Proteomics identification of PGAM1 as a potential therapeutic target for urothelial bladder cancer

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A B S T R A C T
Urothelial bladder cancer (UBC) is a major global health problem. There have been no major advances for the treatment of UBC in the last 30 years. In this study, we attempted to discover novel candidate therapeutic biomarkers for UBC. We utilized a two-dimensional polyacrylamide gel electrophoresis (2-DE) and ESI-Q-TOF MS/MS-based proteomic method to compare and identify differentially expressed proteins in UBC and adjacent normal tissues. Thirty five differentially expressed proteins (over 2-fold, p < 0.05) were identified. Further cluster analysis revealed these proteins were mainly involved in metabolism, apoptosis regulation, calcium ion binding and so on. Among them, phosphoglycerate mutase 1 (PGAM1), significantly up-regulated in UBC, was selected for detailed analysis. Immunohistochemical data showed that increased expression of PGAM1 was correlated with the severity of histological grade. Knockdown of PGAM1 expression by RNAi contributed to a marked anti-tumor activity in vivo. Moreover, we found, upon attenuation of PGAM1, its substrate 3-PG (3-phosphoglycerate) was up-regulated and product 2-PG (2-phosphoglycerate) was down-regulated, which consequently inhibited aerobic glycolysis and oxidative pentose phosphate pathway (PPP) that are essential to cancer cell proliferation. Our finding showed that PGAM1 might serve as a promising therapeutic target for UBC.

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1. Introduction
Urothelial bladder cancer (UBC) is a major global health problem with an estimated 429,000 new cases and 165,000 deaths annually [1]. There have been no major advances for the treatment of UBC in the last 30 years. Even after optimal treatment with chemotherapy and surgery, 5-year survival rates for locally advanced and metastatic UBC are 33% and 5%, respectively [1,2]. In this study, we attempted to discover novel candidate therapeutic biomarkers for UBC.

Recently, the development of genome-wide screening have displayed that the pathophysiological mechanism of UBC was associated with multiple regions of somatic copy number alteration, including amplification of PPARG, E2F3, EGFR and CCND1, as well as loss of RB1 and CDKN2A [3,4]. Recurrent somatic mutation, including TP53, FGFR3, PIK3CA, TSC1, RB1 and HRAS, has also been identified by sequencing of candidate pathways [5]. However, mRNA expression level does not necessarily correlate with protein level, and posttranslational modifications, such as phosphorylation, can not be predicted from transcription assay result [6,7]. Thus, it is believed that analysis of the cancer proteome can be more informative than genomics alone [8].

2-DE based proteomics approach provides an efficient tool to simultaneously analyze the expression patterns of proteins in tissue samples and has been successfully used in the investigation of various diseases. In this study, a 2-DE/MS based proteomics approach was utilized to compare differentially expressed proteins between UBC and adjacent normal tissues. PGAM1, significantly up-regulated in UBC, was selected for detailed analysis in the thoughts of its important biological functions in cell metabolism. Further data unraveled PGAM1 could be developed as a potential therapeutic target for UBC. Finally, we found that attenuation of PGAM1 could inhibit aerobic glycolysis and oxidative pentose phosphate pathway, which are essential to cancer cell proliferation.

2. Materials and methods
2.1. Clinical specimens

Fresh human UBC and adjacent normal tissues were obtained from 9 patients suffering muscle-invasive UBC who underwent surgical resections. The specimens were diagnosed histological after staining with H&E. Information of the patients was shown in Table 1. This study was approved by the Institutional Ethics Committee of Sichuan University.

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Table 1
The clinical and pathologic data of patients with urothelial bladder cancer for 2-DE.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Gender</th>
<th>Age</th>
<th>Histological type</th>
<th>TNM classification</th>
<th>Clinical stage</th>
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<tr>
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<td>Female</td>
<td>53</td>
<td>HG</td>
<td>T4bN1MO</td>
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2.2. 2-DE and image analysis

