Receptor-like protein tyrosine phosphatase κ negatively regulates the apoptosis of prostate cancer cells via the JNK pathway

PING-HUI SUN, LIN YE, MALCOLM D. MASON and WEN G. JIANG

Metastasis and Angiogenesis Research Group, Institute of Cancer and Genetics, Cardiff University School of Medicine, Cardiff CF14 4XN, UK

Received May 20, 2013; Accepted June 25, 2013

DOI: 10.3892/ijo.2013.2082

Abstract. Receptor-like protein tyrosine phosphatase κ (PTPRK) has been indicated as a putative tumour suppressor in primary central nervous system lymphomas and colorectal cancer. The present study investigated the expression of PTPRK in prostate cancer and the biological impact of PTPRK on prostate cancer cells. The expression of the PTPRK protein and transcript in prostate cancer was examined using IHC and PCR. Knockdown of PTPRK in prostate cancer cells was performed using a specific anti-PTPRK transgene. The impact of PTPRK knockdown on prostate cancer cells was evaluated using in vitro cell models and the apoptosis was analysed using flow cytometry. PTPRK expression was increased in prostate cancer tissues and knockdown of PTPRK in PC-3 cells suppressed the in vitro cell growth in which an increased apoptotic population was seen. Accompanied with the knockdown of PTPRK, increased expression of caspase-3, caspase-8 and p53, and a decreased ID1 expression were evident in the cells. Furthermore, an increased tyrosine phosphorylated c-Jun N-terminal kinase (JNK) was seen in the PTPRK knockdown cells. The effect on apoptosis was diminished by a JNK inhibitor. In conclusion, PTPRK knockdown resulted in increased apoptosis leading to the inhibition of in vitro growth of prostate cancer cells. PTPRK is a key factor in coordinating apoptosis via the regulation of MAPK pathways, in particular the JNK pathway in prostate cancer cells.

Introduction

Apoptosis, also known as programmed cell death, can be induced by both intrinsic and extrinsic pathways. The intrinsic pathway is also known as mitochondria pathway and is regulated by different molecules such as XIAP (inhibitors of apoptosis) and Bcl-2 family. The extrinsic pathway is activated by tumour necrosis factors (TNFs) and its down-stream transcription factors are also involved in intrinsic pathway of apoptosis. The extrinsic pathway involves the promotion of apoptosis via the ligand-activation of death receptors (1). For example, TNF-α binding to its receptors leads to form two complexes; one can lead to activation of pro-survival NF-κB pathway and another one activates the apoptosis pathway through Fas associated death domain (FADD) and activation of caspase-8 (2). Protein tyrosine phosphatases (PTPs) play crucial roles in regulation of cell functions such as proliferation and survival. PTPs deficiency leads to several physiologic abnormalities and dysregulation of apoptosis resulted by PTP deficiency may play a profound role in these disorders. For example, PTP-PEST (tyrosine-protein phosphatase non-receptor type 12, PTPN12) makes cells more sensitive to the anti-Fas and TNF-α induced apoptosis. PTP-PEST is cleaved by caspase-3, which increases its catalytic activity and changes its protein structure. Furthermore, the PTP-PEST proteolysis facilitates cellular detachment during apoptosis (3). Unlike PTP-PEST, PTP1B (PTPN1) has no caspase cleavage site and it is not cleaved by caspas during apoptosis. However, there is a report showing activated PTP1B contributes to STAT3 dephosphorylation and induces apoptosis in human glioma cells (3,4). PTP1B and its catalytic activity are required for inositol-requiring kinase 1 (IRE1) signalling which activates JNK and p38 MAPK. Moreover, endoplasmic reticulum (ER)-induced apoptosis is decreased in cells lacking PTP1B and PTP1B null mice are resistant to Fas-induced liver damage because of absence of PTP1B-mediated suppression of pro-survival NF-κB and ERK signalling (5,6). In addition, p53 is required for T cell protein tyrosine phosphatase (TC-PTP, PTPN2) overexpression induced apoptosis. In breast cancer cells, MCF-7, the accumulation of p53 in response to TC-PTP overexpression directly increases expression of Apaf-1 and pro-apoptotic α-isoformal of caspase-1 (7,8).

Currently, the role played by PTPRK in cancer remains largely unknown. The present study examined the expression of PTPRK in prostate cancer and the impact of this molecule on prostate cancer cell apoptosis.

