than in plasma cells from healthy donors (Fagerli et al., 2008). Critical dose-dependent effects of PRL-3 expression in both positive and negative regulation of cell-cycle progression were highlighted in a recent report (Basak et al., 2008), which provided insight into PRL-3's role in cancer progression, emphasizing the importance of proper modulation of PRL-3 protein levels to affect its role in cell cycle regulation. PRL-3 expression might be coordinately regulated at transcriptional-translational levels, and in a temporospatial-dependent manner.

Increasing evidence suggest that PRL-3 is likely a multitasking phosphatase involved in multiple steps of cancer progression. PRL-3 overexpression was sufficient to promote cell migration, invasion, and metastasis (Zeng et al., 2003). PRL3 promotes cell invasion and proliferation by the downregulation of Csk leading to Src activation, which initiates a number of signal pathways culminating in the phosphorylation of ERK1/2, STAT3, and $p130^{Cas}$ (Liang et al., 2007). PRL-3 downregulates PTEN expression and signals through PI3K to promote epithelial-mesenchymal transition (EMT) (Wang et al., 2007a). Endothelial cell recruitment and new blood vessel formation both play central roles in tumor progression and cancer metastasis. Importantly, PRL-3 could initiate tumor angiogenesis by recruiting endothelial cells in vitro and in vivo (Guo et al., 2006). Despite its multiple tasks in triggering cancer metastasis, little is known about the molecular mechanisms underlying the regulation of PRL-3 expression in physiological or pathological conditions.

Because an excess of PRL phosphatase activity is clearly a key alteration contributing to the acquisition of metastatic properties in tumor cells, understanding how PRL-3 is regulated will be essential to its exploitation as a therapeutic target to retard cancer progression. Here, we investigate the translational regulation of PRL-3 by the poly(C)-binding protein 1 (PCBP1 also known as hnRNPE1), which is a member of the hnRNP family of RNA- and/or DNA-binding proteins (Makeyev and Liebhaber, 2002). PCBP1 is known to participate in the regulation of RNA transcription, pre-mRNA processing, maturation, and mRNA export. PCBP1 has been reported to play crucial roles in a broad-spectrum of transcriptional and translational events (Huo and Zhong, 2008), but its molecular mechanism in cancer development and progression remains to be defined.

Table 1. PRL-3 Protein Expression in Human Cancers

Types of Cancer	PRL-3$^+$/Total Samples	PRL-3$^+$ %
Colon	62/301	20.6%
Breast	22/173	12.7%
Lung	45/155	29.0%
Pancreas	42/126	33.3%
Squamous	26/69	37.7%
Prostate	5/53	9.4%
Brain	2/20	10.0%
Esophagus	5/18	27.8%
Stomach	3/18	16.7%
Cervix	3/15	20.0%
Bladder	3/14	21.4%
Liver	2/14	14.3%
Ovary	1/12	8.3%
Kidney	1/12	8.3%
Skin	3/8	37.5%
All cancer types	225/1008 in total	22.3% in average

The percentages of PRL-3-positive cancers as detected with immunohistochemistry are summarized.

RESULTS

PRL-3 Protein Is Upregulated in Many Types of Human Cancer

To expand previous observations that upregulation of PRL-3 correlates with metastasis of human cancers, we used two specific PRL-3 mouse monoclonal antibodies (mAbs) (clones 223 and 318) (Li et al., 2005) to analyze >1000 multiple human cancer samples for PRL-3 protein expression by immunohistochemistry (IHC). The percentages of PRL-3 positive cancer types are summarized in Table 1. PRL-3 protein levels were elevated in an average of 22.3% of cancer samples (n = 1008). Selected images were shown in Figure 1A. With six human tissue samples (five tumors and one normal), a full western blot with PRL-3 mAb (clone 318) shows that PRL-3 protein is expressed in three tumor samples (Figure 1B).

PRL-3 mRNA and Protein Levels Are Not Strictly Correlated in Cancer Cell Lines

To study how PRL-3 expression is regulated, we analyzed its expression in 12 cancer cell lines. The results showed that the PRL-3 mRNA levels in these cell lines were comparable (Figures 2A and 2B). However, PRL-3 protein was only detected in a subset of these cancer cell lines by using two different PRL-3 mAb detecting different epitopes (Figures 2C and 2D). These results indicate that regulation of PRL-3 levels not only occurs at the transcription level (Saha et al., 2001) but may also present at the translational level.

GC-Rich Motifs in the 5′ UTR of PRL-3 mRNA Negatively Regulate the Luciferase Reporter

We searched the expressed sequence tags (EST) database and found that PRL-3 mRNA has a relatively long 5′ UTR (855 b) and 3′ UTR (540 b) compared to its coding region (519 b) (Figure 3A,

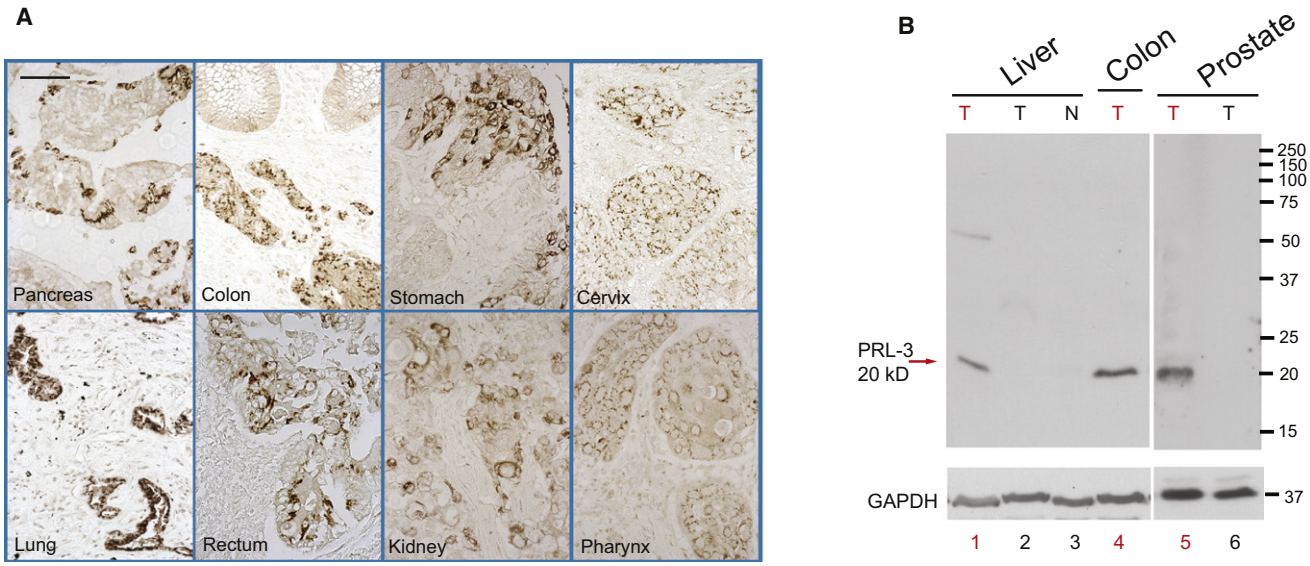

Figure 1. PRL-3 Protein Is Overexpressed in Human Cancers

(A) PRL-3 mAbs (clone 223 and clone 318) were used to detect PRL-3 protein expression in multiple human cancer samples. PRL-3-positive signals (DAB chromogen in brown) were mainly detected at the plasma membrane and the Golgi- or endosomal-like subcellular structures in the cytoplasm. Selected images of PRL-3-positive cancer samples are shown. The scale bar represents100 μm.