Tissues were ground into powder in liquid nitrogen and lysed in lysis buffer (7 M urea, 2 M thiourea, 4% CHAPS, 100 mM DTT, 0.2% P3–10 ampholyte, Bio-Rad) containing protease inhibitor cocktail 8340 (Sigma). 2-DE samples were prepared by mixing equal amount of proteins from the 9 clinical patients after protein extraction. Mixed protein samples (2 μg) were applied to IEF strip (17 cm, pH 3–10 NL, Bio-Rad) using a passive rehydration method. After 12–16 h of rehydration, the strips were transferred to an IEF Cell (Bio-Rad). IEF was performed as follows: 250 V for 30 min, linear; 1000 V for 1 h, rapid; linear ramping to 10 000 V for 5 h and finally 10 000 V for 4 h. The second dimension was performed using 12% SDS-PAGE at 30 mA constant current per gel after equilibration. The gels were stained using CBB R-250 (Merck, Germany) and scanned with a Bio-Rad GS-800 scanner. Three independent runs were made for mixed sample to ensure the accuracy of analyses. The maps were analyzed by PDQuest software Version 6.1 (Bio-Rad). The quantity of each spot in a gel was normalized as a percentage of the total quantity of all spots in that gel and evaluated in terms of OD. Only spots that showed consistent and significant differences (± over two fold, P < 0.05) were selected for analysis with MS.

2.3. Mass spectrometry analysis and protein identification

Mass spectra were acquired using a Q-TOF mass spectrometer (Micromass, Manchester, UK) fitted with an ESI source (Waters). Tryptic digests were dissolved in 18 μl 50% ACN. For ESI-Q-TOF analysis, the automatic scan rate was 1.0 s with an inter-scan delay of 0.02 s, and the system was operated at 3.0 kV. Spectra were accumulated until a satisfactory S/N had been obtained. Parent mass peaks with the range ±0.05 Da; and an allowance of one missed cleavage. Fixed modifications of cysteine carboxidimethylation, and variable modifications of methionine oxidation were allowed. The data format was selected as Micromass PKL and the instrument was selected as ESI-Q-TOF. Proteins with probability based MOWSE scores, derived from ions scores as a non-probabilistic basis for ranking protein hits when using Mascot searching engine, exceeding their threshold (p < 0.05), and with the molecular weight and pl consistent with the gel regions from which the spots were excised, were considered to be positively identified.

Protein cluster map was generated by Cluster software. Undetected in N corresponds to +10 and Undetected in C corresponds to -10. Proteins up-regulated in cancer tissue are in red, and the down-regulated proteins are in green. The intensity of the color green or red corresponds to the degree of alteration, respectively.

2.4. RT-PCR

The following primer sequences were used to detect PGAM1 transcripts: sense (5′-GACCCACTCCCTTCAATAG-3′) and antisense (5′-ACCCAGCTTATACCCTCC-3′). Total RNA was extracted using Trizol Reagent (Invitrogen, USA). RT-PCR reaction was performed as follows: reverse transcription at 45 °C for 30 min and denaturation at 94 °C for 2 min; then amplification for 30 cycles at 94 °C for 30 s, annealing at 54 °C for 1 min, and extension at 72 °C for 1.5 min, followed by a terminal elongation step at 72 °C for 10 min and a final holding stage at 4 °C. PCR products were resolved by 1% agarose gel electrophoresis.

2.5. Western blot

Proteins were extracted in RIPA buffer (50 mM Tris-base, 1.0 mM EDTA, 150 mM NaCl, 0.1% SDS, 1% Triton X-100, 1% sodium deoxycholate, 1 mM PMSF) and quantified by the DC protein assay kit (Bio-Rad, USA). Samples were separated by 12% SDS-PAGE and transferred to PVDF membranes (Amersham Biosciences). After blocking with 5% non-fat milk in Tris-buffered saline, 0.1% Tween 20 for 1 h, the membranes were incubated overnight at 4 °C with anti-PGAM1 antibody (ab129191). After that, the blots were incubated with secondary antibody conjugated to HRP for 2 h at room temperature. Target proteins were detected by enhanced chemiluminescence reagents (Amersham Pharmacia Biotech, Piscataway, NJ, USA).

