Phosphatase of regenerating liver 3 (PRL3) is overexpressed in a variety of tumors, and high levels of PRL3 expression are associated with tumorigenesis and metastasis. Consistent with an oncogenic role for PRL3, we show that ectopic PRL3 expression promotes cell proliferation and invasion. However, little is known about the molecular basis for PRL3 function. Obtaining this knowledge is vital for understanding PRL3-mediated disease processes and for the development of novel anticancer therapies targeted to PRL3. Here we report that up-regulation of PRL3 activates the Src kinase, which initiates a number of signal pathways culminating in the phosphorylation of ERK1/2, STAT3, and p130Cas. The activation of these pathways likely contributes to the increased cell growth and motility of PRL3 cells. We provide evidence that PRL3 induces Src activation through down-regulation of Csk, a negative regulator of Src. Importantly, Src activation and Csk down-regulation are also observed in colon cancer cells expressing a higher level of PRL3. Thus, we have revealed a biochemical mechanism for the PRL3-mediated cell invasion and proliferation in which elevated PRL3 expression causes a reduction in Csk level, leading to Src activation.

Protein-tyrosine phosphatases (PTPs) are key regulatory enzymes in various signal transduction pathways. Defective or inappropriate regulation of PTP activity leads to aberrant tyrosine phosphorylation, which contributes to the development of many human diseases, including cancer (2, 3). The PRL phosphatase represents a novel subfamily of PTPs, which is comprised of three members (PRL1, -2, and -3) sharing a high degree (>75%) of amino acid sequence identity (4–6). PRL1 was originally identified as an immediate early gene in regenerating liver (4). Subsequently, the PRL phosphatases have been implicated in the development of a number of tumorigenesis and metastasis processes (7).

PRL3 has attracted much attention because of its involvement in tumor metastases (7, 8). PRL3 is consistently and massively overexpressed in liver metastases of colorectal cancer, and its expression in primary tumors and normal colorectal epithelium is undetectable (9). Subsequently, PRL3 mRNA is found to be elevated in nearly all metastatic lesions derived from colorectal cancers, regardless of the sites of metastasis (liver, lung, brain, or ovary) (10, 11). High PRL3 expression has also been reported in cancer types other than colorectal cancers. For example, PRL3 is highly expressed in a Hodgkin lymphoma cell line (12) and in liver carcinoma samples (13); high PRL3 expression has been detected in invasive breast tumor vasculature (14), and overexpression of PRL3 is associated with ovarian cancer progression (15). In addition, PRL3 promotes invasion and metastasis of human gastric carcinomas (16) and mouse melanoma (13). Moreover, cells (Chinese hamster ovary and B16) stably transfected with PRL3 exhibit enhanced motility and invasion activity and induce metastatic tumor formation in mice (13, 17), whereas knockdown of endogenous PRL3 in cancerous cells using small interfering RNA abrogates cell motility and the ability to metastasize in a mouse model (11, 18). Collectively, these studies suggest that an excess of PRL3 is a key alteration contributing to the acquisition of metastatic and proliferative properties of tumor cells.

Although considerable evidence has now accumulated suggesting that PRL3 may play key causal roles in the development of tumorigenesis and metastasis, little is known about the underlying mechanism(s) by which PRL3 promotes cell invasion and growth. Obtaining this knowledge is vital for the development of novel anticancer therapies targeted to PRL3. In this study, we seek to define the signaling pathways that are regulated by PRL3. Our results indicate that PRL3 down-regulates the C-terminal Src kinase (Csk), which in turn leads to Src activation. Activated Src then initiates a number of signal pathways, including the extracellular signal-regulated protein kinase 1 and 2 (ERK1/2), signal transducer and activator of transcription 3 (STAT3), and p130Cas, providing a likely mechanism by which PRL3 promotes cell migration and proliferation.