(B) A full western blot using PRL-3 mAb (clone 318) showed that PRL-3 protein was expressed in three of five tumor samples. T, tumor; N, normal, Ts in red are PRL-3-positive tumors.

the mRNA sequence provided in Figure S1A available online). Given that PRL-3 mRNA and protein levels are not correlated in cancer cell lines (Figure 2), we hypothesized that the 5′ UTR (or the 3′ UTR) of PRL-3 mRNA might harbor some *cis*-regulatory elements affecting PRL-3 translation. We then linked 2.4 Kb of the upstream genomic sequence (assumed to contain promoter and enhancer sequences) of the PRL-3 gene to a series of 3′ end deletions of the 5′ UTR, followed by the luciferase reporter to make eight constructs (Figure 3B, a–h). The eight constructs were then respectively transfected into the human colorectal cancer cell line DLD-1, which expresses the PRL-3 mRNA but not the protein (Figure 2, lane 3) making it a suitable system to study the regulation of PRL-3 translation. Seven constructs (Figure 3C, a–g) showed relatively low luciferase activities. Importantly, when the 5′-GCCCAG-3′ sequence at position +49 to +55 was deleted (Figure 3C, h), there was a robust increase (5- to 6-fold) in luciferase activity. This was independent of mRNA level as RT-PCR showed similar luciferase mRNA abundance after normalizing to Renilla for all the eight constructs (quantitative mRNA abundance shown in Figure S1B). Intriguingly, the same GC-motif was also found at two more positions in the 5′ UTR: +72 to +77, and +155 to +160 (Figure S1A, underlined sequences). This raised the question of whether the three GC-motifs play an equal role in modulating PRL-3 repression in a dose-dependent manner. We found that the translational repression activity is not proportional to the copy numbers of GC-motif as we did not see significant difference in luciferase activities among constructs harboring with one, two, or three GC-motifs (Figure 3C, a–g). A single GC-motif at position +49 to +55 was sufficient to suppress luciferase expression (Figure 3C, g).

To confirm this was a general phenomenon, we again respectively transfected the same eight constructs (Figure 3B, a–h) into another human colorectal cancer cell line HCT-116 that

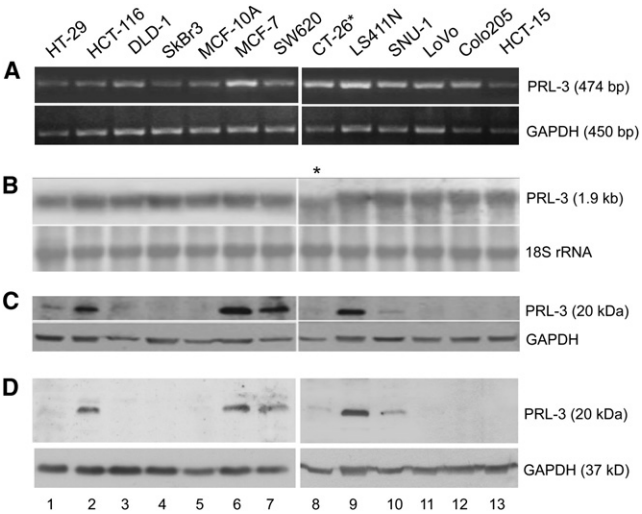

Figure 2. PRL-3 mRNA and Protein Levels Are Not Strictly Correlated in Cancer Cell Lines

(A and B) Total RNAs were extracted from 12 human cell lines and 1 mouse cell line-CT26*. The expression of PRL-3 mRNAs was assayed by semiquantitative RT-PCR using GAPDH mRNA as a control (A) or by Northern blot analysis with 18S rRNA as a loading control (B). Mouse PRL-3 mRNA (*) is smaller than human PRL-3 mRNA.

(C and D) The levels of PRL-3 protein expression in the indicated cell lines were assessed by immunoblot analysis with PRL-3 mAb (C) clone 223 or (D) clone 318.

expresses both PRL-3 mRNA and protein (Figure 2C, lane 2). Compared with construct g (1-GC-motif; Figure 3B), a 2.5-fold induction of enzyme activity was observed for construct h (0-GC-motif; Figure 3D) although RT-PCR showed the eight constructs (Figure 3B, a–h) expressed similar levels of mRNA in HCT-116 cells (quantitative mRNA abundance shown in Figure S1C). The lesser induction (2.5-fold) in HCT-116 cells compared to higher induction (5- to 6-fold) in DLD-1 cells indicates that the degree of repression could be cell-type specific. These results suggest that the GC-motif is at least partly responsible for the translational repression of PRL-3 seen in DLD-1 and to a lesser extent, in HCT-116 cells. To exclude the possibility that deletion of putative *cis*-acting elements may affect mRNA stability, we looked at the levels of luciferase mRNA in the presence of actinomycin D, which prevents the synthesis of new mRNAs. By quantitative real-time RT-PCR (qRT-PCR), although luciferase transcript levels for constructs a–h (see Figures S1D and S1E) showed no major difference in both DLD-1 and HCT-116 cells, a specific increase in luciferase activity was seen in construct h (Figures S1F and S1G) in DLD-1 and HCT-116 cells. The lack of correlation between mRNA levels and luciferase activity among these eight constructs suggested that the increase in luciferase activity was not due to changes in mRNA stability. The above results illustrate that the 6-base GC-motif negatively regulates translation.

GC-Rich Motifs in the 5′UTR of PRL-3 mRNA Negatively Regulate PRL-3 Protein Translation

To investigate whether the coding sequence of PRL-3 mRNA is also subject to translational regulation by its 5′ UTR, we replaced the above mentioned luciferase open reading frame (ORF) with the PRL-3 coding sequence to make another set of eight corresponding PRL-3 mini-genes (Figure 3E, a′–h′) and then transfected them respectively into the DLD-1 cell line. By western blot, we found a minimum of two GC-motifs (Figure 3F, construct f′) were required to completely suppress exogenous PRL-3 protein expression. A single copy of the GC-motif (Figure 3F, construct g′) showed partial suppression of PRL-3 translation compared with complete loss of suppression in construct h′ that contained no GC-motifs. This suggested that the GC-motifs were responsible for the regulation of PRL-3 translation in DLD-1 cells. We then transfected the eight PRL-3 mini-genes (Figure 3E, a′–h′) into HCT-116 cells. Consistent with observations in Figure 3F, a clear increase in PRL-3 level was observed when the last GC-motif was removed (Figure 3G, h′ with red circle), which was probably due to the translation of exogenous PRL-3. The quantitative mini-PRL-3 mRNA abundance is shown in Figures S1H and S1I, respectively, for DLD-1 and HCT-116 cells. The rate of translation efficiency of PRL-3 differs between cell types with DLD-1 showing a higher rate of repression compared to HCT-116 colon cancer cells.

PCBP1 Binds Specifically to the GC-Rich Motif

To study how the translational repression was rendered by the 6-base GC-motif *cis*-element, we predicted the secondary structure of the 5′ UTR of human PRL-3 mRNA with or without the six-base GC-motif by using MFOLD (http://mfold.bioinfo.rpi.edu/cgi-bin/rna-form1.cgi). There was no difference in secondary structure with or without the presence of the six-base sequence (data not shown). We therefore anticipated that a *trans*-acting factor might act on these six-base GC-motifs. To explore this possibility, we synthesized two biotin-conjugated single strand RNA probes mimicking part of the 5′ UTR of PRL-3 mRNA: one with the GC-motif (RNA-GC+) and another without the GC-motif (RNA-GC−). Cytoplasmic protein extract from DLD-1 cells was incubated with either RNA-GC+ or RNA-GC− and then the RNA-protein complexes formed were affinity pulled-down using streptavidin beads. Eluted proteins were resolved on a gradient SDS-PAGE gel. A specific protein band pulled down only by RNA-GC+ is indicated with a red arrow (Figure 4A). The band was excised for mass spectrometry analysis and revealed to be PolyC-RNA-binding protein 1 (PCBP1). To validate the specific binding of PCBP1 to the GC-motif, we incubated purified PCBP1 with biotin-labeled RNA-GC+ or RNA-GC− probes in a RNA gel mobility shift assay. As shown in Figure 4B, PCBP1 bound only to RNA-GC+ (but not to RNA-GC−) in a dose-dependent manner; causing a band shift indicated with arrowhead (Figure 4B). These results show that PCBP1 specifically binds to RNA that contains the GC-motif.

PCBP1 Regulates PRL-3 Protein Levels and AKT Activity

To examine the role of PCBP1 in regulating PRL-3 expression, we assessed the expression of both proteins in four colon cancer cell lines. An inverse correlation between PCBP1 and PRL-3 protein expression was observed (Figure 4C). These results suggest that PCBP1 may be functioning in these cells to repress PRL-3 mRNA translation. Consistently, expression of exogenous EGFP-PCBP1 in HCT-116 and A2780 human cancer cell lines reduced their endogenous PRL-3 protein expressions (Figure 4D, lanes 2 and 4). The downregulation of PRL-3 was accompanied by the downregulation of the phosphorylated active form of AKT (pSer473). Conversely, knockdown of endogenous PCBP1 in HCT-116 (using shRNA) and in A2780 cells (using siRNA) results in a robust upregulation of PRL-3 protein levels as well as an increase in the levels of the active form of p-AKT (Ser473) (Figure 4E, lanes 2 and 4). The data supports our previous observation that overexpression of PRL-3 enhances the activity of AKT in DLD-1 cells (Wang et al., 2007a). Thus, PCBP1 negatively modulates PRL-3 translation and reduces AKT activity.

