PTP1B Is an Androgen Receptor–Regulated Phosphatase That Promotes the Progression of Prostate Cancer

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Abstract

The androgen receptor (AR) signaling axis plays a key role in the pathogenesis of prostate cancer. In this study, we found that the protein tyrosine phosphatase PTP1B, a well-established regulator of metabolic signaling, was induced after androgen stimulation of AR-expressing prostate cancer cells. PTP1B induction by androgen occurred at the mRNA and protein levels to increase PTP1B activity. High-resolution chromosome mapping revealed AR recruitment to two response elements within the first intron of the PTP1B encoding gene PTPN1, correlating with an AR-mediated increase in RNA polymerase II recruitment to the PTPN1 transcriptional start site. We found that PTPN1 and AR genes were coamplified in metastatic tumors and that PTPN1 amplification was associated with a subset of high-risk primary tumors. Functionally, PTP1B depletion delayed the growth of androgen-dependent human prostate tumors and impaired androgen-induced cell migration and invasion in vitro. However, PTP1B was also required for optimal cell migration of androgen-independent cells. Collectively, our results established the AR as a transcriptional regulator of PTPN1 transcription and implicated PTP1B in a tumor-promoting role in prostate cancer. Our findings support the preclinical testing of PTP1B inhibitors for prostate cancer treatment. Cancer Res; 72(6); 1529–37. ©2012 AACR.

Introduction

Despite early diagnosis and efficient treatment of most early-stage tumors, prostate cancer remains the second leading cause of cancer-related deaths in North American men (1). This discrepancy is the result of the lack of biomarkers able to discriminate between indolent and aggressive tumors, as well as the absence of curative options for castration-resistant metastatic prostate cancer (CRMPC; ref. 2). The androgen receptor (AR) plays a key role in the development and progression of prostate cancer, and androgen deprivation therapy and/or AR blockade continues to be the standard treatment for advanced or recurrent tumors, even though the majority of patients eventually develop CRMPC (3).

Remarkably, most castration-resistant tumors still depend on the AR for their growth and survival, a feature that underscores the “addiction” of prostate cancer cells to AR signaling (4, 5). In primary tumors, this concept is further supported by the presence of common genetic alterations that directly and/or indirectly affect AR-dependent transcription. For instance, the loss of NKX3.1 and PTEN tumor suppressor genes has been associated with Akt-mediated ligand-independent AR activity (6, 7), although recent findings challenge this concept and suggest that PI3K-Akt and AR signaling pathways regulate each other through reciprocal feedback (8, 9). Besides, the prostate-specific AR-induced TMPRSS2-ERG fusion protein has also been shown to regulate AR transcriptional activity (10). The characterization of signaling pathways acting upstream and downstream of the AR is therefore of paramount importance to identify new therapeutic targets that could interfere with AR signaling not only in CRMPC but also at earlier stages of the disease.

One largely unexplored mechanism in prostate tumors is the regulation of tyrosine phosphorylation by classical protein tyrosine phosphatases (PTP). Instead, the vast majority of studies have addressed the contribution of receptor and non-receptor tyrosine kinases, as important mediators of tumor-promoting signals responsible for the induction and/or enhancement of AR activity, as well as inducers of AR-independent survival mechanisms (11, 12). But abnormal regulation of tyrosine phosphorylation–dependent signaling in cancer cells can also be caused by altered PTP signaling. In fact,
mutations and/or aberrant expression of several PTPs have been reported in different cancer types and shown not only to counteract oncogenic tyrosine kinases but as well to directly promote tumor development and progression (13). With respect to prostate cancer, however, only a limited number of classical PTPs have been investigated and their relationship with AR-dependent signaling remains largely unknown (13).

To address this issue, we first profiled the expression of classical PTPs in the context of AR-dependent signaling. Unexpectedly, we found that the protein tyrosine phosphatase 1B (PTP1B) gene PTPN1, previously reported as being induced in androgen-deprived conditions (14), is actually induced after androgen stimulation and is a direct transcriptional target of the AR. In primary prostate tumors, we observed a positive correlation between PTP1B expression, AR nuclear localization, and Ki-67 nuclear index. We also found that PTPN1 is frequently amplified in metastatic tumors and a subset of high-risk primary tumors. Finally, we provide evidence that PTP1B depletion decreases LNCaP tumor growth rates in vivo and abrogates androgen-induced cell migration and invasion in vitro. These findings support a tumor-promoting role for PTP1B in prostate cancer and suggest that PTP1B inhibition may be of use in the treatment of the disease.

