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Downregulation of Protein Tyrosine Phosphatase PTPL1 Alters Cell Cycle and Upregulates Invasion-related Genes in Prostate Cancer Cells

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Abstract

PTPL1, a non-receptor type protein tyrosine phosphatase, has been involved in the regulation of apoptosis and invasiveness of various tumour cell types, but its role in prostate cancer remained to be investigated. We report here that downregulation of PTPL1 by small interfering RNA in PC3 cells decreases cell proliferation and concomitantly reduces the expression of cell cycle-related proteins such as cyclins E and B1, PCNA, PTTG1 and phospho-histone H3. PTPL1 downregulation also increases the invasion ability of PC3 cells through Matrigel coated membranes. cDNA array of PTPL1-silenced PC3 cells versus control cells showed an upregulation of invasion-related genes such as uPA, uPAR, tPA, PAI-1, integrin α6 and osteopontin. This increased expression was also confirmed in PTPL1-silenced DU145 prostate cancer cells by quantitative real time PCR and Western blot. These findings suggest that PTPL1 is an important mediator of central cellular processes such as proliferation and invasion.

Keywords: PTPL1, PTPN13, cell cycle, invasion, uPA system

Introduction

Prostate cancer is the most common non-skin cancer among men in most western populations [1]. Death from prostate cancer is primarily due to its metastasis to distal sites, predominantly to the bone [2]. The metastatic process consists of detachment of cancer cells from the primary tumor, adhesion to and degradation of the extracellular matrix to infiltrate the surrounding tissue, the invasion of blood and lymphatic vessels, and finally the establishment of metastases at secondary sites [3]. A prerequisite for invasion into surrounding tissue is a change in the overall cellular machinery controlling these processes and phosphorylation of tyrosine residues is usually a critical step for regulating activities of certain cellular proteins and subsequent activation/deactivation of their downstream signaling events. The family of protein tyrosine phosphatases (PTPs) is divided into two major subtypes: the receptor-like and the non-receptor subtype. Protein tyrosine phosphatase L1 (PTPL1, also known as PTPN13, FAP-1, PTP-BAS or hPTP1E) is a non-receptor tyrosine phosphatase. Besides its carboxy-terminal catalytic domain, the protein structure comprises a kinase non-catalitic C-lobe (KIND) domain and two major domains: a FERM domain involved in plasma membrane and cytoskeleton binding and five PDZ domains. Those latter are protein-protein interaction domains, with an important role in the assembly of supramolecular protein complexes [4, 5]. The role of PTPL1 in cancer remains controversial. There are data to support PTPL1 acting as both tumor promoter and suppressor in a variety of cancer models [5, 6]. In relation to its function as a tumor suppressor, a study of the tyrosine phosphatome of colorectal cancers identified PTPN13 gene as among the most frequently mutated PTPs [7]. Data regarding PTPL1 and cell cycle are scanty. Herrmann *et al.*, [8] demonstrated that PTPL1 is capable to interact with the spindle midzone microtubules and that its localization is regulated in a cell cycle-dependent

manner. Moreover, PTPL1 overexpression induces defects in cytokinesis. Also, it has been shown that PTPL1 functions downstream of cAMP signaling and plays an essential role in controlling meiotic resumption in *Xenopus* oocytes [9]. In relation to invasion, there are several reports addressing a role of PTPL1 in this process. In SKOV3 ovarian carcinoma cells infected with recombinant lentivirus carrying shRNA against PTPL1, Her2 phosphorylation induced by EGF and the invasive potential were increased compared to SKOV3 infected with virus carrying empty vector. PTPL1 inhibits Her2 activity by dephosphorylating the signal domain of Her2 and plays a role in attenuating invasiveness and metastasis of Her2-overexpressing tumor cells [10]. Other study has shown that overexpression of PTPL1 reduced lysophosphatidic acid-induced transwell cell migration and that suppression of endogenous PTPL1 expression significantly enhanced lysophosphatidic acid-induced cell migration in SKOV3 cells [11]. Also, it has been reported that HPV 16-mediated PTPL1 loss synergizes with ErbB2 activity during invasive growth in HPV-related head and neck cancers [12]. Recently, another study has addressed the role of PTPL1 in invasion in MCF7 breast cancer cells. In this setting, PTPL1 downregulation increased tumor growth in athymic mice and also enhanced several parameters associated with tumor progression, including cell proliferation on extracellular matrix components and cell invasion [13].