PCBP1 Affects the Efficiency of PRL-3 mRNA Translation

To understand how PCBP1 affects the translation of PRL-3 mRNA, we determined its ribosome occupancy in cells that did or did not express PCBP1. A representative distribution profile of the ribosomes is shown (Figure 5A). In HCT-116 parental cells that express low levels of PCBP1 protein but high levels of endogenous PRL-3 protein (Figure 4C, lane 2), PRL-3 mRNA can be found in the heavier polysome fractions (Figure 5B, a: 9–11, red circle), indicating it was highly translated. When PCBP1 protein was overexpressed in HCT116 cells, PRL-3 mRNA was shifted into lighter polysome fractions (Figure 5B, b: 6–8, red circle). In agreement with this observation, in DLD-1 cells that have high levels of PCBP1 protein but low levels of PRL-3 protein (Figure 4C, lane 3), the PRL-3 mRNA was found in the lighter polysome fractions (Figure 5C, a: 5–8, red circle).

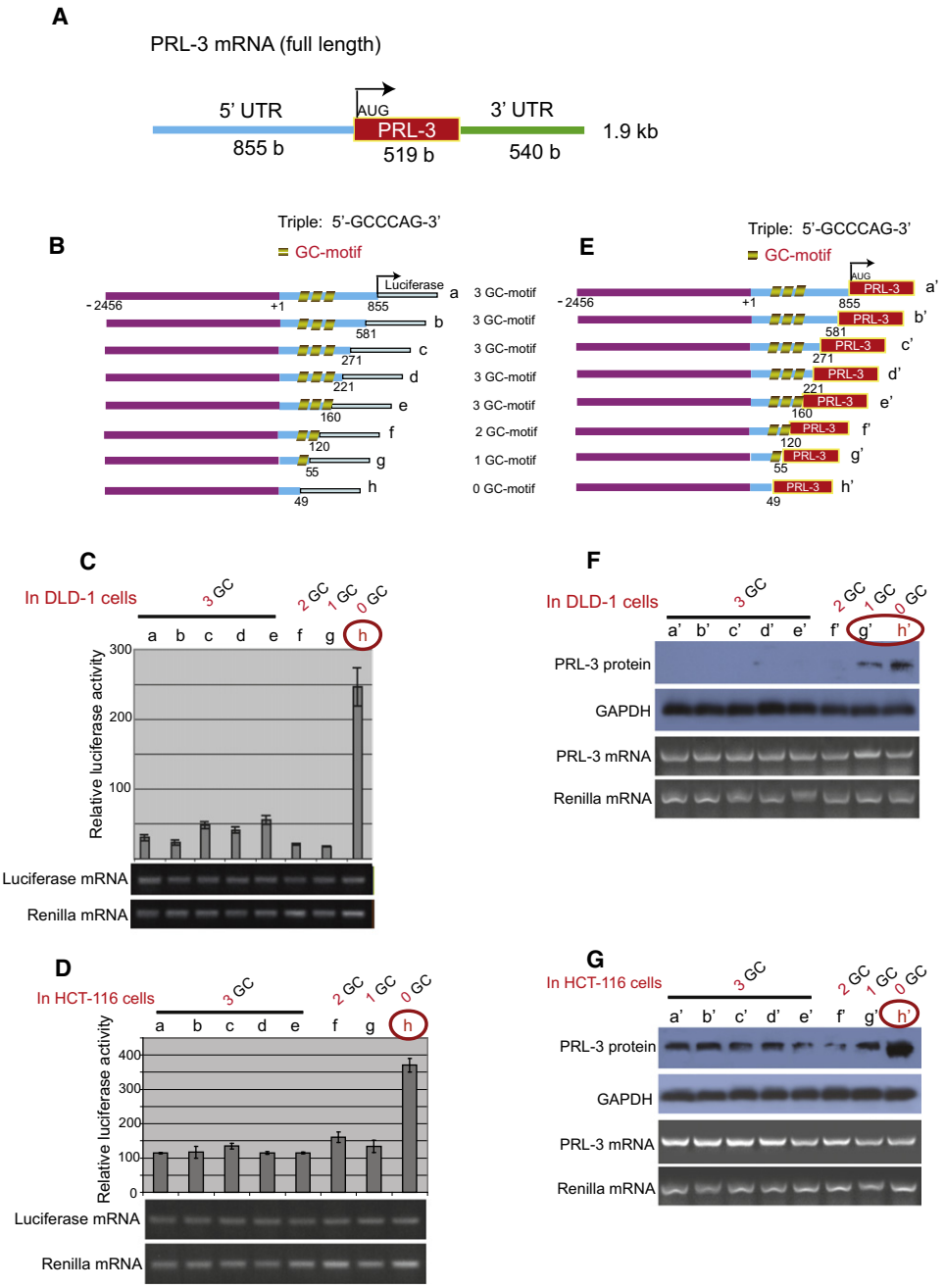

Figure 3. Identification of Three GC-Motifs, GCCCAG, at the 5′ UTR of PRL-3 mRNA

(A) A schematic diagram of human PRL-3 mRNA illustrates a long 5′ UTR (855 bases), encoding region (519 bases), and a long 3′ UTR (540 bases) (sequences shown in Figure S1A).

(B) A 2.4 kb genomic fragment (in purple) together with the first exon region corresponding to different 3′-deletions of the 5′ UTR (in blue) were inserted at the 5′ of the luciferase gene (in light blue) in the pGL3-Basic luciferase reporter vector. Constructs are designated a–h, respectively (primer sequences provided in Supplemental Experimental Procedures).

(C and D) Equal amounts of the indicated luciferase reporter plasmid DNA (a–h) were transiently cotransfected with pRL-TK encoding the Renilla luciferase as an internal normalized control into DLD-1 or HCT-116 cells respectively. Data are the mean ± standard deviation (SD) of three independent experiments; bottom panels, RT-PCR showed similar mRNA abundance among eight constructs (a–h) in DLD-1 and HCT-116 cells (for quantitative RT-PCR, also see Figures S1B and S1C).

(E) The luciferase ORF from constructs a–h was replaced with the PRL-3 coding region, the new set of constructs are designated respectively as a′–h′. The positions of the 5′ UTR deletions relative to the start site (+1) of transcription are shown. The start codon ATG is shown with an arrow, and the coding region of PRL-3 is shown in red.

(F and G) Equal amounts of the indicated PRL-3 mini-gene plasmid DNAs (a′–h′) were transiently cotransfected with pRL-TK into DLD-1 cells or HCT-116 cells.

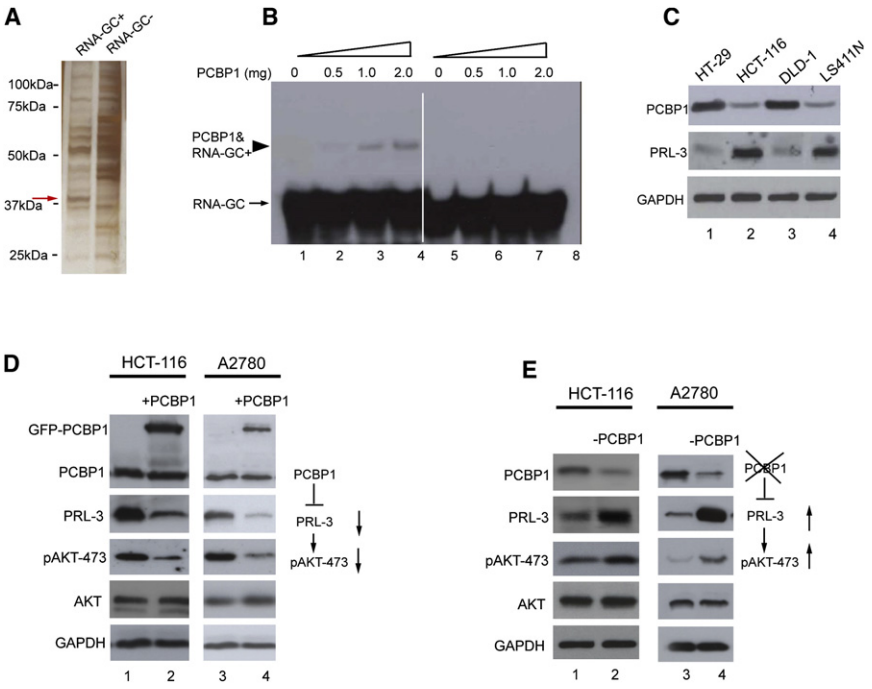

Figure 4. Identification of PCPB1 as a Binding Protein for the GC-Motif in the 5′ UTR of PRL-3 mRNA

(A) RNA affinity pull-down assay was used to identify specific GC-motif binding proteins that were then resolved on a 9%–16% SDS-PAGE gradient gel with silver stain. The red arrow indicates a band that was pulled down by the single stranded RNA bait RNA-GC+ but not by the RNA bait RNA-GC−.