2.6. Immunohistochemistry and correlation analysis

Sections were stained by Envision System-HRP method (DakoCytomation Inc., Carpinteria, CA), according to kit manufacturer’s instructions. Specific antibodies performed included goat anti-human PGAM-1 antibody (ab22220) and rabbit anti-human PCNA antibody (ab15497). Saturation and intensity of immunostained cells was evaluated over 8 visual fields at a power of ×400 under a light microscope (Olympus Optical, Tokyo, Japan). In statistical analysis, with reference to Jeffrey’s study [9], total staining of PGAM-1 was scored as the product of the staining intensity (on a scale of 0–3: negative = 0, weak = 1, moderate = 2, strong = 3) × the percentage of cells stained (positively recorded on an ordered categorical scale: 0 = zero, 1 = 1–25%, 2 = 26–50%, 3 = 51–100%), which resulted in a scale of 0–9. The evaluation was performed by two independent investigators, without any prior knowledge of each patient’s clinical information and outcome. Any discrepancy between the two evaluators was resolved by reevaluation and careful discussion until agreement was reached.

2.7. Tumor xenograft model and shRNA treatment

The sequence of shRNA used for knockdown of PGAM1 is as follows [10]: 5′-CCGCGAAGAAACTTGAAGCCTATCAACTCGAGTTGATAGGCTTACAAGTICCTGTTTTTTG-3′. HK sequence, which has no homology with any mammalian sequence, was used as negative control (NC group).

Human bladder cancer cells 3 × 10⁶ T24 or 5 × 10⁶ EJ cells were subcutaneously injected into the right dorsal flank of athymic nude BALB/c mice. The tumor-bearing mice were randomly assigned into the following four groups and each mouse received the corresponding treatment by caudal vein injection: (a) PBS group, 100 μl of PBS; (b) Lipo group, liposome 25 μg (volume = 100 μl); (c) Negative control (NC group), HK shRNA 10 μg complexed with liposome 25 μg (volume = 100 μl); (d) SHRNA group, PGAM1 shRNA 10 μg complexed with liposome 25 μg (volume = 100 μl). Injections were performed every three days, and tumor volumes were evaluated according to the following formula: tumor volume (mm³) = 0.52 × length × width². Side effects of treatment and the weight, appetite, and behavior of the mice were
recorded for 27 days, after which the mice were sacrificed. Tumor net weight of each mouse was measured. The dissected tumors were fixed in neutral buffered formalin and embedded in paraffin, and sections (5 μm) were used for histologic analysis.

2.8. TUNEL

Presence of apoptotic cells within tumor sections was evaluated by TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling) technique using the DeadEnd Fluorometric TUNEL System (Promega, Madison, WI) following the manufacturer's protocol. Percent apoptosis was determined by counting the number of apoptotic cells and divided by the total number of cells in the field (5 high power fields/slide).

2.9. 3-PG and 2-PG concentration

Cellular metabolites were extracted and spectrophotometrically measured as described previously [10]. To determine cellular concentration of 2-PG and 3-PG, 100 μL of packed cell pellets were homogenized in 1.5 ml of hypotonic lysis buffer (20 mM HEPES (pH 7.0), 5 mM KCl, 1 mM MgCl2, 5 mM DTT, and protease inhibitor cocktail). The homogenates were centrifuged in a cold room at 4 °C for 10 min at maximum speed, and the supernatants were applied to Amicon Ultra tubes with 10KDa cut off filter (Millipore). The flow through containing the metabolites was used for the measurement.

2.10. Glycolytic rate assay

Glycolytic rate was measured by monitoring the conversion of 5-3 H-glucose to 3H2O. Cells were washed once in PBS prior to incubation in 1 ml of Krebs buffer without glucose for 30 min at 37 °C. The Krebs buffer was then replaced with Krebs buffer containing 10 mM glucose spiked with 10 μCi of 5-3H-glucose. Following incubation for 1 h at 37 °C, triplicate 50 μl aliquots were transferred to uncapped PCR tubes containing 50 μl of 0.2 N HCl, and a tube was transferred into an eppendorf tube containing 0.5 ml of H2O for diffusion. The tubes were sealed, and diffusion was allowed to proceed for a minimum of 24 h at 34 °C. The amounts of diffused 3H2O were determined by scintillation counting.

2.11. Cell viability assay

For the proliferation assay, cells were seeded at 5 x 10^3 cells per well in 96-well plates. Afterward, medium containing respective agents were added to each well and cells were further cultured at 37 °C for 48 h. Cell viability was examined by MTT test. The results were from three independent experiments of each group.