**EXPERIMENTAL PROCEDURES**

Materials—Anti-phosphotyrosine (PY20), Csk, p130Cas, FAK, and vinculin antibodies were from BD Biosciences. Polyclonal anti-STAT3 and pSTAT3/Tyr-705 antibodies (Santa Cruz Biotechnology) were generous gifts from Dr. Xin-Yuan Fu (Indiana University School of Medicine) and Dr. Hua Yu (City of Hope). Anti-Src, anti-Src-pY416, and anti-Src-pY527 antibodies were from BIOSOURCE. Polyclonal anti-ERK1/2 and anti-pERK1/2 (Thr-202/Tyr-204) antibodies were purchased.
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from Cell Signaling (Beverly, MA). Src inhibitor SU6656 was purchased from Calbiochem.

Cell Culture and Stable Clone Selection—HEK293 cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (Invitrogen), penicillin (50 units/ml), and streptomycin (50 μg/ml) under a humidified atmosphere containing 5% CO₂. Human PRL3 was inserted into pcDNA3 expression vector. HEK293 cells were seeded at 40% confluency in antibiotic-free medium and grown overnight. Transfection was performed using Lipofectamine (Invitrogen) according to the manufacturer's recommendations. 24 h after transfection, 0.5 mg/ml G418 was added to the culturing medium. Stable clones were picked after 2 weeks of selection. For Csk rescue experiments, HEK293/PRL3 cells were transfected with pcDNA4-Csk, and final stable clones were established by selection with Zeocin (400 μg/ml). mRNA Extraction and RT-PCR—mRNA from different cell lines was prepared using Trizol reagent (Invitrogen). mRNA was treated by Dnase and quantified by absorbance at 260 and 280 nm. RT-PCR was performed using the Invitrogen SuperScript one-step RT-PCR kit. Reverse transcription was done at 50 °C for 30 min, and cDNA was amplified for 36 cycles (94 °C, 30 s; 55 °C, 1 min; 72 °C, 90 s). The sequences of specific primers were as follows: PRL3 sense, 5'-CTTCCTCATCACCACACCC-3' and antisense: 5'-GTCTTTGCTGTGGGTGCCTC-3'; 18 S ribosome sense, 5'-CGCCGCTAGAGGTGAAATTC-3', and antisense, 5'-TTGGCAAATGCTTTCGCTC-3'. The PCR products were separated by 2% agarose gel and visualized by staining with ethidium bromide.

Real Time Quantitative RT-PCR Analysis—The PRL3 mRNA level was determined with a two-step quantitative RT-PCR protocol using the fluorescent intercalating dye SYBR-Green RT-PCR kit (Invitrogen) and an ABI Prism 7700 sequence detection system (Applied Biosystems). 250 ng of RNA was used for the first strand cDNA synthesis. Primer sequences for PRL3 and 18 S were the same as those used for the RT-PCR experiments. 18 S rRNA was used as an internal control. The cycle threshold (Cₘ) value, defined as the PCR cycle at which a statistically significant increase of reporter fluorescence is first detected, was used as measure for the starting copy numbers of target genes. The ratio of the Cₘ value of PRL3 mRNA to that of 18 S in each cell lines was determined as relative PRL3 mRNA level.

Cell Migration Assay—Cells were serum-starved overnight, harvested with trypsin/EDTA, and washed twice with serum-free DMEM. Cells (5 × 10⁵) were then suspended in 1 ml of serum-free DMEM and then added to the upper insert of the haptotaxis chamber (Corning Costar, Cambridge, MA). The lower well was added with 3 ml of DMEM medium plus 10% fetal bovine serum. The migration was allowed for 16 h at 37 °C. Cells on the upper chamber surface were mechanically removed with a cotton swab, and migratory cells were collected and counted with a hemocytometer.