In this study, we investigate whether PTPL1 influences cell cycle and invasion in prostate cancer cells. We also identify cell cycle and invasion-related downstream targets induced upon PTPL1 silencing that point towards a role for PTPL1 in modulation of cell proliferation and negative regulation of invasion in prostate cancer.

Materials and methods

Cell culture

Human prostate cancer cell lines PC3 and DU145 were obtained from the Interlab Cell Line Collection (Genoa, Italy) and routinely grown in RPMI 1640 supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 50 µg/ml streptomycin, HEPES buffer 10 mM and 1 mM glutamine in a 37°C, humidified incubator under 5% CO_2. Cells were harvested by trypsinization.

Small interfering RNA

Small interfering RNA (siRNA) transfections were carried out using the Dharmafect reagent (Thermo Fisher Dharmacon, Lafayette, CO) according to the manufacturer's instructions. PTPL1-specific siRNA and the negative control siRNA were from Qiagen (Hilden, Germany) and used at 100 nM. Transfection efficiency was tested using PTPL1-specific siRNA labeled with Alexa Fluor 488 (Qiagen). At 24 h and 72 h after silencing, the percentage of transfected cells were 61.7% and 26.7%, respectively.

Western blotting

Cells were lysed in Nonidet P-40 (NP40) lysis buffer (10 mM Tris-HCl (pH 7.5), 150 mM NaCl, 10% glycerol and 1% NP40). Equal amounts of total protein, as determined by using BCA protein assay (Pierce, Rockford, IL), were separated by SDS-PAGE on 4-20% gradient polyacrylamide gels (Invitrogen, Carlsbad, CA). Gels were

electroblotted onto nitrocellulose membranes (GE Healthcare, Europe GmbH, Cerdanyola, Spain). Membranes were stained with Ponceau S to ensure that protein amounts were comparable. For immunodetection, blots were blocked in 1% blocking reagent (Roche, Mannheim, Germany) in 0.05% Tween 20-PBS for 1 h and incubated with primary antibody overnight at 4°C diluted in blocking buffer. Blots were then washed in 0.05% Tween 20-PBS and incubated with either goat anti-mouse (1:10,000; GE Healthcare) or goat anti-rabbit (1:20,000; GE Healthcare) peroxidase-labeled antibodies in blocking buffer for 1 h. Enhanced chemoluminescent system was applied according to the manufacturer's protocol (GE Healthcare). Chemoluminiscent signal was detected on BioMax light films (Kodak, Rochester, NY) at different exposure times. Scanning densitometry was performed with QuantiScan software (Biosoft, Cambridge, UK). Arbitrary densitometric units of the protein of interest were corrected for those of β-actin.

Antibodies

Mouse monoclonal anti-β-actin was from Sigma (St. Louis, MO). Rabbit polyclonal anti-PTPL1, anti-phospho-histone H3 (Ser 10) and anti-uPA were from Santa Cruz Biotechnology (Santa Cruz, CA). Mouse monoclonal anti-PCNA was from Dako (Glostrup, Denmark). Rabbit polyclonal anti-PTTG1 was previously described [14]. Mouse monoclonal anti-cyclin B1 was from BD Biosciences (San Jose, CA), mouse monoclonal anti-cyclin E was from Monosan (Uden, The Netherlands), mouse monoclonal anti-active β-catenin (clone 8E7) was from Millipore (Temecula, CA, USA), rabbit polyclonal anti-integrin α6 was from Cell Signaling (Danvers, MA) and rabbit polyclonal anti-osteopontin was from Neomarkers (Fremont, CA) Dilutions used

in Western blots were anti- PTPL1 (1:500); anti-cyclin E (1:100); anti-PCNA (1:10000); anti-cyclin B1 (1:1000); anti-PTTG1 (1:1000); anti-phospho-Histone H3 (Ser 10) (1:800); anti-uPA (1:500); anti- integrin α6 (1:500); anti-active β-catenin (1: 300); anti-osteopontin (1:100); anti-β-actin (1:20000).

Flow cytometric analysis of cell cycle

Cells were trypsinized and fixed in 70% ethanol. Propidium iodide staining of nuclei was performed with a CycleTest Plus DNA reagent kit (BD Biosciences), and the DNA content was measured with a FACScan instrument (BD Biosciences). Data were acquired with CellQuest Pro software (BD Biosciences). ModFit LT2 software (Verity Software, Topsham, ME) was used to assess cell cycle.