Materials and Methods

Cell culture

LNCaP, 22rv1, and DU145 cells were purchased from American Type Culture Collection and maintained in RPMI-1640 medium (Wisent) supplemented with 10% FBS, L-glutamine, and 50 μg/mL gentamycin. C4-2 cells were obtained from MD Anderson (Houston, TX) and maintained in the same conditions. The synthetic androgen analog R1881 was obtained from Perkin Elmer. For androgenic stimulation assays, cells were first androgen deprived in phenol-free RPMI-1640 supplemented with 5% charcoal-stripped FBS, L-glutamine, and 50 μg/mL gentamycin. After 48 hours, medium was refreshed and R1881 or ethanol (vehicle) was added for different time periods. For short interfering RNA (siRNA) experiments, cells were incubated for 24 hours with 100 nmol/L siRNA or nontargeting control before androgen deprivation.

Analysis of gene and protein expression

Total RNA extraction, reverse transcription, and quantitative real-time PCR (qPCR) were done as already described (15). The primer sequences used can be found in Supplementary Table S1. Threshold cycle numbers were calculated using the second derivative maximum obtained with the LightCycler®480 software version 3.5 (Roche). Data was normalized according to both PPIB and ARBP levels (Supplementary Table S1).

Immunoblotting procedures were done as previously described (15). Membranes were probed with the following antibodies according to manufacturer's instructions: mouse monoclonal anti-PTP1B (BD Transduction Laboratories), mouse monoclonal anti-AR (Lab Vision), rabbit polyclonal anti-calnexin (Cell Signaling Technology), and mouse monoclonal anti-P5A (Lab Vision). Densitometry analyses were done with ImageJ (U.S. NIH, Bethesda, MD; http://imagej.nih.gov/ij/).

Phosphatase assay

Cells were lysed in ice-cold radioimmunoprecipitation assay (RIPA) buffer supplemented with 3 mmol/L DTT and EDTA-free complete protease inhibitor cocktail (Roche). PTP1B was immunoprecipitated 2 hours at 4°C using 200 μg of protein lysate, 1 μg of mouse monoclonal anti-PTP1B clone AE4-2J (EMD4 Biosciences), and 30 μL of Protein G agarose beads (Fisher Canada). Beads were then washed 3 times in RIPA buffer and once in assay buffer (50 mmol/L HEPES, 3 mmol/L DTT, and 0.1 mg/mL bovine serum albumin). The phosphatase assay was done as previously described (16) using DFMUP (Invitrogen) as the PTP1B substrate and, where indicated, 50 μmol/L of a PTP1B inhibitor (kind gift from Brian Kennedy, Merck Frosst, Pointe-Claire, Qc).

siRNA and stable miRNA expression systems

Detailed experimental procedures are reported in Supplementary Materials and Methods and in Supplementary Table S1.

ChiP assays and ChiP-on-chip on chr.20 tiled array

Chromatin was prepared from LNCaP cells exposed to 1 nmol/L R1881 or vehicle for 4 hours following pretreatment with 20 μmol/L bicalutamide (BIC) or vehicle for 30 minutes. Chromatin immunoprecipitation (ChiP) was done as described previously (17) using antibodies specific to AR (mouse monoclonal anti-AR from Lab Vision and BD Biosciences) or the activated RNA polymerase II (RNA Pol II; rat monoclonal anti-RNA Pol II subunit B1 (phospho CTD Ser-2) from Millipore). Quantification of ChiP enrichment by qPCR was carried out using the LightCycler®480 instrument (Roche). Amplification and labeling of AR-bound ChiP fragments was done as described previously (18). Hybridization was carried out on custom designed chr.20 Agilent tiled arrays (150 bp resolution) and analyzed using Feature Extraction 10 and ChiP Analytics 3.1 (Agilent). The primers used for standard ChiP quantification and validation are listed in Supplementary Table S1.