Materials and methods

Cell lines and cells culture. PC-3 (human prostate cancer cell line) and MRC-5 (fibroblast cell line) were obtained from the European Collection of Animal Cell Cultures (ECACC,
Table I. Primer sequences used in the current study.

<table>
<thead>
<tr>
<th>Molecular</th>
<th>Forward primers (5'-3')</th>
<th>Reverse primers (5'-3')</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTPRK</td>
<td>AATTACAATGTTAGGGGAGA</td>
<td>CCACCTTTCCACCTGAAGTA</td>
</tr>
<tr>
<td>PTPRK (Q-PCR)</td>
<td>AATTACAATGTTAGGGGAGA</td>
<td>ACTGAACCCTGACCGTACATATTGTGTGACGATGAAAGC</td>
</tr>
<tr>
<td>GAPDH</td>
<td>GGCCTGTTTTAATCTCTGGTA</td>
<td>GACTGGTGCTAGTGCTCCTT</td>
</tr>
<tr>
<td>GAPDH (Q-PCR)</td>
<td>CTGAGTACGTGCTGAGGAC</td>
<td>ACTGAACCCTGACCGTACATGATCCAGACTCTGACCTTT</td>
</tr>
<tr>
<td>c-Myc</td>
<td>TGCTCCATGAGGAGACAC</td>
<td>TTTGATTTTTTCCACACTC</td>
</tr>
<tr>
<td>c-Myc (Q-PCR)</td>
<td>TGCTCCATGAGGAGACAC</td>
<td>ACTGAACCCTGACCGTACATGATCCAGACTCTGACCTTT</td>
</tr>
<tr>
<td>ID1</td>
<td>TCAACGGCCAGATCTG</td>
<td>ACTGAACCCTGACCGTACATGATCCAGACTCTGACCTTT</td>
</tr>
<tr>
<td>p53</td>
<td>ATCCCTCACATCATCACACT</td>
<td>ACTGAACCCTGACCGTACATGATCCAGACTCTGACCTTT</td>
</tr>
<tr>
<td>Caspase-3</td>
<td>GGGGTGTCAAAATACTACCG</td>
<td>ACTGAACCCTGACCGTACATGATCCAGACTCTGACCTTT</td>
</tr>
<tr>
<td>Caspase-8</td>
<td>AGAAAGGAGGAGTGGGAAG</td>
<td>ACTGAACCCTGACCGTACATGATCCAGACTCTGACCTTT</td>
</tr>
<tr>
<td>Caspase-9</td>
<td>AAGCCCAAGCTCTTTTC</td>
<td>ACTGAACCCTGACCGTACATGATCCAGACTCTGACCTTT</td>
</tr>
</tbody>
</table>

Bold text indicates the specific Z sequence for Real-time PCR.

Salisbury, UK). DU-145, LNCaP, CA-HPV-10 and PZ-HPV-7 were obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA) and PNT-1A and PNT-2C2 were generously given by Professor Norman Maitland (University of York, York, UK). Cells were routinely cultured with Dulbecco’s modified Eagle's medium containing 10% fetal calf serum and antibiotics at 37°C with 5% CO₂.

*Human prostate specimens.* Fresh tissue samples were collected immediately after surgery and stored at -80°C until use, with approval of the Bro Taf Health Authority local research ethics committee. All patients were informed and participated with written consent. All the specimens were verified by a consultant pathologist.

*Immunohistochemical staining.* Frozen specimens of mammary tissues were cut at a thickness of 6 µm using a cryostat (Leica CM 1900, Leica Microsystems UK Ltd., Buckinghamshire, UK). The sections were mounted on super frost plus microscope slides, air dried and then fixed in the mixture of 50% acetone and 50% methanol for 15 min. After 10 min air-drying, the slides were placed into OptiMax Wash Buffer (BioGenex, San Ramon, CA, USA) for 5 min to rehydrate. The slides were incubated in blocking buffer with 10% horse serum for 20 min and probed anti-PTPRK antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) for 1 h. After three times washes, the slides were incubated with biotinylated secondary antibody (MultiLink swine anti-goat/mouse/rabbit immunoglobulin, Dako Inc., Carpinteria, CA, USA). After washing, slides were placed in avidin-biotin complex (ABC, Vector Labs) for 30 min. Diaminobenzidine chromogen (Vector Labs) was then added to the slides and incubated in the dark for 5 min. The slides were counterstained with Mayer’s haematoxylin for 1 min and dehydrated in ascending grades of ethanol before clearing in xylene and mounting with a cover slip.