Proliferation assay

Cells were seeded in a 24-wells plate and silenced with control siRNA or PTPL1 siRNA. Cells were tripsinized and counted, using a hemocytometer, after 24 h, 48 h, 72 h and 96 h of silencing respectively.

BrdU incorporation assay

Cells were labeled for 2 h with 10 μM bromodeoxyuridine (BrdU), harvested, and fixed in glycine:ethanol (3:7) at 4°C. A cell proliferation kit (Roche) was used to detect BrdU incorporation into cellular DNA by flow cytometry. Fluorescence data were acquired by using CellQuest Pro software.

Tumor cell migration and invasion assays

To assess cell migration, a wound healing assay was performed. Cells were grown to near confluency on a 24-well plate. 24 h after silencing, a scratch was made on the cell monolayer using a sterile 200 µl pipette tip. The monolayer was washed twice and incubated for another 36 h. Cells were photographed at 0 h and 36 h under a Zeiss inverted microscope with Nomarski optics using a 16× objective and the wound areas were measured with ImageJ software (Rasband, W.S., U.S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2011).

The invasion chamber consists of a 24-well plate with control inserts containing an 8-µm pore-size polyethylene terephthalate (PET) membrane and Matrigel inserts (BD Biosciences) containing an 8-µm pore-size PET membrane coated with a thin layer of extracellular matrix (ECM). Cells silenced during 24 h were tripsinized and suspended in serum-free RPMI 1640 and added to the upper chamber at 2×10^4 cells/insert. The lower chamber was filled with medium containing 5% fetal bovine serum as chemoattractant. After 48 h of culture, the upper surface of the inserts were wiped with cotton swabs, and the inserts were stained with Quick Panoptic (QCA, Tarragona, Spain) and evaluated under light microscopy.

Reverse-transcription and quantitative PCR

Total RNA was extracted using the Purescript RNA Isolation Kit (Gentra, Minneapolis, MN) according to the manufacturer's protocol. 1 µg of total RNA was subjected to DNase I (Invitrogen) digestion and subsequently processed to cDNA by reverse

transcription with Transcriptor First Strand cDNA Synthesis Kit (Roche) according to the manufacturer's protocol. PCR reactions were performed in a 25 µl reaction volume on the SmartCycler II Real-Time PCR Detection System (Cepheid, Sunnyvale, CA) using the QuantiMix Easy SyG kit (Biotools, Madrid, Spain) and 500 nM of each specific primer. The levels of PTPL1 and the housekeeping gene HPRT1 in each sample were quantified by measuring the Ct values in duplicate. These mean Ct values were transformed to quantities using the delta-Ct method [15]. The sample with the lowest value was assigned the value 1. The quantity of PTPL1 transcript was divided by the quantity of HPRT1 to obtain a normalized value.

Microarray hybridization and analysis

Total RNA was extracted from siRNA control and siRNA PTPL1 PC3 cells with RNeasy® Mini kit (Qiagen). Three micrograms of total RNA were biotin-labeled using GEArray AmpoLabeling LPR Kit (SABiosciences, Frederick, MD) and used to hybridize 96-gene cDNA pathway-specific arrays (Human extracellular & adhesion molecules GEArrays, SABiosciences), following manufacturer's instructions. Chemoluminiscent signal was detected on BioMax light films at different exposure times. Films were scanned for densitometric analysis by using QuantiScan software. Raw densitometric values were corrected by substracting the average background of negative controls. Normalization was completed with the average of non-saturated signals from four different housekeeping genes.

Statistics

Data comparing differences between two conditions were statistically analyzed, when indicated, using paired Student's *t* test. Differences were considered significant when $P < 0.05$.