(B) EMSA of PCPB1 showed that it bound specifically to RNA-GC+, but not to RNA-GC− probe in vitro. Free RNA probes (arrows) and PCPB1-RNA complex (arrowhead) are indicated. The amount of PCPB1 protein is indicated on the top of each lane. All RNA probes are labeled with biotin.

(C) Immunoblot analysis shows the levels of PRL-3 and PCBP1 protein in four different colon cancer cell lines, with GAPDH as a loading control.

(D) Overexpression of PCPB1 inhibits PRL-3 expression and reduces phosphorylation of AKT at pSer473 (lanes 2 and 4). Lanes 1 and 3 represent HCT-116 and A2780 parental cells.

(E) Knockdown of PCPB1 increases PRL-3 expression and upregulates AKT phosphorylation (lanes 2 and 4). Lanes 1 and 3 represent HCT-116 and A2780 parental cells.

The data suggest that the low PRL-3 protein levels in DLD-1 cells are due to translational repression. This level of shift in ribosomal load would account for the differences in protein level seen in HCT-116 and DLD-1 cells (Figure 4C). Importantly, knockdown of PCBP1 in DLD-1 cells resulted in a clear shift of PRL-3 mRNA localization toward heavier polysomal fractions (Figure 5C, b: 8–11, red circle), suggesting that knockdown of PCBP1 could release the inhibition of PCBP1 on PRL-3 translation. To further confirm the role of PCBP1 in the translational repression of PRL-3, we expressed luciferase reporter construct g (1-GC-motif; Figure 3B) and construct h (no GC-motif; Figure 3B) in DLD-1 cells and found that in the presence of the PCBP1 binding region-GC-motif (construct g, Figure 3B), the luciferase mRNA was found in the lighter fractions (Figure 5C, c: 5–8, red circle), similar to endogenous PRL-3 mRNA (Figure 5C, a: 5–8, red circle). In contrast, the luciferase mRNA (construct h, Figure 3B) that had no PCBP1 binding GC-region was shifted to the heavier polysomal fractions (Figure 5C, d: 8–11, red circle). These results suggest that the translation of PRL-3 protein was regulated by PCBP1 binding to its cognate GC-motif, thus the level of PCBP1 protein can directly affect the association of PRL-3 mRNA with polyribosomes. This finding leads us to propose a working model for the action of PCBP1 in regulating the translation of the PRL-3 mRNA (Figure 5D).

PCBP1 and PRL-3 Protein Levels Were Inversely Correlated in Human Cancer Samples

To verify whether our findings in cancer cell lines were of clinical relevance, we examined 47 human cancer samples (ten stomachs, ten colon, ten breast, five lung, five brain, five liver, and two prostate) and found common discordance between PRL-3 mRNA and PRL-3 protein levels (Figure 6A and Figure S2). More importantly, in agreement with our cell culture data, we found a strong inverse correlation between PCBP1 and PRL-3 protein levels in the ten stomach tumor samples (Figure 6Ab). Results from analyzing serial sections of a colon sample by various methods are presented in Figure 6B. By in situ hybridization, a PRL-3 sense probe gave no signal (Figure 6Ba), whereas an antisense probe detected PRL-3 mRNA in both normal and cancerous colon epithelia (Figure 6Bb). IHC analysis revealed that PRL-3 protein was only expressed in cancerous but not normal epithelia (Figures 6Bc and 6C), whereas PCBP1 protein was only expressed in normal but not cancerous epithelia (Figure 6Bd). Similar results were obtained by IHC analysis of 63 lung and 72 colon cancer tissue arrays in that overexpression of both PRL-3 and PCBP1 was rarely observed in the same cancer tissues or cells (summarized in Figure 6D). Taken together, these data demonstrate that PRL-3 and PCBP1 protein levels showed an inverse correlation in that PCBP1 and PRL-3 proteins are detected predominantly/respectively in normal and cancerous epithelium of tissue samples.

Overexpression PCBP1 Abrogated Tumor Formation whereas Knockdown PCBP1 Enhanced Tumorigenesis

To directly examine the function of PCBP1 in tumor progression, we knocked down endogenous PCBP1 or overexpressed exogenous PCBP1 in HCT-116 cells and then injected these cell pools into the right or left hip areas, respectively, of four nude mice to compare their ability to form tumors. Knockdown

Western blot shows the protein levels of PRL-3 and GAPDH; bottom panels, RT-PCR showed similar mRNA abundance among eight constructs (a′–h′) transfected into DLD-1 cells or HCT-116 cells (for quantitative RT-PCR; see also Figures S1H and S1I).

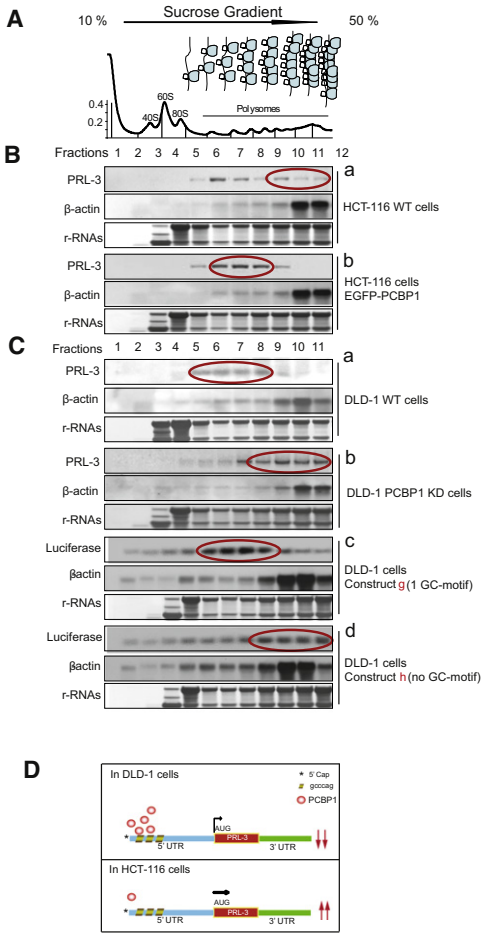

Figure 5. PCBP1 Causes PRL-3 mRNA to Shift into the Lighter Polysomal Fractions

(A) A representative distribution profile of ribosomes from the lightest to the heaviest fractions in the sucrose gradient. The marked numbers match to equivalent collected fractions. Fraction 1 represents the top (10%) of the gradient, fraction 11, the bottom (50%) of the gradient.

(B) As shown in (Ba), a significant amount of PRL-3 mRNA in HCT-116 cells is associated with the heavy polysomal fractions (9–11, red circle). As shown in (Bb), overexpression of PCPB1 in HCT-116 cells results in a shift of PRL-3 mRNA to the lighter polysome fractions (6–8, red circle). β-actin mRNA polysomal distribution is unaffected by PCBP1 protein. rRNAs are shown to demonstrate polysome integrity.

(C) As shown in (Ca), PRL-3 mRNA is associated with the lighter polysomal fractions in DLD-1 cells (5–8, red circle). As shown in (Cb), Knockdown of endogenous PCBP1 in DLD-1 cells causes a shift of PRL-3 mRNA localization toward heavier polysomal fractions (8–11, red circle). As shown in (Cc), The luciferase construct g (1-GC-motif) mRNA is associated with the lighter polysomal fractions (5–8, red circle) in DLD-1 cells. As shown in (Cd), the luciferase construct h (0-GC-motif) mRNA is predominantly associated with the heavier polysomal fractions (8–11, red circle) in DLD-1 cells. rRNA and β-actin levels are shown.