*Reverse transcription-PCR.* Total RNA extraction from frozen tissues and culture cells was performed using Tri Reagent (Sigma-Aldrich Inc., Saint Louis, MO, USA). Following reverse transcription into cDNA, PCR was carried out using ReadyMix PCR Reaction Mix (Sigma-Aldrich Inc.). Primer sequences are shown in Table I. Reactions were carried out at the following conditions: 94°C for 5 min, 30 cycles of 94°C for 30 sec, 55°C for 30 sec and 72°C for 30 sec, followed by a final extension of 7 min at 72°C. PCR products were separated on a 1.5% agarose gel and photographed after staining with ethidium bromide.

*Real-time quantitative PCR.* The level of PTPRK transcripts in the breast cancer cohort was determined using a real-time quantitative PCR, based on technology which was modified from a method reported previously (9). Primer sequences are shown in Table I. The reaction was carried out on an iCycler iQ™ (Bio-Rad, Hertfordshire, UK) which is equipped with an optical unit that allows real-time detection of 96 reactions. The reaction conditions were: 94°C for 12 min, 90 cycles of 94°C for 15 sec, 55°C for 40 sec (the data capture step) and 72°C for 20 sec. The levels of the transcripts were generated from an internal standard that was simultaneously amplified with the samples.

*Construction of ribozyme transgene targeting human PTPRK and the establishment of corresponding stable transfectants.* Anti-human PTPRK hammerhead ribozymes were designed based on the secondary structure of the gene transcript and generated using the Zuker RNA mFold program (10). The ribozymes were synthesized and then cloned into a pEF6/V5-His TOPO vector (Invitrogen, Paisley, UK). The verified ribozyme transgenes and empty plasmids were transfected into PC-3 (PC-3PTPRKkd and PC-3PTPRKkdV5His) cells, respectively, using an EasyjetPlus electroporator (EquiBio, Kent, UK). After a period of selection with 5 µg/ml blasticidin (up to 10 days), the verified transfectants were cultured in maintenance medium containing 0.5 µg/ml blasticidin. Primer sequences of the ribozymes were 5’-CTGCAGTTTGCTCTTCTTTTCAATTAATATCTGTAGTGCCGTAGGA-3’ and 5’-ACTAGTTCTATCCTCTTCTCTATGTTTTCGTCTTTCACGGACT-3’.
**Table II. Primary antibodies used in the current study.**

<table>
<thead>
<tr>
<th>Protein target</th>
<th>Cat. no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse anti-GAPDH</td>
<td>SC-47724</td>
</tr>
<tr>
<td>Rabbit anti-PTPRK</td>
<td>SC-28906</td>
</tr>
<tr>
<td>Mouse anti-p38</td>
<td>SC-7972</td>
</tr>
<tr>
<td>Rabbit anti-JNK</td>
<td>SC-571</td>
</tr>
<tr>
<td>Rabbit anti-caspase-3</td>
<td>SC-7148</td>
</tr>
<tr>
<td>Mouse anti-caspase-8</td>
<td>SC-70501</td>
</tr>
<tr>
<td>Mouse anti-caspase-9</td>
<td>SC-17784</td>
</tr>
<tr>
<td>Mouse anti-phosphotyrosine</td>
<td>SC-508</td>
</tr>
</tbody>
</table>

**Cell growth assay.** Prostate cancer cells (3,000 cells/well) were plated into 96-well plates. Cells were fixed in 4% formaldehyde after 1 and 5 days of culture. The cells were then stained with 0.5% crystal violet. Absorbance was determined at a wavelength of 540 nm using a spectrophotometer (BioTek, ELx800). Growth rate at day 5 (%) = absorbance of day 5/absorbance of day 1 x 100.

**Analysing apoptosis using flow cytometer.** Cells (1x10^4) were seeded into flasks and underwent different treatments with 200 nM p38 inhibitor (SB203580), 200 nM JNK inhibitor (SP600125) and serum free control for three days then both the adherent cells and floating cells were collected. Following cell collection, experiments were carried out using the Annexin V kit (Santa Cruz Biotechnology) and analysed using PartecCyFlow® SL flow cytometry and the accompanying FloMax software package (Partec GmbH, Münster, Germany).