Results

PTPL1-silenced PC3 cells exhibit changes in cell cycle profile

We examined if PTPL1 silencing could modify the cell cycle distribution in prostate cancer PC3 cells. We therefore analyzed the DNA content of propidium iodide stained siRNA control and siRNA PTPL1 PC3 cells by flow cytometry at two time points, and percentage of cells in different phases of the cell cycle was computed. Cells silenced for PTPL1 during 72 h (Fig. 1A) showed an increase of 10.1% in G_0/G_1 phase cells and a concomitant decrease of 7.0% in S phase and 3.1% in G_2/M phase populations respect to siRNA control cells. Moreover, the difference in G_0/G_1 phase cells between siRNA control and siRNA PTPL1 cells was statistically significant ($P < 0.05$ from Student's *t* test) (Fig. 1B). Similarly, PTPL1 silenced cells during 96 h (Fig. 1A) showed an increase of 8.9% in G_0/G_1 phase cells respect to siRNA control cells ($P < 0.05$ from Student's *t* test) and a decrease of 6.6% and 2.3% in S and G_2/M phase populations respectively (Fig. 1B). In order to confirm these results, a proliferation assay was realized by counting the cell number after 24 h, 48 h, 72 h and 96 h of PTPL1 or siRNA control silencing. At all time points, cell number was lower in PTPL1-silenced cells than in siRNA control cells, and the doubling time from 0 h to 96 h for siRNA control cells was 30.4 h and for siRNA PTPL1 cells was 38.2 h. Moreover, the difference between siRNA control and siRNA PTPL1 cells at 72 h and 96 h was statistically significant ($P < 0.05$ from Student's *t* test) (Fig. 1C). To further confirm these results,

we realized a proliferation assay by means of detection of BrdU incorporation in DNA synthesizing cells by flow cytometry. According to cell cycle results, PC3 cells silenced for PTPL1 during 72 h showed a decrease of 8.3% in proliferating cells compared with siRNA control cells (20.5% ± 3.3 *versus* 28.8% ± 4.0), also in a statistically significant way (Fig. 1D). Finally, a Western blot analysis of several cell cycle-related proteins was performed after 72 h and 96 h of PTPL1 silencing. As presented in figure 1E, PC3 cells silenced for PTPL1 at both time points showed decreased cyclins E and B1, PTTG1, phospho-histone H3 and PCNA expression levels, according to diminished proliferation found in these conditions.

PTPL1 silencing induces a more invasive phenotype in PC3 cells

Next, a wound healing assay was performed to test the effect of PTPL1 silencing on cell migration (Fig. 2A). The difference between siRNA control and siRNA PTPL1 cells was not statistically significant, suggesting the absence of a migratory phenotype. Then, to test the invasion ability of PC3 cells silenced for PTPL1, we realized a Matrigel assay. PC3 siRNA control and siRNA PTPL1 cells were allowed to migrate through uncoated or Matrigel-coated inserts. Cells that migrated to the lower surface of each type of insert were photographed and counted under a light microscope. Percent of invasion was calculated as the number of cells migrating through the Matrigel insert (+ECM) relative to the migration through the control insert (–ECM). We observed that silencing of PTPL1 increases the percentage of invading cells with 13.7% (23.6% ± 6.1 *versus* 37.3% ± 4.2) (Fig. 2B), in a statistically significant way ($P < 0.05$). Therefore, downregulation of endogenous PTPL1 expression increases the invasive potential of PC3 cells.

PTPL1-silenced PC3 and DU145 cells show increased expression of invasion-related genes

To confirm matrigel results, we performed a cDNA array of invasion-related genes. The gene expression profile of PTPL1-silenced PC3 cells as compared to siRNA control PC3 cells showed a set of differentially expressed genes. We focused on upregulated genes that were the following (fold increase between parentheses): Osteopontin (2,21), integrin α6 (2,16), PAI-1 (2,01), integrin β5 (1,95), uPA (1,74), tPA (1,73), uPAR (1,54) and laminin γ1 (1,51). Then, some of these upregulated genes were validated by quantitative real-time PCR (qPCR). Fig. 3A shows downregulation of PTPL1 mRNA in PC3 cells. qPCR results completely agree with those from the cDNA array. We observed an upregulation of uPA, uPAR, tPA, PAI-1 and integrin α6 in PTPL1 silenced cells as compared with siRNA control PC3 cells (Fig. 3B). This increased expression was statistically significant. To examine whether the results obtained in PC3 cells could be extended to another prostate cancer cell line, we perform the same qPCR analysis with control and PTPL1-silenced DU145 cells. Percentage of PTPL1 downregulation in DU145 cells is shown in Fig. 3A. uPA, uPAR and integrin α6 were also upregulated in PTPL1-silenced DU145 cells, in a statistically significant way (Fig. 3B).