2.12. Flow cytometry

Flow cytometric analysis was performed to identify sub-G1 cells/apoptotic cells and to measure the percentage of sub-G1 cells after propidium iodide (PI) staining in hypotonic buffer. Briefly, cells were suspended in 1 ml hypotonic fluorochrome PI solution (50 mg/ml PI in 0.1% sodium citrate plus 0.1% Triton X-100) containing 0.5 mg/ml RNase A, and the cells were analyzed by the use of a flow cytometer (ESP Elite, Beckman Coulter, Fullerton, CA, USA). Apopotic cells appeared in the cell cycle distribution as cells with DNA content of less than that of G1 cells.

2.13. NADPH/NADP+ ratio and oxidative PPP flux assay

NADPH/NADP+ kit (BioAssay Systems) was used to measure cellular NADPH/NADP+ ratio. Subconfluent cells seeded on a 10 cm dish were collected by a scraper, washed with PBS, and lysed with 200 μL of NADP+ (or NADPH) extraction buffer. Heat extract was allowed to proceed for 5 min at 60 degrees before adding 20 μl of assay buffer and 200 μl of the counter NADPH (or NADP+) extraction buffer to neutralize the extracts. The extracts were spun down and the supernatants were reacted with working buffer according to the manufacturer's protocol. The absorbance at 565 nm from the reaction mixture was measured with plate reader.

Cells were seeded on 6-cm dishes that are placed in a 10-cm dish with 2 sealed pinholes on the top. 14CO2 released from cells was collected by completely sealing the 10-cm dish, in which the cells on the 6-cm dish were incubated in 2 ml of medium containing [1-14C]- or [6-14C]-glucose, respectively, at a final specific activity of 10 μCi/ml glucose at 37 °C for 3 h. The oxidative PPP flux was stopped by injecting 0.3 ml of 50% TCA through one of the holes on the top, and at the same time 14CO2 released was trapped by injecting 0.3 ml of Hyamine Hydroxide into a small cup placed on the 10-cm dish through the second hole. Krebs cycle measurements, obtained in parallel samples incubated with [6-14C]-glucose, were used to correct the oxidative PPP flux measurements obtained from samples incubated with [1-14C]-glucose. Each dish was completely re-sealed with parafilm and incubated overnight at room temperature. Hyamine Hydroxide in the small cup was dissolved into 60% methanol and directly subjected to scintillation counting.

3. Results

3.1. 2-DE and ESI-Q-TOF-MS/MS analysis

A pair of representative 2-DE pictures was shown in Fig. 1A. 35 differentially expressed proteins (over 2-fold, p < 0.05) were identified (arrows in Fig. 1A and listed in Table S1). The identified proteins were categorized into four groups, depending on their subcellular locations. 52% of the total proteins were located in the cytoplasm, and the remainder was situated in nuclear (31%), cell membrane (6%) and secreted protein (11%) (Fig. 1B). Moreover, cluster analysis displayed that the changed proteins were involved in different biological processes, including metabolism, apoptosis regulation, calcium ion binding and so on (Fig. 1C). Notably significant alterations were found in a group of metabolic proteins (48.6% of total 35 proteins). These proteins work in diverse metabolic processes, such as glycolysis, tricarboxylic acid cycle and oxidative phosphorylation. Cluster maps (Fig. 1D) showing altered expression of these proteins were generated by Cluster software. Among them, PGAM1 was identified with obvious alteration. It was up-regulated 4.1-fold in UBC compared with adjacent normal tissues (P < 0.05). Furthermore, ESI-Q-TOF-MS/MS analysis showed that PGAM1 has 13 matched peptides and a MASCOT score of 145, as shown in Table S1.

3.2. Over-expression of PGAM1 in UBC

To confirm the altered expression of PGAM1 in UBC, RT-PCR was conducted in human high grade UBC tissues, human low grade UBC tissues and adjacent normal tissues, respectively. Results showed increased PGAM1 was observed in both high grade UBC tissues and low grade UBC tissues when compared with adjacent normal tissues (adjacent normal tissues, 19.5 ± 5.8; low grade UBC tissues, 45.3 ± 6.2; high grade UBC tissues, 89 ± 8.4; Student's t test, p < 0.01). Further Western blot analysis was performed, and overexpression of PGAM1 was also observed in both high grade UBC tissues and low grade UBC tissues when compared with adjacent normal tissues (adjacent normal tissues, 24.5 ± 3.1; low grade UBC tissues, 53.2 ± 5.3; high grade UBC tissues, 98.2 ± 11.2 Student's t test, p < 0.01). Taken together our data showed that PGAM1 was overexpressed in high grade and low grade UBC tissues at both the mRNA and protein levels, which was consistent with the observation made in the 2-DE analysis. Representative pictures
from high grade UBC tissues and adjacent normal tissues were shown in Fig. 1E (Left: RT-PCR; Right: Western blot).