Computational motif discovery

De novo and known motif discovery was carried out using the MEME Suite (http://meme.nbcr.net/meme/intro.html). Motif discovery and chromosomal location mapping were done using the Genomatix Software Suite (http://www.genomatix.de/index.html).

Immunohistochemistry

Detailed experimental procedures and PTP1B scoring scale are reported in Supplementary Materials and Methods and in Supplementary Table S2, respectively.

PTPN1 copy number alteration in prostate cancer

Analysis of copy number alteration at the PTPN1 and known motif discovery was carried out using the MEME Suite (http://meme.nbcr.net/meme/intro.html). Motif discovery and chromosomal location mapping were done using the Genomatix Software Suite (http://www.genomatix.de/index.html).
between amplification of the PTPN1 locus or its increased RNA expression and the indicated events (Fig. 4E and F) was identified based on the 2-tailed Fisher exact test. For RNA expression data, a z-score threshold of 1.5 SDs was used to separate low (≤ −1.5) or high (≥1.5) from normal expression.

**Migration and invasion assays**

LNCap, C4-2, and DU145 cells were treated for siRNA experiments as described in the cell culture section, except for DU145 cells that were not androgen deprived before the experiment. Details about the migration procedure are reported in Supplementary Materials and Methods.

**Mouse experimental procedures**

Six-week-old male severe combined immunodeficient (SCID) mice (Jackson Laboratories) were subcutaneously injected with 2 × 10⁶ shCTRL1a, shCTRL1b, shPTP1B1a, or shPTP1B2 cells resuspended in a 200 μL 70% PBS/30% Matrigel (BD Biosciences) solution. Ten mice per group were monitored daily for their physical health and once a week for tumor occurrence. Tumor volume was measured using the following equation: length × width × depth × 0.5236 (20). The animal protocol followed the Canadian Council on Animal Care ethical regulations and was approved by the McGill University Research and Ethics Animal Committee.

**Statistics**

Statistical analyses were carried out with the Prism 5.0 GraphPad Software. The Mann–Whitney or the Kruskal–Wallis tests were used to compare the distributions between 2 or more groups, respectively. The Spearman's rho correlation test was used to measure correlation coefficients between clinicopathologic and molecular variables. Kaplan–Meier and Log-Rank analyses were used to compare time of tumor onset between shCTRL and shPTP1B mice.

**Results**

**Androgen treatment induces PTP1B expression in AR-expressing prostate cancer cells**

We screened for classical PTP transcripts that could be modulated by androgen stimulation. Androgen-sensitive LNCap cells were maintained in steroid-depleted medium for 48 hours before stimulation with the synthetic androgen analog R1881. We carried out gene expression profiling after 24 hours of treatment and detected changes in mRNA levels for 11 classical PTPs (Supplementary Fig. S1). Surprisingly, we noted an AR-dependent increase in the levels of the PTPN1 transcript, despite previous findings linking the induction of PTPN1 expression to androgen deprivation (14). Using qPCR, we confirmed a time- and dose-dependent increase in PTPN1 mRNA levels in LNCap cells treated with R1881 (Fig. 1A and B). In AR-expressing androgen-independent C4-2 and 22rv1 cells, the upregulation of PTPN1 mRNA was also significant (Fig. 1C and Supplementary Fig. S2A). In contrast, PTPN1 levels were not affected by R1881 treatment in AR-depleted or AR-negative cells (Fig. 1C and D and Supplementary Fig. S2B–D). Notably, basal PTPN1 mRNA levels did not decrease in untreated AR-depleted cells (Fig. 1C and D) despite a PTPN1 mRNA half-life of about 8 hours (Supplementary Fig. S3), indicating that other transcription factors can also regulate basal PTPN1 expression. Ultimately, the AR-mediated activation of PTPN1 led to an increase in PTP1B protein levels (Fig. 2A and B), which translated into a dose-dependent increase in total PTP1B phosphatase activity (Fig. 2C). Interestingly, substrate-trapping experiments revealed differential binding of PTP1B to

![Figure 1](https://www.aacrjournals.org/canceres/72/6/1531/Graphics/001.png)