**Immunoprecipitation (IP) and western blot analysis.** Protein was extracted from 75-cm^2 flask which were initially seeded with 4x10^6 cells and cultured overnight. The protein samples were incubated with primary antibodies (Table II) at 4°C for 1 h then incubated for another hour after the addition of conjugated A/G protein agarose beads (Santa Cruz Biotechnology). The samples were washed twice with SDS-free lysis buffer before being boiled with 1X sample buffer (Sigma-Aldrich Inc.).

Equal amounts of protein were separated by SDS-PAGE and blotted onto nitrocellulose membranes. The membrane was then probed with the respective primary antibodies and corresponding peroxidase-conjugated secondary antibodies. Protein bands were visualised using a chemiluminescence detection kit (Luminata, Millipore) and photographed using UVITech imager (UVITech Inc.).

**Statistical analysis.** Statistical analysis was performed using SigmaPlot 11 (SPSS Inc., Chicago, IL, USA). Data were calculated as the mean ± SD and the Student's t-test was used for normally distributed data. Fisher's exact test was used for comparison of two independent groups. Each assay was performed at least three times independently. p<0.05 was considered statistically significant.

**Results**

**PTPRK expression in prostate tissues and cell lines.** There is a higher PTPRK expression level in prostate cancer tissues compared with normal prostate tissues using IHC staining (Fig. 1A). Fig. 1B shows that PTPRK is likely to be more frequently expressed in prostate cancer tissues (12/18, 66.7% positive, p=0.143 vs. normal using Fisher's exact test) than normal prostate tissues at mRNA level (4/11, 36.7% positive). Furthermore, the expression of PTPRK was also examined in the prostate cell lines PC-3, DU-145, LNCaP, CA-HPV-10, PZ-HPV-7, PNT-1A, PNT-2C2 and the fibroblast cell line MRC-5. Fig. 1C shows that PTPRK is consistently expressed in all the prostate cell lines, except PNT-1A, where it appears to have a lower level of PTPRK mRNA.

**Verification of PTPRK knockdown in PC-3 cells.** The expression of PTPRK was knocked down using ribozyme transgenes targeting human PTPRK mRNA. This was performed in the prostate cancer cell line PC-3, which expressed PTPRK (Fig. 1C). The knockdown of PTPRK was verified in the transfectants using RT-PCR (Fig. 2A), real-time quantitative PCR (Fig. 2B), western blot analysis (Fig. 2C) and PTPRK protein band volume of three repeats which was normalised against corresponding internal control (Fig. 2D). Decreased expression of PTPRK was seen in PC-3ΔPTPRKkd cells which were transfected with ribozyme transgenes compared to their corresponding empty plasmid control.

**Knockdown of PTPRK reduces in vitro cell growth assay.** Knockdown of PTPRK in PC-3 cells exhibited an impact on cell growth. The PC-3ΔPTPRKkd cells showed a decreasing growth rate at day 5 (634.33±58.76, p<0.001) compared with PC-3ΔEF (739.35±24.14) (Fig. 3A).

**PTPRK knockdown affects apoptosis in prostate cancer cells.** There was an increased proportion of apoptotic cells (both early and late) in PC-3ΔPTPRKkd (34.90%) compared to the PC-3ΔEF control (16.14%) and PC-3 wild-type (8.05%) cells (Fig. 3B).

**Expression of caspases in the PTPRK knockdown cells.** Caspase-3 is an indicator of apoptosis at the end-stage. Caspase-8 and caspase-9 are up-stream key factors of apoptosis; caspase-8 is normally activated by external signalling, and caspase-9 is activated by internal signalling. Therefore, in order to further determine whether PTPRK knockdown has an effect on prostate cancer cell apoptosis, levels of caspase-3, -8 and -9 were examined in the transfected PC-3 cells using PCR, Q-PCR, and western blot analysis. PC-3ΔPTPRKkd cells demonstrated significantly higher expression levels of caspase-3 and caspase-8, but not caspase-9, compared with PC-3ΔEF controls cells in both mRNA and protein levels (Fig. 4).

**Expression of other genes/molecules relevant to apoptosis and/or the cell cycle.** As PTPRK knockdown was shown to promote the progression of apoptosis, the expression of a number of relevant genes was examined using RT-PCR. An upregulation of p53 was also seen in the PTPRK knockdown cells, whilst a downregulation of ID1 appeared in the same cells.
Figure 1. Expression of PTPRK in prostate cancer. (A) The immunohistochemical staining revealed an increase staining of PTPRK in prostate cancer tissues (n=17) compared with normal tissues (n=8). (B) PTPRK is more frequently expressed in prostate cancer tissues compared with normal tissues. (C) Expression of PTPRK mRNA in cell lines using RT-PCR.