Cell Adhesion Assay—HEK293 cells were grown to 70% confluence in 37 °C 5% CO₂ incubator. Cells were released from the cell culture dishes with trypsin/EDTA. Cells were then incubated in DMEM with 10% fetal bovine serum to neutralize trypsin. 5 × 10⁶ cells in 2 ml of DMEM were applied to a 6-well plate and incubated for 2 h. Cell adhesion was monitored by photographing under ×200 magnitude.

Soft Agar Colony Formation Assay—Culture dishes (6 cm) were covered with a layer (7 ml) of 0.5% agar in medium supplemented with 20% fetal bovine serum to prevent the attachment of the cells to the plastic substratum. Cell suspensions (5000 cells per well) were prepared in 1.5 ml of 0.3% agar and poured into the dishes. Agar was allowed to harden for 20 min before being returned to the culturing incubator and kept for 2 weeks until colonies appeared. The dishes were then scanned with a scanner, and the number of colonies with diameters bigger than 1 mm was counted.

Wound-healing Assay—Cells were grown to 90% confluency in a 6-well plate in a 37 °C 5% CO₂ incubator. A wound was created by scratching cells with a sterile 200-μl pipette tip. Cells were washed with phosphate-buffered saline to remove the floating cells, and fresh culturing medium was added. Photos of the wound were taken under ×10 magnification microscope.

Immunoblotting and Immunoprecipitation—Cells were grown to 70% confluency, washed with ice-cold phosphate-buffered saline, and lysed on ice for 30 min in 1 ml of lysis buffer (50 mM Tris-HCl, pH 7.5, 5 mM EGTA, 150 mM NaCl, 10 mM sodium phosphate, 10 mM sodium fluoride, 1 mM sodium pervanadate, 1 mM benzamidine, 1 mM Triton X-100, 10 μg/ml leupeptin, and 5 μg/ml aprotinin). Cell lysates were cleared by centrifuging at 15,000 rpm for 15 min. Lysate protein concentration was estimated using BCA protein assay kit (Pierce). For immunoprecipitation, 10 μg of antibody was added to 1 mg of cell lysate and incubated at 4 °C for 2 h. 20 μl of protein A/G-agarose beads was then added and incubated for another 2 h. After extensive washing, protein complex was boiled with sample buffer, separated by SDS-PAGE, transferred electrophoretically to nitrocellulose membrane, and immunoblotted with appropriate antibodies followed by incubation with horseradish peroxidase-conjugated secondary antibodies. The blots were developed by the enhanced chemiluminescence technique (ECL kit, Amersham Biosciences). Band intensities were analyzed with the software ImageJ developed by the National Institutes of Health. Data shown represented the results of at least three independent experiments.

Src Kinase Assay—Cell lysate (5 mg) from each cell line was incubated with anti-Src antibody (Upstate Biotech) (1 mg of lysate/10 μg of antibody) for 2 h at 4 °C. Src immunocomplexes were recovered by incubating 20 μl of protein A/G-agarose with the lysate for another 2 h. Half of the immunocomplex was subjected to immunoblotting to determine the levels of pSrc527, pSrc416, and Src protein. The other half was used to assay for Src kinase activity using enolase as a substrate as described previously (45). The immunoprecipitate was washed three times with the lysis buffer and twice with the kinase buffer (40 mM HEPES, pH 7.4, 10 mM MgCl₂, 3 mM MnCl₂). The immunoprecipitate was then incubated with 10 μCi of [γ-³²P]ATP, 50 μM ATP, 1 mM dithiothreitol, and 5 μg of acid-treated enolase in a 30-μl volume at 30 °C for 10 min. The reaction was terminated by adding 10 μl of 4× SDS loading.
buffer. The sample was analyzed by SDS-PAGE with 10% gel. 