Invasion-related proteins are upregulated in PC3 and DU145 cells silenced for PTPL1

Finally, to further verify array and qPCR results, we performed a Western blot analysis of uPA, integrin α6 and osteopontin proteins in control and PTPL1 silenced PC3 and DU145 cells during 72 h and 96 h. We also examined the expression level of active β-

catenin, another protein involved in tumor invasion and a transcriptional regulator of uPA and uPAR in colorectal carcinoma [16, 17]. uPA and integrin α6 proteins were increased in both PTPL1-silenced PC3 and DU145 cells during 72 h and 96 h, confirming previous qPCR results. β-catenin was more active in both cell lines silenced for PTPL1 at both time points, and osteopontin expression was also elevated in both cell lines after 96 h of PTPL1 silencing (Fig.4). All these data point towards a role of PTPL1 in the regulation of invasion-related proteins such as uPA, integrin α6, osteopontin or β-catenin.

Discussion

Prostate carcinogenesis involves a multistep progression from precancerous cells to cells that proliferate locally and then metastasize. The influence of protein tyrosine phosphatase PTPL1 in cell cycle and proliferation has not been extensively studied. Available data point towards a role in cytokinesis in HeLa cells [8] and in meiosis in *Xenopus* oocytes [9]. We have shown here that downregulation of endogenous PTPL1 by small interfering RNA induces changes in cell cycle profile. The expression level of several regulators of cell cycle also supports this result. Cyclin E is essential to drive G_1/S transition [18] and PCNA plays a critical role in DNA replication [19]; thus, according to diminished S phase, cyclin E and PCNA expression level are also diminished in PTPL1-silenced PC3 cells. Moreover, cyclin B1 and PTTG1 proteins are elevated in mitosis [18, 20] and histone H3 becomes phosphorylated at Ser 10 at the end of prophase [21]. Also in agreement with the G_2/M phase cells decrease, cyclin B1, PTTG1 and phospho-histone H3 protein levels were diminished in PTPL1-silenced cells. These data could indicate a role for PTPL1 in cell cycle regulation, as PTPL1 silencing impairs progression through S and G_2/M phases. In relation to invasion, it has

been reported that PTPL1 inhibit invasiveness of different cancer cells [10-13]. In line with these results, PTPL1 silencing in PC3 cells induces an increase in the percentage of invading cells (13.7%), supporting a role of PTPL1 in inhibition of invasion. This role was further supported by the cDNA array and qPCR results. Between the differentially upregulated genes in the array performed with siRNA control and PTPL1 PC3 cells, we found several components of the urokinase plasminogen activator (uPA) system, osteopontin and integrin α6. The uPA system consists of urokinase-type plasminogen activator (uPA), its receptor (uPAR), tissue-type plasminogen activator (tPA), plasminogen, and plasminogen activator inhibitors (PAIs). uPA is secreted as a zymogen (pro-uPA), and activation of pro-uPA is accelerated by its binding to uPAR [22]. The active uPA catalyzes the conversion of plasminogen to plasmin, which in turn degrades a variety of extracellular matrix components. Therefore, uPA and its receptor are considered to be regulators of tumor metastasis at different stages and represent an important therapeutic target. The activity and turnover of uPA are regulated by another member of the system, PAI-1, which is the primary endogenous inhibitor of uPA. According to its inhibitory function, PAI-1 was originally predicted to suppress cancer proliferation and metastasis. However, high levels of PAI-1 indicate a poor prognosis for survival in some human cancers [23, 24]. Additionally, it has been shown that both tumor derived uPA and tumor-stroma-induced PAI-1 play important roles in intraosseous metastatic prostate cancer growth through regulation of uPA-uPAR-PAI-1 axis by autocrine/paracrine mechanisms [25]. Therefore, the uPA system is particularly associated with the process of metastasis. Specifically in prostate cancer, overexpression of both uPA and PAI-1 is associated with adverse pathologic features and higher risk of overall and aggressive disease recurrence in men treated with radical prostatectomy for clinically localized cancer [26]. Moreover, it has been demonstrated in PC3 cells that

RNA interference-directed knockdown of uPA and uPAR inhibits invasion, survival, and tumorigenicity *in vivo* [27]. Integrin α6, a laminin receptor, is also associated with an increased invasive potential of human prostate cancer cells in vitro and with the progression of human prostate carcinoma in human tissue biopsy material [28, 29]. Integrin α6 exists in the classical form (140 kDa) and in a smaller form (70 kDa) called α6p. This variant, present in prostate cancer tissue but absent in normal prostate, is produced by proteolytic cleavage of the integrin α6 by uPA in a plasmin-independent manner, enhancing cell invasion and migration on laminin [30]. Integrin α6 cleavage also allows extravasation of human prostate cancer cells from circulation to bone [31]. Osteopontin is overexpressed in a variety of cancers and is involved in invasion and metastasis [32]. Increased osteopontin expression correlates with Gleason score and decreased survival in prostate cancer patients [33]. In prostatectomy specimens, osteopontin expression is independently associated with biochemical recurrence [34]. Interestingly, osteopontin stimulates the secretion of uPA in breast cancer cells [35].