Figure 2. Confirmation of PTPRK knockdown in PC-3 cells. Knockdown of PTPRK was seen in PC-3 PTPRKkd cells compared with empty plasmid control (PC-3 pEF cells) using (A) RT-PCR, (B) real-time quantitative PCR and (C) western blot analysis. (D) PTPRK protein band volume of three repeats, which was normalised against corresponding internal control. *p<0.05.
No effect on the expression of c-Myc was observed in the PC-3 \( \text{PTPRKkd} \) cells compared with the control. The role of JNK in PTPRK knockdown-associated apoptosis. The mitogen-activated protein kinase (MAPK) pathway is the major signalling pathway involved in cellular proliferation and it affects both apoptosis and the cell cycle. As knockdown of PTPRK has been shown to impact apoptosis, its effect on the expression and activations of p38, JNK and ERK in PC-3 cells was analysed. The PC-3 \( \text{PTPRKkd} \) cell showed a similar level of protein expression overall in p38, JNK and ERK compared with their pEF control. Furthermore, levels of phosphorylated p38, JNK and ERK were analysed using immunoprecipitation and western blot analysis. A marked increase active p-JNK (Tyr) was seen in the PTPRK knockdown cells and an increased level of active p38, suggesting that JNK and p38 may play a role in the regulation of apoptosis in the PC-3 \( \text{PTPRKkd} \) cells (Fig. 6A).

Additionally, PC-3 cells were treated with p38 and JNK inhibitors for 72 h and analysed for apoptosis using flow cytometry. The PC-3 \( \text{pEF} \) control cells showed no effect on treatment of cells with p38 and JNK inhibitors, but apoptosis of untreated PC-3 \( \text{PTPRKkd} \) cells (26.70±2.87%) was dramatically increased compared with PC-3 \( \text{pEF} \) cells (14.61±1.74%), \( p<0.001 \). The PC-3 \( \text{PTPRKkd} \) cells treated with p38 inhibitor (26.75±4.80%) exhibited similar apoptotic levels to the untreated cells (13.36±2.69%). However, the PC-3 \( \text{PTPRKkd} \) cells treated with JNK inhibitor (18.76±4.28%) exhibited significant reduction of apoptotic cells compared with untreated PC-3 \( \text{PTPRKkd} \) cells, \( p<0.001 \) (Fig. 6B). The addition of the p38 inhibitor did not inhibit the effect on apoptosis, suggesting that p38 was unlikely to be involved. However, the addition of the JNK inhibitor diminished the apoptotic effect of PTPRK knockdown. This suggests that PTPRK knockdown may utilise a signalling pathway via JNK to impact apoptosis thereby inhibiting cell proliferation.

(Fig. 5). No effect on the expression of c-Myc was observed in the PC-3 \( \text{PTPRKkd} \) cells compared with the control.

The role of JNK in PTPRK knockdown-associated apoptosis. The mitogen-activated protein kinase (MAPK) pathway is the major signalling pathway involved in cellular proliferation and it affects both apoptosis and the cell cycle. As knockdown of PTPRK has been shown to impact apoptosis, its effect on the expression and activations of p38, JNK and ERK in PC-3 cells was analysed. The PC-3 \( \text{PTPRKkd} \) cell showed a similar level of protein expression overall in p38, JNK and ERK compared with their pEF control. Furthermore, levels of phosphorylated p38, JNK and ERK were analysed using immunoprecipitation and western blot analysis. A marked increase active p-JNK (Tyr) was seen in the PTPRK knockdown cells and an increased level of active p38, suggesting that JNK and p38 may play a role in the regulation of apoptosis in the PC-3 \( \text{PTPRKkd} \) cells (Fig. 6A).
Figure 4. The effect of PTPRK knockdown on caspase-3, -8 and -9 expressions in both mRNA and protein levels. (A) PCR results of caspase-3, -8 and -9 mRNA expressions. Expression of (B) caspase-3 and (C) caspase-8 was increased in PC-3 PTPRKkd cells vs. PC-3 pEF control cells using Q-PCR. However, there is no significant difference of (D) caspase-9 seen in PC-3 PTPRKkd cells. (E) The protein expression of caspase-3 and -8 was also increased in PC-3 PTPRKkd cells, no similar change was seen in the caspase-9 expression. Intensity of the bands from three western blots was analysed using Image J software for (F) caspase-3, (G) caspase-8 and (H) caspase-9. The bar graphs show quantification of three western blots of each molecule. The intensity shown is integrated band intensity (intensity x area) and was normalised against the corresponding GAPDH signal. "p<0.01.
Discussion