**Figure 1.** PTPN1 mRNA expression is induced upon R1881 treatment. A and B, PTPN1 expression in LNCaP cells was quantified as described in Materials and Methods after treatment with R1881 (10 nmol/L or the specified concentration) or the vehicle. PTPN1 mRNA levels were significantly increased in a time- (A) and dose-dependent (B; 24 hours treatment) manner (Kruskal–Wallis test, P < 0.01 and P < 0.05 respectively; representative experiment, N = 3, ± SEM). Relative PSA gene (KLK3) expression levels were monitored as treatment control. C and D, PTPN1 mRNA levels were quantified after 24 hours of R1881 treatment in cells transfected with siRNAs against the AR (siAR) or control sequences (siSC). The PTPN1 mRNA increase in siAR-transfected C4-2 (C) or LNCaP (D) cells was significant (Kruskal–Wallis test, P < 0.05 for both cell lines; representative experiment, N = 3, ± SEM). No R1881-dependent PTPN1 induction was observed in siAR conditions.
phosphotyrosine substrates in androgen-depleted and R1881-treated cells (Supplementary Fig. S4). Taken together, these results supported a connection between AR-dependent signaling and PTP1B activity in prostate cancer cells.

The *PTPN1* gene is a direct AR transcriptional target

We assessed whether the AR could regulate *PTPN1* expression through direct recruitment on androgen-responsive elements (ARE) located in the vicinity of *PTPN1*. Because the location of AREs is not restricted to gene promoter regions and often contains degenerated motifs (21, 22), we designed a high-resolution tiled microarray spanning the entire human 20q chromosome enclosing *PTPN1* to carry out location analysis of AR by ChIP hybridized to the array (ChIP-on-chip) in R1881-treated LNCaP cells. The AR-bound segments identified by ChIP-on-chip were submitted to de novo binding site identification. The most enriched sequence (P value $4.3 \times 10^{-5}$; Fig. 3A) was significantly associated to the known glucocorticoid-responsive element (GRE/ARE; P value $1.7 \times 10^{-4}$). No significant AR recruitment was detected within the *PTPN1* promoter although 2 AR-associated segments were identified in the first intron of *PTPN1* located at +6.10 kb and +28.22 kb downstream of the transcription start site (TSS), respectively (Fig. 3B). The AR-bound region located at +28.22 kb harbors a putative GRE/ARE binding site. To test whether the recruitment of AR at these sites was modulated by androgen, we pretreated LNCaP cells with the AR antagonist bicalutamide (BIC) for 30 minutes before R1881 stimulation. BIC treatment prevented the R1881-dependent recruitment of AR at the +6.10 kb and +28.22 kb enhancer sites (Fig. 3C), suggesting that the recruitment of AR at these enhancers is directed by androgen. Interestingly, modest AR occupancy was also detected at the *PTPN1* TSS when cells were treated with R1881 (Fig. 3C), concomitant with a significant R1881-dependent increase in the recruitment of transcriptionally active RNA polymerase II (RNAPol II; Fig. 3D). BIC pretreatment also abrogated the R1881-dependent AR and RNAPol II recruitment at the *PTPN1* TSS (Fig. 3C and D), suggesting that activation of AR is necessary to contribute to the recruitment of RNAPol II for transcriptional activation of the gene. Collectively, these findings validated AR as a transcriptional regulator of *PTPN1* expression in androgen-dependent prostate cancer cells.

Elevated PTP1B expression in prostate primary tumors correlates with AR nuclear localization and the tumor proliferative index

The AR-dependent regulation of PTP1B in androgen-dependent cells prompted us to characterize PTP1B expression in androgen-dependent primary prostate tumors. We analyzed PTP1B expression by immunohistochemistry on a tissue microarray (TMA) regrouping more than 300 cores from 62 patients that had undergone radical prostatectomy. We detected PTP1B expression in both normal adjacent and cancerous luminal cells, as well as in prostatic intraepithelial neoplasia lesions (PIN). Most normal and PIN tissues displayed weak cytoplasmic PTP1B levels, with occasional strong punctate staining of luminal cells (Fig. 4A). In contrast, tumor cells showed a marked increase in PTP1B expression (Fig. 4A and B). The overexpression of PTP1B in primary tumors was not related to the degree of histologic differentiation (Fig. 4C and Supplementary Table S3) or to other clinicopathologic parameters, except for a negative correlation with the surgical margin status (Supplementary Table S3). At the molecular level, however, PTP1B expression positively correlated with AR nuclear localization (Fig. 4D), thus providing in vivo evidence for a link between AR activation and PTP1B expression. Interestingly, both nuclear AR and PTP1B also correlated with the tumor proliferative index, as determined by Ki-67 staining (Fig. 4D). This suggested that PTP1B may not only be regulated by the AR but may also exert tumor-promoting effects in prostate tumors.