**RESULTS AND DISCUSSION**

**Ectopic Expression of PRL3 Promotes Cell Invasion, Proliferation, and Transformation**—To begin to investigate the molecular mechanism(s) by which PRL3 promotes cell invasion and proliferation, we employed human embryonic kidney 293 (HEK293) cells, an epithelium-derived cell line. A number of stable lines ectopically expressing wild-type PRL3 and the catalytically inactive mutant PRL3/C104S, in which the active site Cys is replaced with a Ser, were established. The levels of ectopically expressed PRL3, determined by both RT-PCR and real time quantitative RT-PCR, were in general 3–5-fold higher than that of the endogenous PRL3 in vector control cells. A, PRL3 mRNA determined by RT-PCR. The levels of ectopically expressed PRL3 in PRL3 clone 1, PRL3 clone 2, and PRL3/C104S cells are 3.3-, 4.8-, and 4.9-fold higher than that of the endogenous PRL3 in vector control cells. B, PRL3 mRNA determined by real time quantitative RT-PCR. Compared with the vector control cells, the PRL3 mRNA levels in PRL3 clone 1 and PRL3 C104S cells are increased by 3.9- and 5.4-fold, respectively. The level of PRL3 mRNA was expressed as fold changes relative to the 18S control. The means and S.D. of three independent experiments are shown.

PRL3 expression on cell adhesion and spreading. As shown in Fig. 2D, 2 h after plating the cells in fresh culture dishes, the majority of the PRL3(1) cells assumed a spreading morphology, although very few vector control or the PRL3/C104S cells did so. Taken together, the results show that elevated expression of PRL3 promotes EMT and increases cell motility. The acquisition of these motile and invasive properties is consistent with involvement of PRL3 in tumor metastasis.

PRL3-expressing cells also displayed accelerated growth rates versus both vector control and the PRL3/C104S cells (Fig. 3A). The doubling time of the PRL3(1) and PRL(2) cells was 16.2 and 20.4 h, whereas those of the vector control and PRL3/C104S were 26.5 and 26.0 h, respectively. In addition, the PRL3(1) and PRL3(2) cells grew to a higher density of $3.31 \times 10^5$ cells/cm$^2$ and $2.86 \times 10^5$ cells/cm$^2$, whereas the vector control and mutant cells could only reach a density of $1.24 \times 10^5$ cells/cm$^2$ and $1.35 \times 10^5$ cells/cm$^2$, respectively. We also determined the ability of PRL3 to promote anchorage-independent growth in a soft agar colony-forming assay. As shown in Fig. 3B, PRL3(1) cells gained the capacity to form colonies in soft agar, a hallmark for transformation, whereas no visible colonies were found for both the
mock control and PRL/C104S cells. Collectively, these results are in complete agreement with previous observations in a number of cell lines, including HEK293 (13, 17, 19), that ectopic expression of PRL3 enhances cell growth, causes cell transformation, and promotes tumor metastasis and that the catalytic activity of PRL3 is required for the observed phenotypes. Consequently, the established PRL3 HEK293 cell lines serve as an excellent model system to study the mechanism(s) by which PRL3 promotes cell invasion and proliferation. Because the phenotypes were more prominent in the lower expressing PRL3 clone 1, the rest of the work was focused on this clone.

Up-regulation of PRL3 Leads to Src Activation—As a first step toward elucidating the cellular pathways mediated by PRL3, we measured the total protein tyrosine phosphorylation of HEK293 cells expressing vector alone, PRL3, and PRL3/C104S using anti-Tyr(P) antibodies. Dramatic increases in tyrosine phosphorylation of a number of proteins were observed in PRL3 cells in comparison with the control or PRL3/C104S cells (Fig. 4). This is an unusual observation because PRL3 is a protein phosphatase. We hypothesized that the increased tyrosine phosphorylation in PRL3 cells may result from a PRL3-mediated dephosphorylation of an inhibitory component(s) in signaling pathways, leading to activation of one or more tyrosine kinases. Given the pleiotropic role of Src kinase in controlling cell growth, adhesion, and invasion (20–22), we suspected that Src may be activated in PRL3-expressing cells. Src activity is regulated by phosphorylation at two distinct tyrosine residues. Autophosphorylation of Tyr-416 in the kinase domain is important for maintaining Src activity. In contrast, phosphorylation of Tyr-527 in the C-terminal tail by Csk inactivates Src because of an intramolecular Tyr(P)-SH2 interaction (23, 24). Indeed, removal of the inhibitory phosphate from Tyr(P)-527 results in an increase in Src activity (25, 26), whereas a decrease in Tyr(P)-527 phosphorylation because of ectopic expression of PTPα produces a transformed phenotype in embryonic fibroblasts (27). Moreover, the oncogene v-SRC, which lacks the C-terminal Tyr-527, is constitutively active and causes transformation (28). These findings indicate that the transforming potential of Src is suppressed by Tyr-527 phosphorylation.