(D) Schematic model depicting how PCBP1 can negatively regulate translation of PRL-3 mRNA. When PCPB1 protein level is high, it binds to the GC-rich motifs at the 5′ UTR of PRL-3 mRNA to reduce PRL-3 translation efficiency (upper panel). When PCPB1 protein level is low, the GC-motifs are freed of PCBP1, resulting in increased PRL-3 translation (lower panel).

of endogenous PCBP1 significantly enhanced tumorigenesis (Figure 7A, left hip), whereas overexpression of exogenous PCBP1 markedly inhibited tumorigenesis (Figure 7A, right hip). To make a more quantitative comparison, we repeated the same experiment with four additional mice. The combined results from all eight mice showed that the weights of tumors formed by PCBP1 knocked-down cells were significantly heavier than the weights of tumors formed by cells overexpressing PCBP1 ($p = 0.0019$, paired t test). These results show clearly that PCBP1 acts as a tumor suppressor in vivo.

DISCUSSION

The PRL-3 phosphatase is becoming important both as a biomarker for multiple human cancers and as a therapeutic target for metastatic tumors. Consistent with earlier studies (Bardelli et al., 2003; Kato et al., 2004; Peng et al., 2004; Wang et al., 2007b), our analysis confirmed that PRL-3 protein is often upregulated in human cancers. Accumulating evidence indicates that inhibiting PRL-3 overexpression may reduce cancer metastasis. Knowledge of how PRL-3 is regulated is important to understand how PRL-3 spurs cancer progression. Currently, upstream regulators and downstream targets of PRL-3 are not well defined. In our current study, we have identified triple six-base GC-rich *cis*-elements in the 5′ UTR of the PRL-3 mRNA that are targeted by PCBP1 to mediate translational repression. The three copies of the GC-motif may serve to triple-check at translational levels. Binding of PCBP1 to the six-base GC-motifs resulted in dramatic downregulation of PRL-3 translation as demonstrated by both a decrease in ribosomal occupancy and protein levels. We found that overexpression of exogenous PCBP1 caused marked decrease in the levels of endogenous PRL-3 protein with a concomitant decrease in AKT activation, whereas knockdown of endogenous PCBP1 led to upregulation of PRL-3 protein expression and promoted AKT activation. These results support our previous data that overexpression of PRL-3 results in the activation of AKT in DLD-1 cells (Wang et al., 2007a). AKT mediates several well-described PI3K responses, including growth, metabolism, and glucose homeostasis (Manning and Cantley, 2007), and activation of AKT is known to enhance tumorigenesis. Although PRL-3 was previously shown to downregulate PTEN to influence AKT activation (Wang et al., 2007a), we did not see obvious changes in PTEN protein levels in response to the perturbation of PCBP1 levels here (data not shown), suggesting that the regulation of PTEN levels is complex. Future experiments are needed to uncover additional mechanistic aspects in the relationship between PRL-3 and PTEN. Our data suggest the existence of a PCBP1 – PRL-3 – AKT signaling pathway. PCBP1 thus functions to fine-tune PRL-3 levels by regulating the rate of translation, and keep metastatic genes such as PRL-3 under control.

These data clearly demonstrate a critical role for PCBP1 in regulating PRL-3 translation and provide mechanistic insight into how PRL-3 protein expression can be regulated at the translational level. Expression of PCBP1 does not appear to induce a complete inhibition of translation, but rather a reduction in its efficiency. Previous elegant studies have described PCBP1 functioning to inhibit 80S ribosome assembly (Ostareck et al., 2001; Ostareck et al., 1997), and it is possible that PCBP1 could

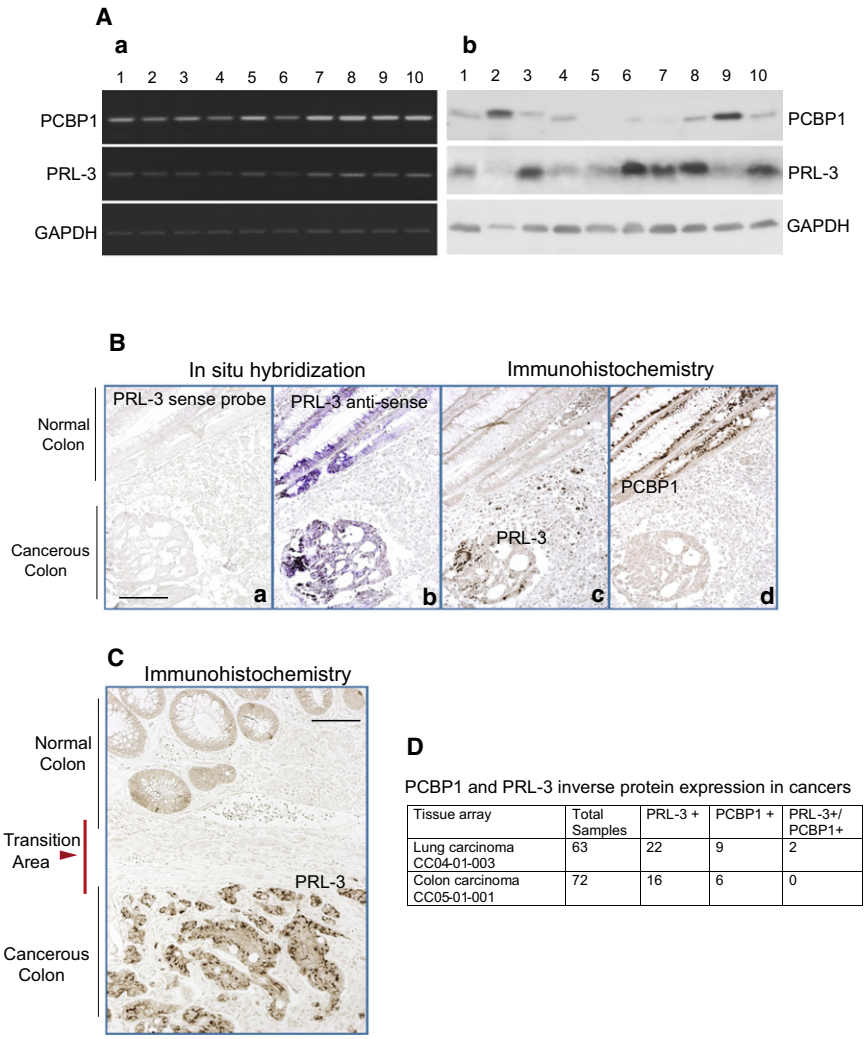

Figure 6. An Inverse Correlation between PCBP-1 and PRL-3 Protein Levels in Human Cancer Samples

(A) As shown in (Aa), RT-PCR shows PCBP1, PRL-3, and GAPDH mRNAs; in (Ab), western blot shows PCBP-1, PRL-3, and GAPDH protein levels. An inverse correlation between PCBP1 and PRL-3 protein levels is observed in eight of ten tumors (lanes: 1–3 and 6–10).

(B) Detection of PRL-3 mRNA in normal and cancerous colon tissue by in situ hybridization using a PRL-3 sense probe as a negative control (Ba) or a PRL-3 antisense probe (Bb). PRL-3 protein was detected in cancerous but not normal epithelia (Bc) and PCBP-1 protein was detected in normal but not cancerous epithelia (Bd) using immunohistochemistry. The scale bar represents 200 μm.

(C) The section showed that PRL-3 protein was expressed in cancerous but not normal epithelia. A red arrowhead indicates the transition area between cancerous and normal colon tissue. The scale bar represents 100 μm.

(D) Summary of the expressions of PCBP1 and PRL-3 protein by IHC on tissue array.

D

PCBP1 and PRL-3 inverse protein expression in cancers

Tissue array	Total Samples	PRL-3 +	PCBP1 +	PRL-3+/ PCBP1+
Lung carcinoma CC04-01-003	63	22	9	2
Colon carcinoma CC05-01-001	72	16	6	0

function in a similar manner on PRL-3 mRNA. Recently, critical dose-sensitive effects of PRL-3 expression in both positive and negative regulation of cell-cycle progression provided insight into PRL-3's role in cancer progression, emphasizing the importance of proper and precise modulation of PRL-3 protein levels during the cell cycle (Basak et al., 2008). Given such sensitivity of cells to the levels of PRL-3 protein, together with previous data on PRL-3 mRNA expression (Saha et al., 2001), we suggest that PRL-3 is under dual regulatory control at both mRNA and protein levels to ensure fidelity of its expression.