3.3. Over-expression of PGAM1 was correlated with the severity of histological grade

In order to study its prognostic value, IHC and H&E staining were conducted to assess PGAM1 expression in paraffin-embedded UBC tissues (Fig. 2A). 60 UBC tissue specimens (including 30 high grade UBC and 30 low grade UBC) and 30 adjacent normal tissues recruited from the archives of the pathology. As described above, total staining of PGAM1 was scored as the product of the staining intensity (on a scale of 0–3: negative = 0, weak = 1, moderate = 2, strong = 3) × the percentage of cells stained (positively recorded on an ordered categorical scale: 0 = zero, 1 = 1–25%, 2 = 26–50%, 3 = 51–100%), which resulted in a scale of 0–9. As shown in Table 2, positive staining of PGAM1 was rarely detected and total staining score was only 0.73 ± 0.78 in 30 adjacent normal tissues. However, low grade UBC and high grade UBC showed an obvious increasing trend of positive staining of PGAM1, with 3.63 ± 1.92 and 6.1 ± 1.95 total staining score, respectively (P < 0.05). Thus, increased expression of PGAM1 was more likely to be present with poor differentiation.

3.4. Down-regulation of PGAM1 significantly inhibited bladder cancer xenograft growth in vivo

The in vivo anti-tumor effects were studied in T24 and EJ bladder cancer models, because PGAM1 was up-regulated in both T24 and EJ bladder cancer cells. As shown in Fig. 3, Down-regulation of PGAM1 by shRNA significantly inhibited tumor growth, when compared with the controls (P < 0.05, respectively), which was evaluated by measuring...
3.5. Down-regulation of PGAM1 decreased cell proliferation and increased apoptosis in vivo

To obtain additional insight into the in vivo anti-tumor effects, cell proliferation and apoptosis were assessed by PCNA IHC analysis and TUNEL assay (P < 0.05, respectively). As shown in Fig. 4, PGAM1 shRNA clearly reduced percentages of PCNA-positive nuclei. Moreover, a significantly greater percentage of TUNEL-positive nuclei could be observed in PGAM1 shRNA treated group when compared with control groups (P < 0.05, respectively).

3.6. Down-regulation of PGAM1 affected intracellular 3-PG and 2-PG levels, and thus inhibited glycolysis and oxidative pentose phosphate pathway

In this study, Western blot and RT-PCR data showed that PGAM1 was up-regulated in both T24 and EJ bladder cancer cells, when compared with immortalized human normal bladder epithelial cells SV-HUC-1 (Fig. 5A). In addition, we found that attenuation of PGAM1 by shRNA could lead to increased 3-PG and decreased 2-PG, compared with corresponding control groups in T24 and EJ cell lines (Fig. 5B and C). Moreover, PGAM1 shRNA contributed to decreased glycolytic rate in T24 and EJ cell lines (Fig. 5D). Corresponding to decreased glycolytic rate, inhibited proliferation and increased apoptosis were observed in PGAM1 shRNA treated group (Fig. 5E and F). In addition, addition of 2-PG into cell culture media could affect inhibited rate of PGAM1 shRNA (Fig. 5E and F).

Since glycolytic intermediates including glucose-6-phosphate (G6P) can be diverted into the oxidative PPP, which contributes to macromolecular biosynthesis and thus supports cancer cell proliferation, we next examined whether attenuation of PGAM1 by shRNA could affect PPP flux and NADPH/NADP+ ratio. As shown in Fig. 5G and H, oxidative PPP flux and NADPH/NADP+ ratio were decreased in PGAM1 knockdown T24 and EJ cell lines.

4. Discussion

Cancer cells undergo extensive oncogene-directed metabolic reprogramming. It has been reported that aerobic glycolysis and other metabolic alterations observed in cancer cells supply the anabolic requirements associated with cell growth and proliferation [11]. The alterations to cellular metabolism in cancer cells have been increasingly recognized as a crucial hallmark of cancer. Recent research has focused on ways to target the increased dependence of cancer cells on glycolysis. Several glycolytic targets are currently being explored, such as HK, PKM2, PDK4 [12–14].