Amplification of the *PTPN1* gene is associated with metastatic prostate cancer and a subgroup of high-risk primary tumors

To gather additional evidence for a role of PTP1B in prostate cancer pathogenesis, we examined the status of the *PTPN1* gene in an independent cohort of 218 patients (19).
Interestingly, PTPN1 amplification was found in 6.6% (12 of 181) of primary tumors and enriched in 21.6% (8 of 37) of the metastatic tumor samples (Fig. 4E). In addition, the PTPN1 amplification in primary tumors was significantly associated with a previously identified pattern of copy number alterations [cluster 5 as defined by Taylor and colleagues (19)], found predictive of early recurrence after radical prostatectomy. PTPN1 amplification and mRNA expression also significantly correlated with AR gene amplification and transcript levels, respectively (Fig. 4E and F). These findings complemented our ChIP-on-chip and immunohistochemical results and are consistent with the AR-mediated induction of PTPN1 in human prostate cancer. Moreover, given the special role of AR signaling in prostate cancer and its exclusive amplification in late-stage disease, our identification of a positive correlation between AR and PTPN1 amplification and expression strongly supports a role for PTP1B in prostate cancer progression.

PTP1B plays a tumor-promoting role in prostate cancer cells

To address the role of PTP1B in vivo, stable LNCaP-derived clones expressing short hairpin RNAs (shRNA) against PTP1B (shPTP1B), or a nontargeting control shRNA (shCTRL), were injected in the right flank of male SCID mice (Fig. 5A). Subsequent weekly monitoring revealed that PTP1B depletion significantly delayed tumor occurrence, with a median time to onset of 9 weeks for shPTP1B tumors, compared with 6 weeks for shCTRL xenografts (Fig. 5B). On average, shPTP1B tumors also grew more slowly at a rate of approximately 35 mm³ per week, compared with approximately 175 mm³ a week for shCTRL tumors (Fig. 5C). Importantly, analysis of PTP1B expression in endpoint tumors showed no significant difference between shPTP1B and shCTRL specimens (Supplementary Fig. S5), suggesting that the restoration of endogenous PTP1B levels was required for the growth of shPTP1B clones.

We next attempted to identify the mechanism(s) by which PTP1B promotes tumor growth. Although PTP1B silencing did not affect cell proliferation (Supplementary Fig. S6), it significantly impaired the migratory and invasive properties of LNCaP cells (Fig. 6A and Supplementary Fig. S7 and S8). Importantly, this was exclusively observed in androgen-stimulated conditions, which provides a functional link between AR-induced PTP1B expression and PTP1B function. Combined with our observations in primary tumors, these results clearly supported an AR-dependent tumor-promoting role for PTP1B in androgen-dependent prostate cancer cells.

We also examined the role of PTP1B in androgen-independent prostate cancer cells. In contrast to parental LNCaP cells, PTP1B silencing in C4-2 cells affected migration both in the presence and absence of androgen stimulation (Fig. 6B and Supplementary Fig. S8). Importantly, the AR remains transcriptionally active in androgen-deprived C4-2 cells (23), which suggests that the AR-dependent regulation of PTP1B activity is maintained in AR-expressing androgen-independent cells. Besides, AR depletion in C4-2 cells also triggered an R1881-independent increase in PTP1B levels, an event that was not observed for the PSA gene (Fig. 1C). This indicates that in the context of AR downregulation, other transcription factors can substitute for the AR to promote PTP1B expression and activity in androgen-independent cells. In line with this, PTP1B is highly expressed in AR-negative DU145 cells and PTP1B knockdown also impaired cell migration (Fig. 6C and Supplementary
Figure 4. PTP1B and AR are coexpressed and coamplified in prostate tumors. A, immunohistochemical analyses of a prostate tumor TMA showed a strong PTP1B expression in tumor tissue (top), a strong PTP1B expression in tumor cells (middle, red arrow), and weak expression in normal glands (middle, black arrow). Punctate staining was often observed in normal gland (bottom). B and C, expression of PTP1B is elevated in cancer tissues compared with normal or PIN glands (B; Mann–Whitney, *P < 0.01; Median ± SD), but does not vary according to the degree of histologic differentiation (C; Gleason score). D, Spearman correlations between PTP1B expression and the nuclear localization of AR, Ki-67, and cyclin D1 (green boxes represent P < 0.01). E, PTPNI amplification is significantly associated with metastatic (N = 37) but not primary prostate tumors (N = 181). It significantly correlates with AR amplification (N = 217), an event exclusively observed in metastatic samples. PTPNI amplification is also associated with a subset of genomic alterations (cluster 5), which displays early recurrence after radical prostatectomy (19). F, PTPNI mRNA expression in prostate cancer tumors positively correlates with AR mRNA expression (samples with available mRNA expression data; N = 150).