In conclusion, our data support a role for PTPL1 in cell cycle and invasion. PTPL1 downregulation in PC3 cells decreases cell proliferation, as well as expression of cyclins E and B1, PCNA, PTTG1 and phospho-histone H3 proteins. Suppression of PTPL1 endogenous expression in PC3 cells also elicits a more invasive phenotype in Matrigel assays. The increased invasive potential may be explained, at least in part, by the induction of well known mediators of invasion such as uPA system, osteopontin or β-catenin, that were also induced in PTPL1-silenced DU145 cells. Interestingly, it has been reported that PTPL1 interacts with APC (Adenomatous Polyposis Coli), a β-catenin interacting protein [36], thus the influence of PTPL1 on uPA system,

osteopontin, integrin α6 and β-catenin suggests that PTPL1 acts as a tumor suppressor whose downregulation or absence might relate to prostate cancer progression.

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Disclosure of Potential Conflict of Interest

The authors declare that they have no conflict of interest.

Figure legends

Fig. 1

PTPL1 downregulation induces changes in cell cycle. PC3 cells were silenced with a non-targeting control siRNA or with PTPL1 siRNA during 72 h and 96 h. (A) Downregulation of PTPL1 expression was monitored by RT-PCR. The quantity of PTPL1 transcript was divided by the quantity of HPRT1 to obtain a normalized value. (B) Cell cycle analysis by flow cytometry. Percentage of cells in different phases of the cell cycle was computed with ModFit software. (C) Proliferation assay. Cells silenced with control or PTPL1 siRNA were counted after 72 h and 96 h of silencing. (D) Flow cytometric analysis of BrdU incorporation after 72 h of silencing. (E) Western blot analysis of PTPL1, cyclin E, PCNA, cyclin B1, PTTG1 and phospho-histone H3 proteins is shown. β-actin expression level was assessed to ensure equal protein loading. Densitometric analysis of protein expression levels are shown as histograms. Data represent the mean ± S.E.M. * $P < 0.05$ and ** $P < 0.01$ from Student's *t* test. The experiments were performed at least three times.

Fig. 2

PTPL1 silencing increases the invasion ability of PC3 cells *in vitro*.

(A) Wound healing assay was performed to determine siRNA control and siRNA PTPL1 PC3 cells motility. Quantification was carried out by measuring the wound area at 0 h and 36 h and calculating the percentage of wound closure for each condition. The experiment was performed three times. (B) Invasion assay with PC3 cells silenced with a non-targeting control siRNA or with PTPL1 siRNA during 24 h. Cells were then trypsinized, suspended in serum-free media and added to PET inserts uncoated (–ECM) or coated with Matrigel (+ECM). Cells were allowed to invade during 48 h and inserts

were processed as described in Materials and methods. The experiment was performed five times with each sample in duplicate. Data represent the mean ± S.E.M. * $P < 0.05$ from Student's *t* test, comparing siRNA control *versus* PTPL1 silenced cells. Representative photographs are shown.

Fig. 3

Quantitative real-time PCR analysis of PTPL1 and invasion-related genes in PC3 and DU145 cells silenced with a non-targeting control siRNA or with PTPL1 siRNA during 72 h. (A) Downregulation of PTPL1 expression in PC3 and DU145 cells. The quantity of PTPL1 transcript was divided by the quantity of HPRT1 to obtain a normalized value. Data represent the mean of three experiments ± S.E.M. (B) Analysis of uPA, uPAR, tPA, PAI-1, integrin α6 (ITGA6) and osteopontin (OPN) expression in control and PTPL1-silenced PC3 and DU145 cells. The quantity of each transcript was divided by the quantity of HPRT1 to obtain a normalized value. Data represent the mean of three experiments ± S.E.M. * $P < 0.05$ and ** $P < 0.01$ from Student's *t* test, comparing siRNA control *versus* PTPL1 silenced cells.