PGAM1 is a vital enzyme in the glycolytic pathway that catalyzes the conversion of 3-PG to 2-PG. It has been reported that this enzyme is upregulated in diverse cancers, including lung squamous carcinoma, cholangiocarcinoma and hepatoma [15–17]. Moreover, activity of PGAM1 is known to be important for tumorigenesis, invasion and metastasis. However, few papers demonstrated that PGAM1 was overexpressed in UBC and the mechanisms by which PGAM1 support rapid cancer cellular proliferation remain unclear.

In the present study, the global protein profiles were compared between UBC and adjacent normal tissues using a 2-DE and MS/MS-based approach. A total of 35 differentially expressed proteins were identified and cluster analysis revealed that these altered proteins were involved in diverse biological processes, mainly including metabolism, apoptosis regulation, calcium ion binding and so on. Significant alterations were found in a group of metabolic proteins (48.6% of total 35 proteins). These proteins function in diverse metabolic processes, such as glycolysis, tricarboxylic acid cycle, oxidative phosphorylation and so on.

Among these identified proteins, PGAM1, which was significantly up-regulated in UBC and had 13 matched peptides and a MASCOT score of 145, was selected for detailed analysis. IHC assay displayed overexpression of PGAM1 was correlated with a low degree of differentiation. Furthermore, our results also showed that PGAM1 silence alone could retard tumor cell growth by inducing apoptosis and inhibiting proliferation.

In order to elucidate the mechanisms by which attenuation of PGAM1 could affect cancer cellular proliferation, cellular metabolic pathways and products were investigated. We found down-regulation of PGAM1 by shRNA affected intracellular 3-PG and 2-PG levels, and thus inhibited glycolysis and oxidative PPP.

Oxidative PPP is a major pathway for glucose catabolism. The PPP directs glucose flux to its oxidative branch and produces a reduced form of nicotinamide adenine dinucleotide phosphate (NADPH), an essential reductant in anabolic processes [18]. It has been accepted that the PPP plays an important role in modulating cancer cell growth because PPP not only generates pentose phosphates to supply their high rate of nucleic acid synthesis but also provides NADPH, which is required for...
both the synthesis of fatty acids and cell survival under stress conditions. Previous studies showed targeting the PPP for cancer therapy might be appealing. For example, drug adriamycin resistance could be reversed by treatment with dehydroepiandrosterone (DHEA) or 6-aminonicotinamide (6-AN), which inhibit the first and second step in the oxidative PPP, respectively [18]. These data supported our findings.

Fig. 3. Antitumor effects of attenuation of PGAM1 by shRNA in athymic nude BALB/c mice bearing human bladder cancer T24 or EJ. Effects of PGAM1 shRNA on the growth curves of T24 (A) and EJ (C) and effects on the tumor weight of T24 (B) and EJ (D). Tumor growth was significantly inhibited in the PGAM1 shRNA treated group compared with the controls. The similar results were also found in the tumor weight. Western blot showed PGAM1 shRNA efficiently down-regulated the expression of PGAM1 in T24 and EJ model. *P < 0.05.

Fig. 4. Histological analysis by TUNEL assay and PCNA immunoreactivity analysis in T24 tumor models. (A) Top panel: detection of apoptotic cells using TUNEL analysis. The data revealed that PGAM1 shRNA induced a significant enhancement of apoptotic cells versus control. Bottom panel: proliferated tumor cells were detected by an antibody to PCNA. PGAM1 shRNA treated group showed a significant decrease compared with the controls. (B) Percent apoptosis in each group. (C) Quantified values shown were the average percentage of PCNA-positive nuclei. *P < 0.05; **P < 0.01.
5. Conclusion

In the present study, we have found 35 differentially expressed proteins in human UBC using 2-DE-MS/MS proteomic methods. Pathological analysis showed that PGAM1 is a promising biomarker for UBC. Further in vivo study indicated that silencing of PGAM1 induced significantly antitumor effects, displaying that it may serve as a potential therapeutic target for UBC.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jprot.2015.11.027.

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Conflict of interest

None declared.

Transparency document

The Transparency document associated with this article can be found in online version.

References