Overall, these results showed that PTP1B exerts tumor-promoting functions in both androgen-dependent and androgen-independent prostate cancer cells.

**Discussion**

PTP1B is a well-established regulator of metabolic signaling and has become an attractive drug target for the treatment of diabetes and obesity (24). In these diseases, PTP1B counteracts the action of tyrosine kinases such as the insulin receptor and Jak2, leading to insulin resistance and attenuation of leptin signaling, respectively (25, 26). With regard to cancer, this inhibitory function should intuitively suppress oncogenic signaling, yet accumulating evidence suggests that PTP1B can exert the opposite effect (27). Our results corroborate this trend and are in line with previous studies in breast (28–30), colon (31), and gastric cancer (32) in which PTP1B ablation and/or inhibition abrogated the tumorigenic properties of malignant cells. More specifically, we show here that PTP1B promotes prostate cancer cell migration and invasion in vitro and is associated with tumor growth and metastasis in vivo (Figs. 4–6 and Supplementary Fig. S7), which is not unexpected given the number of PTP1B substrates that play a direct role in these processes such as FAK, CrkII, p130Cas, cortactin, and p62dok (27). We have yet to identify the prostate-specific PTP1B substrate(s) responsible for the observed phenotype, but proteomic analyses are underway to characterize the phosphorysine substrates that differentially interact with PTP1B in androgen-deprived and androgen-supplemented conditions (Supplementary Fig. S4), as well as specific substrates associated with cell migration, invasion, and/or in vivo tumor growth.

The mechanisms that modulate PTP1B activity in tumor cells are still largely undefined, but the transcriptional regulation of PTP1B has long been suspected. Indeed, previous characterization of the PTPNI promoter has identified multiple binding sites for transcription factors that are aberrantly regulated not only in metabolic disorders but also in malignant disease. These include Egr-1, Sp1, Sp3 (33), YB-1 (34), NF-kB (35), and HIF (36) that can be activated by a variety of stimuli such as glucose, insulin, proinflammatory cytokines, oncoproteins, or tumor suppressor loss. In addition, an increase in PTP1B levels and/or stability has been reported in breast cancer cells expressing a constitutively active ErbB2 mutant (28, 37), as well as in a subset of B-cell lymphoma cell lines following IL-4 treatment (38). Here, we show that PTPNI transcription can also be modulated by the AR signaling axis, a vital pathway in prostate cancer biology. We also show that PTPNI and AR are coamplified in metastatic tumors (Fig. 4E) and that PTP1B expression positively correlates with both AR expression (Fig. 4F) and nuclear localization (Fig. 4D). All together, these results clearly establish PTPNI as an
AR-regulated gene and corroborate findings of a previous study that reported the coamplification of PTPN1 and AR in a human prostate cancer xenograft model (39). Interestingly, PTPN1 is located on chromosome 20q13, a region frequently amplified in several tumor types (40). Despite conflicting results that question the relevance of this amplified region in hereditary prostate cancer (41), the fact that PTPN1 is frequently amplified in metastatic tumors as well as in a subset of high-risk primary tumors (Fig. 4E) supports a tumor-promoting role for this region in sporadic prostate cancer development and/or progression.