Fig. 4

Western blot analysis of PTPL1, active β-catenin, uPA, integrin α6 and osteopontin proteins in PC3 (A) and DU145 cells (B) silenced with a non-targeting control siRNA or with PTPL1 siRNA during 72 h and 96 h. β-actin expression level was assessed to ensure equal protein loading. Densitometric analysis of protein expression levels are shown as histograms. Bars represent the mean ± S.E.M. * $P < 0.05$ and ** $P < 0.01$ from Student's *t* test. The experiments were performed at least three times.

Figure 1

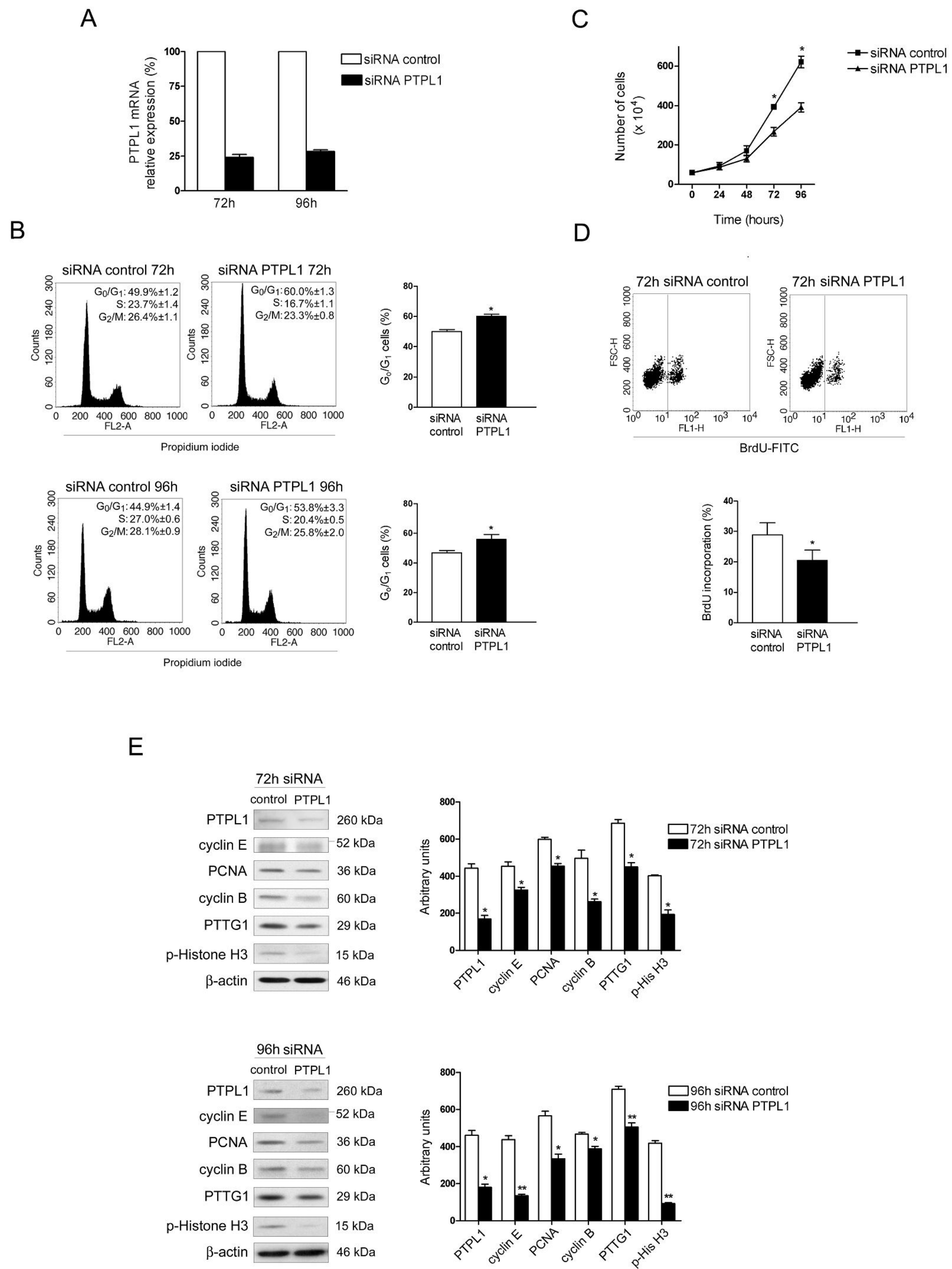

Figure 2

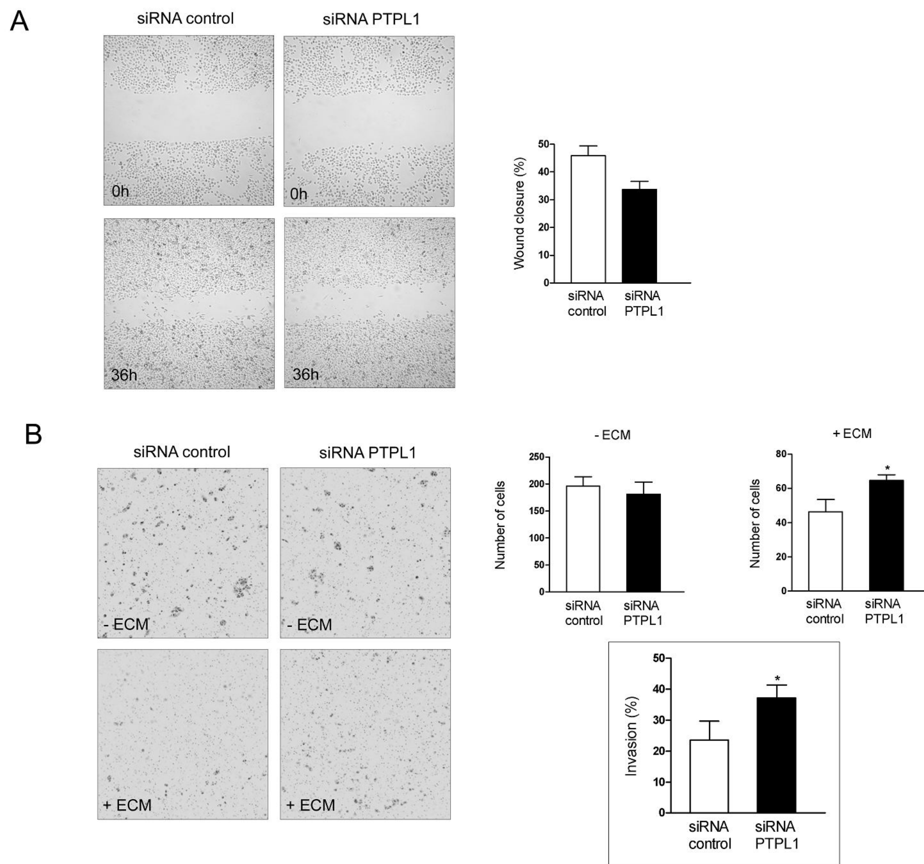

Figure 3

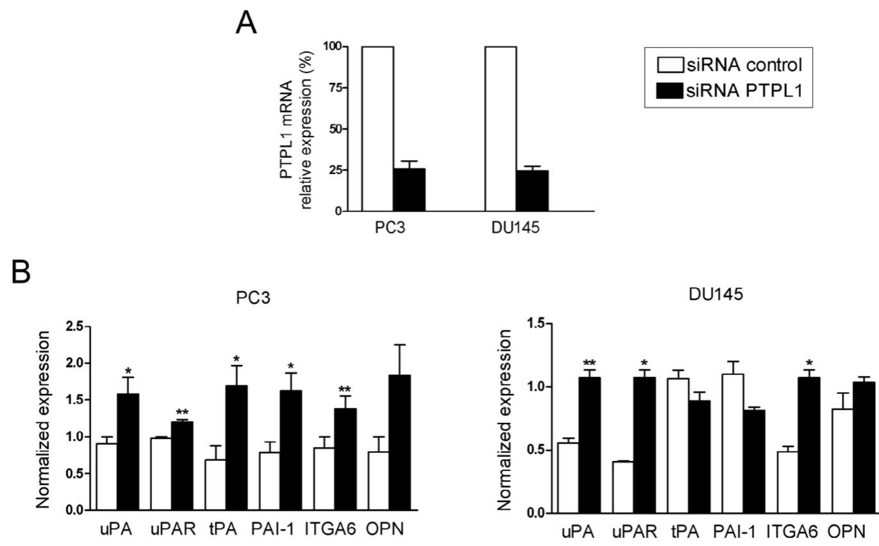

Figure 4