PCBP1 has been reported to be downregulated in metastatic cervical cancers (Pillai et al., 2003) and metastatic breast cancer cells (Thakur et al., 2003). Our evidence that PCBP1 can downregulate PRL-3 translation indicates that PCBP1 could be a tumor suppressor. Consistently, overexpression or knockdown of PCBP1 in HCT-116 cells led to decreased or increased tumorigenesis, respectively, which correlated with PRL-3 levels and AKT activation. Understanding how the PRL-3 gene is regulated will help us to develop strategies to block excess PRL-3 expression or inhibit its activity and thus prevent it from promoting cancer metastasis. Similar studies on the protein level of p53 (Takagi et al., 2005), folate receptor (Sun and Antony, 1996), cyclooxygenase-2 (Fiori et al., 2005), and Alzheimer's disease protease β-site APP-cleaving enzyme 1 (Lammich et al., 2004) have all demonstrated the importance of such translational regulation. We suggest that our current results will open an avenue for further study of the biological functions of PCBP1 on other genes.

The inverse correlation of PCBP1 protein levels with PRL-3 protein levels observed in several types of human cancer suggests that our mechanistic results obtained from analyzing cancer cell lines are of clinical relevance. Besides PCBP1-mediated translational suppression, PRL-3 levels can also be regulated at the mRNA expression level by gene amplification (Saha et al., 2001). It seems that multiple mechanisms may together be responsible for regulating the protein levels under both physiological and pathological conditions. Accordingly, observed upregulation of PRL-3 proteins in diverse human cancers may be due to alterations of any of these or any combination of these various mechanisms. Our results from human cancer cell lines and diverse human primary cancer samples indicate that deregulation of PCBP1-mediated translational suppression may be one of the major mechanisms responsible for PRL-3 protein overexpression in several human cancers.

EXPERIMENTAL PROCEDURES

Histopathologic Analyses with Immunohistochemistry

Human tissue samples were obtained from the National University Hospital-National University of Singapore (NUH-NUS) Tissue Repository with approval of the Institutional Review Board (IRB) of NUH-NUS for research uses. The use of all human tissue samples including commercial samples were approved

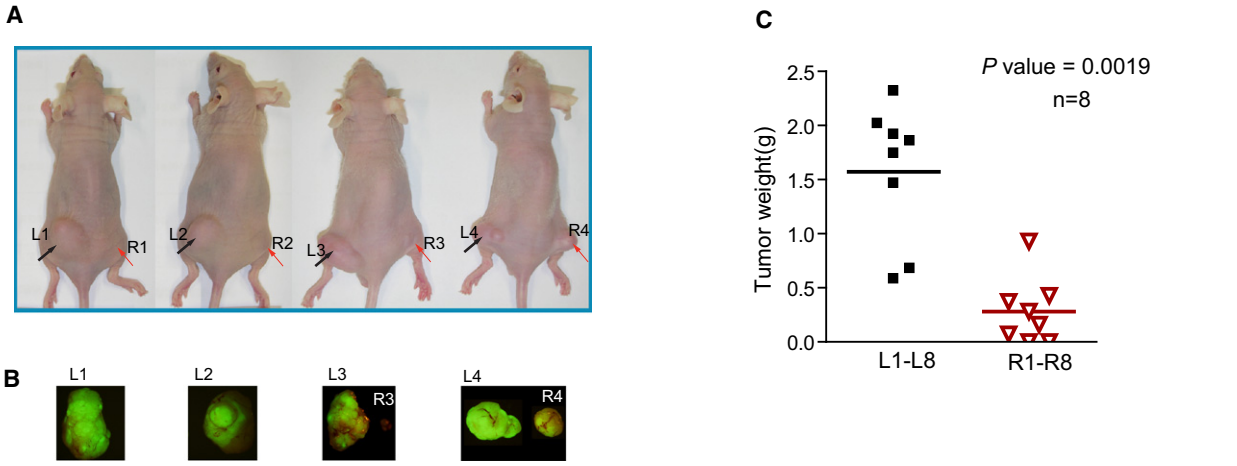

Figure 7. Knockdown of Endogenous PCBP1 Enhanced Tumorigenesis whereas Overexpression of Exogenous PCBP1 Abrogated Tumor Formation

(A) HCT-116 cells in which endogenous PCBP1 was knocked down were injected subcutaneously into the left sides (black arrows) of the hip area of nude mice, whereas HCT-116 cells that overexpress exogenous PCBP1 were injected into the right sides as indicated with red arrows. The arrows indicate the sites of injection. The tumor cells were propagated in vivo for 4 weeks.

(B) Xenograft tumors (L1–L4) were derived respectively from the left sides of nude mice 1–4, respectively, whereas xenograft tumors (R3–R4) were derived from the right sides of nude mice 3 and 4, respectively. Tumors were photographed.

(C) The same experiment was repeated with four additional mice. The graph illustrates the distribution of xenograft tumor weights between two groups.

by Institutional Review Board (IRB) of the Institute of Molecular and Cell Biology. We investigated PRL-3 protein expression on a large collection of human cancer samples. The majority of the tissues were purchased from Cybrdi (Rockville, MD; http://cybrdi.com/index.php). These include a major solid tumor tissue array (CC00-01-006), squamous cell carcinoma (CC00-01-009), lung carcinoma (CC04-01-003 and CC04-01-006), colon adenocarcinoma (Grade I ~III) with normal controls (CC05-01-001), breast carcinoma (CC08-02-001), and pancreatic carcinoma (Duct adenocarcinoma/islet cell carcinoma/mucinous carcinoma) with normal controls (CC14-01-001). Human low density prostate tissue array (TS42081004) was purchased from InnoGenex (San Ramon, CA). In addition, some formalin-fixed and paraffin-embedded surgical specimens of primary multiple cancer samples were collected from the archives of the pathology department of the Henan Medical Hospital and National University Hospital in Singapore. We used Dako EnVision Systems K 1395 (Dako, Carpinteria, CA) to perform IHC analysis (Supplemental Experimental Procedures).

Cancer Cell Lines and Cell Culture

Colon cancer cell lines include the following: HCT-15 (CCL-225), HT-29 (HTB-38), HCT-116 (CCL-247), DLD-1 (CCL-221), LS411N (CRL-2159), LoVo (CCL-229), SW620 (CCL-227), CT-26 (CRL-2638), Colo205 (CCL-222), breast cancer cell lines: SkBr3 (HTB-30), MCF-7 (HTB-22), MCF-10A (CRL-10317, immortalized cell line), and a stomach cancer cell line: SNU-1(CRL-5971) were obtained from ATCC (Manassas, Virginia). Cells were maintained in the appropriate media with 10% heat-inactivated fetal bovine serum and 1% antibiotics at 37°C with 5% CO_2.

Northern Blot Analysis and Reverse Transcription-PCR

Total RNAs were isolated with the RNeasy mini kit (QIAGEN) and separated on a 1.5% formaldehyde-denaturing agarose gel before being transferred onto nitrocellulose membranes. Northern blotting was carried out using DIG-labeled antisense probes corresponding to nucleotides 840–1210 of PRL-3 (accession number NM032611), nucleotides 741–989 of β-actin (accession number X03672), and nucleotides 550–1537 of luciferase (accession number NM001101.3). The bound probes were visualized by incubation with alkaline phosphatase-conjugated anti-DIG antibodies, washed, and exposed to film with CDP-Star substrate (Roche Diagnostics). For amplification of the human PRL-3 cDNA fragment (474 bp) and GAPDH fragment (450 bp) with PCR primers described in the Supplemental Experimental Procedures, the PCR products were analyzed with a 1.3% agarose gel (Figure 3), scanned with GS-800 Calibrated Densitometer (BioRad), and the band images were further analyzed with Quantity One Software (BioRad). Quantitative RT-PCR was shown in Figure S1.