Our high-resolution analysis of AR binding to PTPN1 is the first to show that PTP1B expression is not solely regulated by transcription factors binding to the classical PTPN1 promoter region. In R1881-stimulated LNCaP cells, direct binding of the AR to PTPN1 occurs through 2 enhancers embedded into the large 50.77 kb first intron. In fact, intronic AR binding is quite common: Hu and colleagues recently reported in a genome-wide study that 49% of the AR binding sites from mouse epididymis were intronic, most of them located within the first intron (42), which is also consistent with other studies in LNCaP (22, 43), HPr-1AR (44) and primary human muscle

Figure 5. PTP1B downregulation delays tumor occurrence and decreases tumor growth rates in vivo. A, Western immunoblotting showing PTP1B levels in stable shCTRL and shPTP1B clones (left) relative to Calnexin (right). For the xenograft assays, a total of 40 mice were injected with either shCTRL-1a (n = 10), shCTRL-1b (n = 10), shPTP1B-1a (n = 10), and shPTP1B-2 (n = 10) clones. For data analysis, shCTRL and shPTP1B mice were pooled together. B, tumor development occurred in 15 shCTRL mice and 13 shPTP1B animals. Kaplan-Meier analyses of tumor occurrence show that shPTP1B tumors at a median time of onset (TD50) of 9 weeks compared with 6 weeks for shCTRL clones (Log-rank test, P < 0.05). C, weekly monitoring of tumor growth revealed that shPTP1B tumors grow significantly slower than the shCTRL tumors (Mann-Whitney test; **, P < 0.01).

Figure 6. PTP1B downregulation impedes cell migration in vitro. A and B, LNCaP (A) and C4-2 (B) cells transiently transfected with an siRNA against PTP1B (si1B) or a nontargeting sequence (siSC) were androgen-deprived for 48 hours. Cells were then treated with R1881 or the vehicle for 36 hours before FBS-induced cell migration as described in Materials and Methods. C, DU145 cells were transiently transfected with si1B or siSC for 72 hours before FBS-induced cell migration (Mann-Whitney test; *, P < 0.05; **, P < 0.01 compared with the corresponding siSC; N = 3 ± SEM).
cells (45). Our results also indicate that other transcription factors regulate PTP1B expression in androgen-deprived LNCaP cells, as well as in androgen-independent cells. In fact, AR gene expression programs have been shown to differ between androgen-dependent and androgen-independent cells (46), thus raising the possibility that alternative factors can activate a subset of critical tumor-promoting genes no longer regulated by the AR. This is particularly relevant given recent discoveries showing that frequent prostate cancer alterations, that is, constitutive PI3K signaling and TMPRSS2-ERG expression, can downregulate AR transcriptional activity (8–10). Among potential candidates, the aforementioned NF-κB and MYC factors are particularly attractive given their established roles in prostate cancer progression. For instance, the regulation of PTP1B by NF-κB (35) could reconcile our findings with a previous report linking PTP1B expression to androgen withdrawal-induced neuroendocrine differentiation (14), an aggressive tumor phenotype that has been associated with NF-κB activation (47, 48). Similarly, MYC has been shown to be activated in prostate cancer (36). In fact, AR gene expression programs have been shown to differ between androgen-dependent and androgen-independent DU145 cells (34). Finally, given the location of their respective response elements in the classical PTP1B promoter region, it is also conceivable that these factors cooperate with the AR to regulate PTP1B transcription in low androgen conditions. In line with this, a significant increase in PTP1B mRNA levels was observed in C4-2 cells stimulated with low androgen levels (0.1 nmol/L; Fig. 1C), a phenomenon that was not observed in parental LNCaP cells (Fig. 1D).

In summary, our study uncovers a previously unsuspected regulation of PTP1B by the AR and provides direct evidence for a tumor-promoting role of PTP1B in prostate cancer. The combination of our cellular and in vivo findings advocates for the future preclinical testing of existing antisense or small molecules inhibitors of PTP1B for the treatment of the disease (50). Moreover, our initial profiling of androgen-induced PTPs (Supplementary Fig. S1) justifies further investigation to address the role and significance of other classical PTPs in prostate cancer.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Development of methodology: L. Lessard, D.P. Labbe
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AR-Induced PTP1B Expression in Prostate Cancer


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