To determine the effect of PRL3 expression on Src phosphorylation, total cell lysates from the vector control, PRL3, and PRL3/C104S cells were resolved by SDS-PAGE, electrotransferred to nitrocellulose membranes, and probed with phosphospecific pSrc416 and pSrc527 antibodies. As shown in Fig. 5A, Tyr-527 phosphorylation in PRL3 cells decreased 73% as compared with the vector control and PRL3/C104S cells. No significant changes were observed in the levels of Tyr-416 phosphorylation and Src protein among the different cell lines. According to the discussion above, these results indicate that Src kinase is activated as a result of elevated PRL3 expression. To show directly Src activation, Src protein was immunoprecipitated from cell lysates with anti-Src antibodies and assayed for kinase activity using enolase and [γ-32P]ATP as substrates. As shown in Fig. 5B, Src kinase activity increased by 180% in PRL3 cells as compared with those in vector control and PRL3/C104S cells. To determine whether Src activation is required for the observed phenotypes induced by PRL3 up-regulation, we assessed the effects of Src inhibition using a specific small molecule Src inhibitor SU6656 (Calbiochem). As expected, treatment of PRL3 cells with 2.5 μM SU6656 reduced tyrosine phos-
phorylation of p130Cas, a direct substrate of Src, by 73% and caused a decrease in cell migration by more than 50% (Fig. 5C), indicating that Src activity is required for the PRL3-mediated cell migration. Taken together, the results support the conclusion that up-regulation of PRL3 leads to Src activation.

ERK1/2, STAT3, and p130Cas Are Also Activated in PRL3 Cells—To further corroborate that Src is activated in PRL3 cells, we next examined the status of a number of signaling molecules downstream of Src, including ERK1/2, STAT3, focal adhesion kinase (FAK), and the adaptor protein p130Cas. One of the major signaling pathways regulated by Src is mediated by ERK1/2 (20), which are involved in several fundamental cellular processes in cell proliferation, survival, and motility (29, 30). Not surprisingly, a dramatic increase (410%) in ERK1/2 activity was apparent in the PRL3 cells when compared with the vector control and the PRL3/C104S cells (Fig. 5A). In addition, inhibition of Src activity with SU6656 completely blocked ERK1/2 activation, suggesting that ERK1/2 is downstream of Src (Fig. 5C). The transcription factor STAT3 is a direct substrate of Src, and STAT3 is constitutively activated in Src-transformed cells (31). In fact, STAT3 target genes are implicated in all processes of tumorigenesis, including proliferation, invasion, and migration (32, 33). To determine the activation status of STAT3, we used an antibody directed toward Tyr(P)-705 of STAT3. Consistent with Src activation, a substantial increase (38%) in STAT3 phosphorylation was observed in PRL3-expressing cells (Fig. 5A). The results demonstrate that both the ERK1/2 pathway and STAT3 are activated when PRL3 level is elevated. It is likely that the Src-dependent activation of ERK1/2 and STAT3 is, at least in part, responsible for the PRL3-induced cell proliferation and invasion.