Immunoblotting

Cells were harvested and lysed in cell lysis buffer (50 mM Tris-HCl [pH 7.4], 250 mM NaCl, 0.1% NP-40, 5 mM EDTA, 2 μg/ml leupeptin, 2 μg/ml aprotinin, 4 mM Prefabloc SC, and protein inhibitor cocktail). The cell lysates were subjected to SDS-PAGE. PRL-3 monoclonal antibodies (clones 223 and 318) were used. The detailed immunoblotting procedures had been described previously (Li et al., 2005). PCPB1 and GAPDH antibodies were obtained from Santa Cruz; phospho-AKT (Ser473) and total AKT antibodies were purchased from Cell Signaling Technology.

Plasmid Constructions

For obtaining the promoter region of human PRL-3, genomic DNA was extracted from DLD-1 cells. Primers 5′-GGCCGACGCGTGGCAGGGCAGAGCCACATAG-3′ with a Mlu I restriction site and 5′- CGGCGCTCGAGGGCGCCTCCCGACGGGCCC-3′ with an Xho I site were used to amplify the 3.3 Kb genomic fragment preceding the translation initiation site ATG of the PRL-3 coding sequence. This 3.3 Kb fragment contains two regions: the first corresponding to the upstream promoter sequences (2.4 kb), and the second corresponding to the exon (855 bp) contains the 5′UTR of the PRL-3 mRNA. The 3.3 Kb fragment was doubly digested with MluI and XhoI and subcloned into the pGL3-Basic vector (Promega), which is located upstream of the firefly luciferase (reporter gene) to form pGLR3-luciferase (construct −2456–+855). Primers (Supplemental Experimental Procedures) were used to generate constructs (a–h, Figure 3B). Furthermore, forward primer: 5′-GGCCATCCATGGCTCGGATGAACCGCCCGGCCCCGG-3′ (with NcoI site underlined) and reverse primer: 5′-GGCCATTCTAGACTACATAACGCAGCACCGGGTCTTGTG-3′ (with Xba I site underlined) were used to obtain a PCR fragment for PRL-3 coding region, which was then used to replace the luciferase reporter gene as constructs a′–h′ (Figure 3E).

RNA Affinity Binding Proteins and Mass Spectrometry Analysis

Two different HPLC-grade single-stranded PRL-3 RNA probes corresponding to positions +35 to +65 of 5′ UTR were synthesized, namely RNA-GC+ with the GC-motif at position +49 to +55: 5′-GAGUGGGCCACGGGGCCCAGCCCUAAGCAC-biotin-3′, and RNA-GC- without the GC-motif: 5′-GAGUGGGCCA

CGGGCCCUAAGCAC-Biotin-3′ (Sigma-Proligo). Cytoplasmic cell extracts were isolated from 1 × 10^7 DLD-1 cells and RNA affinity capture was subsequently carried out with streptavidin magnetic MicroBeads (Miltenyi Biotec) as described previously (Takagi et al., 2005). Elutes were separated on a 9%–16% gradient SDS-PAGE and visualized by silver staining. Selected bands that specifically associated with RNA-GC+ (but not RNA-GC-) were excised for LC-MS analysis.

RNA-Electrophoretic Mobility Shift Assays

The encoding region of PCBP 1 was amplified from a cDNA library of DLD-1 cells by RT-PCR, using the primers: 5′-GGATCCCCGGGAATGGATGC CGGTGTGACTGAAAGTGG-3′ and 5′-CCGGTAAGCTTCTAGCTGCACCC CATGCCCTTCTC-3′ (with XmaI and HindIII restriction sites). The PCR fragment was doubly digested with XmaI and HindIII and inserted into the respective sites of pGEX-KG vector to make GST-PCPB1 fusion protein. The insert was confirmed to be free of mutations by sequencing. The purified GST-PCBP 1 fusion protein was cleaved by thrombin (Sigma) to remove the GST tag. RNA-GC+ and RNA-GC− were respectively incubated with the indicated amount of purified PCBP1 in the presence of excess yeast tRNA at 30°C for 20 min. The binding complexes were resolved on a 15% native polyacrylamide gel and then transferred to nitrocellulose membrane (Amersham). The biotin-containing bands were visualized by incubation with streptavidin-HRP conjugates for 1 hr, then washed three times and exposed to film with HRP luminescent substrates (Pierce).

Knockdown or Overexpression of PCBP 1

A2780 cells were transfected with either siRNA targeting human PCPB1 (sc-43843; Santa Cruz Biotechnology) or mock siRNA using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. We also constructed 2 HuSH29-mer shRNAs (5′-TCACCGAGTGTGTCAAGCAGATTTGCCTG-3′ and 5′-TGGTAGGCAGGTTACTATCACTGGCTCTG-3′) in p-GFP-V-RS vector (TG310588, OriGene Technologies, Rockville, MD) to stably knockdown PCPB1 in DLD-1 and HCT-116 cells. For overexpression studies, HCT-116 or A2780 cells were transfected with pEGFP-PCBP1 plasmids, in which PCBP1 is fused to the C-terminus of EGFP protein in a pEGFP-C1 vector (Clonetech). Twenty-four hours after seeding, the cells were harvested and lysed in cell lysis buffer (as noted above) for western blot analysis to examine the expression levels of various proteins.

Measuring the Distribution of mRNA in Ribosome Fractions

For polyribosome isolation (Takagi et al., 2005), cells were incubated with 90 μg/ml cycloheximide (Sigma) for 10 min followed by trypsinization and harvest. Twenty million cells were resuspended in RSB (20 mM Tris-HCl [pH 7.4], 20 mM NaCl, 30 mM $MgCl_2$, RNasin, 100 μg/ml Heparin, and 5 μg/ml cycloheximide). An equal volume of lysis buffer (1.2% Triton X-100, 1.2% deoxycholate) was added and and then incubated on ice for 5 min. The nuclei and cell debris were removed by centrifugation for 3 min at 12,000 rpm. The supernatant was then diluted with an equal volume of dilution buffer (25 mM Tris-HCl [pH 7.4], 25 mM NaCl, 25 mM $MgCl_2$, 0.05% Triton X-100, and 500 μg/ml heparin) and 400 μl of the extract was loaded onto 11.5 ml linear 10% to 50% sucrose gradients and centrifuged at 36,000 rpm for 2 hr in a SW41 rotor (Beckman). Twelve (1 ml each) fractions were collected with the BioComp piston gradient fractionator linked to an EM-1 UV Monitor (BioRad). The fractions were incubated in 1% SDS and proteinase K at 42°C for 30 min. RNA was purified by Phenol Chloroform extraction followed by ethanol precipitation. Equal volumes of RNA from each fraction were loaded onto a formaldehyde-denaturing agarose gel for northern blot analysis.

Extracting Total Proteins from Fresh-Frozen Human Multiple Cancer Samples

Forty-seven multiple human tumor samples were obtained from NUH-NUS (National University of Singapore) Tissue Repository. Small, fresh tumor samples were homogenized and resuspended in lysis buffer (50 mM Tris [pH 7.4], 150 mM NaCl, 1% NP-40, 1 mM EDTA, 2 μg/ml leupeptin, 2 μg aprotinin, 4 mM Prefabloc SC, and protease inhibitor cocktail) for 15 min at 4°C. The supernatant was collected and respun at 55,000 rpm for 40 min at 4°C with a TLA100.2 rotor (Beckman). Each sample was loaded (~40 μg/sample) on a 15% gel for SDS-PAGE analysis.

In Situ Hybridization on Formalin-Fixed and Paraffin-Embedded Surgical Specimens to Detect PRL-3 mRNA Expression

An RT-PCR fragment corresponding to the human PRL-3 mRNA coding region was cloned into EcoR1 and BamH1 sites of the pBluescriptII KS/SK (+) phagemid. For in situ hybridization, the plasmid was cut with BamH1 and the T3 promoter was used to transcribe the PRL-3 sense probe. Alternatively, the plasmid was cut with EcoR1 and the T7 promoter was used for transcribing the PRL-3 antisense probe. We used a DIG RNA Labeling Kit (SP6/T7) to label the RNAs with digoxigenin-UTP by in vitro transcription with the T7 RNA polymerase (Cat. No. 11-175-025-910, Roche) or T3 RNA polymerase (Part 9PIP208, Promega). The protocols were followed according to the manufacturer's instructions.