PTPRK is a poorly studied protein phosphatase in the field of cancer progression. It has been indicated that PTPRK is a potential tumour suppressor in primary lymphoma of central nervous system and associated with colorectal cancer and pancreatic islet tumours (12-15). No study has attempted to define the function of PTPRK in prostate cells and its potential involvement in cancer metastasis.

In this study, we demonstrate the presence of PTPRK expression in 7 prostatic cell lines and 1 human fibroblast cell line. The cell lines have extensively been used as models for in vitro studies on prostate cancer. Its expression in tissues shows that PTPRK is more highly expressed in prostate cancer tissues compared to normal prostate tissue samples in both mRNA and protein levels. According to the mRNA levels of PTPRK, its expression appears at a similar level of that seen in prostate cell lines. The only exception was PNT-1A which had lower PTPRK expression. However, the current assessment of PTPRK expression in prostate cancer is limited and not sufficient to reach any solid conclusion. Hopefully, the implication of PTPRK in disease development and progression of prostate cancer can be elucidated by further investigations using a large clinical cohort of prostate cancer.

Most PTPs play a role in promoting apoptosis. For example, PTP1B has been reported to play a role in the activation of MAPKs. PTP1B activates JNK and p38 pathways via inositol-requiring kinase 1 (IRE1) signalling and lack of PTP1B resulted in decreased levels of ER-induced apoptosis (5,6). However, SHP-1 (PTPN6) dephosphorylates TrkA which in turn inhibits NGF-mediated PLCγ1 and Akt phosphorylation, reducing the TrkA survival signal (19). In contrast, osteoclastic PTP (PTP-oc) has been reported to promote c-Src-mediated activation of NF-κB and JNK leading to protection from apoptosis (20). In this study, the reduction of PTPRK expression...
in PC-3 cells shows promotion of apoptosis and involvement of MAPK signalling pathway. The tyrosine phosphorylation of JNK was increased in PC-3PTPRKkd cells and promotion of apoptosis in PC-3PTPRKkd cells was diminished after treating cells with the JNK inhibitor (SP600125). These data suggest that PTPRK downregulates apoptosis in prostate cancer cells by suppressing the JNK pathway.

We also investigated other apoptosis-related molecules such as p53, ID1 and c-Myc. These molecules play crucial roles in the regulation of cell proliferation. Expression of c-Myc in tumours helps cancerous cells pass through check-points and progress to the G2/M phase of the cell cycle. In addition, expression of ID1 activates NF-κB, which then induces Bcl-2 to inhibit apoptosis. In contrast to these anti-apoptotic factors, p53 acts as a promoter of apoptosis. Furthermore, mRNA expression of p53 was increased and expression of ID1 was decreased. p53 regulates both cell cycle and apoptosis. There are some reports that show that expression of p53 is associated with the induc-
tion of apoptosis in different cancer cells (21), p53 silencing was able to suppress cadmium-induced apoptosis in prostate cells (22). Additionally, activation of JNK can also upregulate p53 expression, leading to the accumulation of Bax which induces cell apoptosis in HeLa cells (23). In contrast, ID1 expression increases NF-κB expression which is associated with the anti-apoptotic pathway. NF-κB activates Bcl-2 to initiate the mitochondrial mediated anti-apoptotic effect and also activates XIAP to inhibit the activities of caspase-3 and -9 (24,25). These results indicate a complex network affected by PTPRK which participates in the coordination of cellular functions, making further investigations into the protein interactions between PTPRK and the network protein an interesting area to explore in future.

PTPRK knockdown resulted in increased apoptosis leading to the inhibition of in vitro growth of prostate cancer cells. PTPRK is a key factor in coordinating apoptosis via regulation of the MAPK pathways, in particular the JNK pathway in prostate cancer cells. Collectively, it is suggested that PTPRK may a key factor to be included in the signature of diagnosis and prediction of prostate cancer, and has great potential in the guidance of personalised treatment of malignancies.

Acknowledgements

The authors would like to thank Cancer Research Wales for their support.

References