Given the importance of PRL3 in tumor metastasis, we decided to gain further insight into the biochemical mechanism for the enhanced motility of the PRL3 cells. We examined two additional Src substrates, FAK and p130Cas, both of which are important mediators of focal adhesion turnover and cell migration (34, 35). Overall, FAK and p130Cas protein levels were similar among the different cell lines. No change in FAK phosphorylation was observed as a result of altered PRL3 expression. In contrast, p130Cas tyrosine phosphorylation increased dramatically (350–460%) in PRL3 cells, when compared with those in the vector control and PRL3/C104S cells (Fig. 5, C and D). p130Cas is a multidomain signaling protein that, upon phosphorylation by Src, relocates to focal adhesions to form multiprotein complexes (35–37). The formation of these complexes in focal adhesions is important for downstream pathways that modulate the migratory response of cells. To determine whether the increased p130Cas phosphorylation is accompanied with increased p130Cas localization in focal adhesions, we measured the amount of FAK and p130Cas associated with vinculin, a known component of focal contacts (38, 39). As can be seen in Fig. 5D, up-regulation of PRL3 promotes localization of p130Cas with vinculin in focal adhesion complexes, most likely because of increased tyrosine phosphorylation by Src. In agreement with the lack of change in FAK phosphorylation, no appreciable difference in the amount of vinculin-associated FAK was noted between the PRL3 and control cells. Taken together, the results suggest that the PRL3-mediated Src activation results in p130Cas phosphorylation and recruitment to focal adhesions, which should contribute to the pro-migratory actions of PRL3, possibly involving up-regulation of the Rho family of small G proteins (40).

PRL3 Induces Src Activation via Down-regulation of Csk—We have shown that the PRL3-mediated cell proliferation and invasion is likely because of activation of a number of Src-dependent signaling pathways, including ERK1/2, STAT3, and p130Cas. This is consistent with Src being activated in a large number of malignancies and cancer metastases. To define the mechanism by which PRL3 activates Src, we first investigated whether pSrc527 is a direct substrate of PRL3. Given that PRL3 is a member of the PTP family and its phosphatase activity is required for the Src-dependent phenotypes, dephosphorylation of pSrc527 by PRL3 would provide the most direct mechanism for Src activation in PRL3 cells. However, we found that purified PRL3 was unable to dephosphorylate Tyr-527 using
either Src immunoprecipitated from HEK293 cells or recombinant Src phosphorylated by Csk (data not shown).

We then investigated the possibility that Csk, which catalyzes Src Tyr-527 phosphorylation, is down-regulated in PRL3-expressing cells. Western blot with monoclonal anti-Csk antibodies (raised against the N-terminal residues 1–156 of Csk; BD Transduction Laboratories) showed that the Csk protein level decreased 81% in PRL3-expressing cells in comparison with the vector control and PRL3/C104S cells (Fig. 6A). Similar results were also obtained with polyclonal anti-Csk antibodies (raised against the C-terminal residues 431–450 of Csk; Santa Cruz Biotechnology) (Fig. 6A). The reduction in Csk level should lead to decreased Tyr-527 phosphorylation, resulting in Src activation. Thus, one likely mechanism for Src activation in PRL3 cells is through down-regulation of the Csk protein. Interestingly, reduced Csk protein/activity has been found to correlate inversely with elevated Src activity in highly metastatic colon and hepatocellular carcinoma (39, 41, 42), whereas overexpression of Csk reduces Src activity and suppresses tumor metastasis in vivo (43).