Tumorigenicity Assays in Mice for Xenograft Tumor Study

One million HCT-116 cells in which PCBP1 was knocked down or overexpressed were injected respectively into the left (*L*, n = 8) or right (*R*, n = 8) side of the hip areas of 8-week-old nude mice (Jackson Labs) to examine the tumorigenicity of the cells in vivo. After 4 weeks, the sizes of the tumor were monitored and photographed. Prism 4 graphing software (GraphPad Software) and the paired t test were used for statistic analysis and presentation. All animal studies were approved by the Institutional Animal Care and Use Committee (IACUC) and were carried out under the policies of Institute of Molecular and Cell Biology's Review Board (IRB), Singapore.

SUPPLEMENTAL INFORMATION

Supplemental Information includes two figures and Supplemental Experimental Procedures and can be found with this article online at doi:10.1016/j.ccr.2010.04.028.

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REFERENCES

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

Bardelli, A., Saha, S., Sager, J.A., Romans, K.E., Xin, B., Markowitz, S.D., Lengauer, C., Velculescu, V.E., Kinzler, K.W., and Vogelstein, B. (2003). PRL-3 expression in metastatic cancers. Clin. Cancer Res. *9*, 5607–5615.

Basak, S., Jacobs, S.B., Krieg, A.J., Pathak, N., Zeng, Q., Kaldis, P., Giaccia, A.J., and Attardi, L.D. (2008). The metastasis-associated gene Prl-3 is a p53 target involved in cell-cycle regulation. Mol. Cell *30*, 303–314.

Bessette, D.C., Qiu, D., and Pallen, C.J. (2008). PRL PTPs: Mediators and markers of cancer progression. Cancer Metastasis Rev. *27*, 231–252.

Diamond, R.H., Cressman, D.E., Laz, T.M., Abrams, C.S., and Taub, R. (1994). PRL-1, a unique nuclear protein tyrosine phosphatase, affects cell growth. Mol. Cell. Biol. *14*, 3752–3762.

Fagerli, U.M., Holt, R.U., Holien, T., Vaatsveen, T.K., Zhan, F., Egeberg, K.W., Barlogie, B., Waage, A., Aarset, H., Dai, H.Y., et al. (2008). Overexpression and

involvement in migration by the metastasis-associated phosphatase PRL-3 in human myeloma cells. Blood *111*, 806–815.

Fiori, A., Perez-Martinez, X., and Fox, T.D. (2005). Overexpression of the COX2 translational activator, Pet111p, prevents translation of COX1 mRNA and cytochrome c oxidase assembly in mitochondria of *Saccharomyces cerevisiae*. Mol. Microbiol. *56*, 1689–1704.

Guo, K., Li, J., Wang, H., Osato, M., Tang, J.P., Quah, S.Y., Gan, B.Q., and Zeng, Q. (2006). PRL-3 initiates tumor angiogenesis by recruiting endothelial cells in vitro and in vivo. Cancer Res. *66*, 9625–9635.

Hanahan, D., and Weinberg, R.A. (2000). The hallmarks of cancer. Cell *100*, 57–70.

Huo, L.R., and Zhong, N. (2008). Identification of transcripts and translatants targeted by overexpressed PCBP1. Biochim. Biophys. Acta *1784*, 1524–1533.

Kato, H., Semba, S., Miskad, U.A., Seo, Y., Kasuga, M., and Yokozaki, H. (2004). High expression of PRL-3 promotes cancer cell motility and liver metastasis in human colorectal cancer: A predictive molecular marker of metachronous liver and lung metastases. Clin. Cancer Res. *10*, 7318–7328.

Lammich, S., Schobel, S., Zimmer, A.K., Lichtenthaler, S.F., and Haass, C. (2004). Expression of the Alzheimer protease BACE1 is suppressed via its 5′-untranslated region. EMBO Rep. *5*, 620–625.

Li, J., Guo, K., Koh, V.W., Tang, J.P., Gan, B.Q., Shi, H., Li, H.X., and Zeng, Q. (2005). Generation of PRL-3- and PRL-1-specific monoclonal antibodies as potential diagnostic markers for cancer metastases. Clin. Cancer Res. *11*, 2195–2204.

Liang, F., Liang, J., Wang, W.Q., Sun, J.P., Udho, E., and Zhang, Z.Y. (2007). PRL3 promotes cell invasion and proliferation by down-regulation of Csk leading to Src activation. J. Biol. Chem. *282*, 5413–5419.

Makeyev, A.V., and Liebhaber, S.A. (2002). The poly(C)-binding proteins: A multiplicity of functions and a search for mechanisms. RNA *8*, 265–278.

Manning, B.D., and Cantley, L.C. (2007). AKT/PKB signaling: Navigating downstream. Cell *129*, 1261–1274.

Miskad, U.A., Semba, S., Kato, H., and Yokozaki, H. (2004). Expression of PRL-3 phosphatase in human gastric carcinomas: Close correlation with invasion and metastasis. Pathobiology *71*, 176–184.

Ostareck, D.H., Ostareck-Lederer, A., Shatsky, I.N., and Hentze, M.W. (2001). Lipoxygenase mRNA silencing in erythroid differentiation: The 3′UTR regulatory complex controls 60S ribosomal subunit joining. Cell *104*, 281–290.

Ostareck, D.H., Ostareck-Lederer, A., Wilm, M., Thiele, B.J., Mann, M., and Hentze, M.W. (1997). mRNA silencing in erythroid differentiation: hnRNP K and hnRNP E1 regulate 15-lipoxygenase translation from the 3′ end. Cell *89*, 597–606.

Peng, L., Ning, J., Meng, L., and Shou, C. (2004). 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. *130*, 521–526.

Pillai, M.R., Chacko, P., Kesari, L.A., Jayaprakash, P.G., Jayaram, H.N., and Antony, A.C. (2003). Expression of folate receptors and heterogeneous nuclear ribonucleoprotein E1 in women with human papillomavirus mediated transformation of cervical tissue to cancer. J. Clin. Pathol. *56*, 569–574.

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

Saha, S., Bardelli, A., Buckhaults, P., Velculescu, V.E., Rago, C., St Croix, B., Romans, K.E., Choti, M.A., Lengauer, C., Kinzler, K.W., and Vogelstein, B. (2001). A phosphatase associated with metastasis of colorectal cancer. Science *294*, 1343–1346.

Stephens, B.J., Han, H.Y., Gokhale, V., and Von Hoff, D.D. (2005). PRL phosphatases as potential molecular targets in cancer. Mol. Cancer Ther. *4*, 1653–1661.

Sun, X.L., and Antony, A.C. (1996). Evidence that a specific interaction between an 18-base *cis*-element in the 5′-untranslated region of human folate receptor-alpha mRNA and a 46-kDa cytosolic trans-factor is critical for translation. J. Biol. Chem. *271*, 25539–25547.

Takagi, M., Absalon, M.J., McLure, K.G., and Kastan, M.B. (2005). Regulation of p53 translation and induction after DNA damage by ribosomal protein L26 and nucleolin. Cell *123*, 49–63.

Thakur, S., Nakamura, T., Calin, G., Russo, A., Tamburrino, J.F., Shimizu, M., Baldassarre, G., Battista, S., Fusco, A., Wassell, R.P., et al. (2003). Regulation of BRCA1 transcription by specific single-stranded DNA binding factors. Mol. Cell. Biol. *23*, 3774–3787.

Wang, H., Quah, S.Y., Dong, J.M., Manser, E., Tang, J.P., and Zeng, Q. (2007a). PRL-3 down-regulates PTEN expression and signals through PI3K to promote epithelial-mesenchymal transition. Cancer Res. *67*, 2922–2926.

Wang, Y., Li, Z.F., He, J., Li, Y.L., Zhu, G.B., Zhang, L.H., and Li, Y.L. (2007b). Expression of the human phosphatases of regenerating liver (PRLs) in colonic adenocarcinoma and its correlation with lymph node metastasis. Int. J. Colorectal Dis. *22*, 1179–1184.

Wu, X., Zeng, H., Zhang, X., Zhao, Y., Sha, H., Ge, X., Zhang, M., Gao, X., and Xu, Q. (2004). Phosphatase of regenerating liver-3 promotes motility and metastasis of mouse melanoma cells. Am. J. Pathol. *164*, 2039–2054.

Zeng, Q., Dong, J.M., Guo, K., Li, J., Tan, H.X., Koh, V., Pallen, C.J., Manser, E., and Hong, W. (2003). PRL-3 and PRL-1 promote cell migration, invasion, and metastasis. Cancer Res. *63*, 2716–2722.

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