To further establish that down-regulation of Csk is responsible for Src activation and the growth and motility-promoting effects of PRL3, we performed a Csk rescue experiment in which Csk was re-introduced into PRL3 cells using a tetracycline-regulated expression system. We reasoned that if a decrease in Csk is the cause for Src activation and the enhanced growth and migration phenotypes, then bringing back Csk to the normal level should restore the properties of the PRL3 cells to those of the control. This prediction was borne out by the outcomes of the experiment. As the Csk level in the PRL3 cells rose, a Csk concentration-dependent increase in Src Tyr-527 phosphorylation was observed with no obvious change in either Tyr-416 phosphorylation or Src protein (Fig. 6B), which is indicative of Src inactivation. Consistent with suppression of Src activity, there was a concentration-dependent decrease in ERK1/2 and p130Cas phosphorylation, and in the amount of p130Cas associated with focal adhesions when the Csk level was increased in PRL3 cells (Fig. 6B). Indeed, when the Csk level in the PRL3 cells was restored to that of the vector control, the Csk-rescued PRL3 cells (100 ng/ml tetracycline) exhibited virtually identical biochemical properties to those of the vector control cells. Consistent with the biochemical data, the Csk-rescued PRL3 cells displayed similar rates of cell proliferation and migration to those of the control cells (Fig. 6C).

These results provide strong evidence that down-regulation of Csk is responsible for Src activation in PRL3 cells.

Our studies with HEK293 cells expressing exogenous PRL3 revealed that PRL3 promotes cell proliferation and migration by down-regulating Csk, leading to Src activation. To ascertain whether this mechanism is unique to HEK293 cells overexpressing PRL3, we also examined two colon cancer cell lines, SW480 and SW620, which originated from the same patient (44). SW480 was from the surgical specimen of the primary colon adenocarcinoma, whereas SW620 was taken from a lymph node biopsy. SW480 was reported to exhibit a higher migration rate than SW620 in three-dimensional matrix. We confirmed that SW480 cells also migrate faster than SW620 in the Transwell assay (Fig. 7A). We then measured PRL3 level by RT-PCR and Csk protein and Src527 phosphorylation by immunoblotting. As shown in Fig. 7B, PRL3 is expressed to a significantly higher level in SW480 cells than in SW620 cells. Interestingly, compared with SW620, SW480 cells also have a

**FIGURE 6.** Down-regulation of Csk is responsible for PRL3-mediated Src activation and cell proliferation and migration. A, Csk protein level is lower in PRL3-expressing cells as compared with the vector control and PRL3/C104S cells. Raising Csk to the normal level restores the biochemical properties (e.g. status of Src, ERK1/2 and p130Cas etc) and cell proliferation and migration (C) of the PRL3 cells to those of the control cells. *mAb*, monoclonal antibody; *IP*, immunoprecipitation.

**FIGURE 7.** Elevated expression of PRL3 in SW480 cells also induces Src activation through Csk down-regulation. A, SW480 cells have a higher migration rate than SW620 cells. B, SW480 expresses a higher level of PRL3, a reduced Csk level, and a higher Src activity in comparison with SW620.
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FIGURE 8. A working model for PRL3-mediated cell invasion and proliferation.

reduced Csk level and decreased Src527 phosphorylation, indicating higher Src activity. Thus, elevated expression of PRL3 in SW480 cells also correlates with Src activation and Csk down-regulation, suggesting that this is likely a more general mechanism for PRL3-mediated cell invasion and growth.

Conclusions—In support for an oncogenic role for PRL3 in tumorigenesis and metastasis, we show that ectopic expression of PRL3 in HEK293 cells promotes cell proliferation and invasion. We provide evidence that up-regulation of PRL3 activates the Src kinase, which then initiates a number of signal pathways culminating in the phosphorylation of ERK1/2, STAT3, and p130Cas. The activation of these pathways likely contributes to the increased cell growth and motility in PRL3 cells. We further demonstrate that PRL3 induces Src activation through down-regulation of Csk, a negative regulator of Src. Importantly, this mechanism may also be operative in colon cancer cell lines, which express a higher level of PRL3 and exhibit a faster migration rate. Collectively, our data establish a biochemical mechanism (Fig. 8) for the PRL3-mediated cell invasion and proliferation in which elevated PRL3 expression causes a reduction in Csk level, leading to Src activation. Experiments are in progress to identify the substrate(s) for PRL3 and to delineate the mechanism by which PRL3 down-regulates Csk.

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