

S100A16 promotes cell proliferation and metastasis via AKT and ERK cell signaling pathways in human prostate cancer

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Abstract S100A16 is a member of the S100 calcium-binding protein family. It is overexpressed in many types of tumors and associated with proliferation, migration, and invasion; however, its function in human prostate cancer is unresolved. Our objective was to determine its effects and the underlying pathways of S100A16 in prostate cancer tissues and cells. We measured S100A16 expression by quantitative real-time polymerase and Western blotting in eight matched prostate cancer and adjacent normal tissues, and in three prostate cancer cell lines, DU-145, LNCaP, and PC-3, compared to a normal prostate epithelial cell line PrEC. DU-145 cells stably overexpressing S100A16 and PC-3 cells with S100A16 knockdown were established by transfection with S100A16 overexpression plasmid or shRNAs. Invasion, migration, and proliferation were analyzed

by transwell assay, wound healing, and colony formation assays, respectively. Western blotting and invasion assays were performed to determine expressions and activation of AKT, ERK, p21, and p27. S100A16 was significantly overexpressed in both prostate cancer tissues and cells lines compared to normal controls ($P < 0.05$). Overexpression of S100A16 significantly promoted invasion, migration, and proliferation in prostate cancer cells in vitro, whereas silencing S100A16 showed the converse effects ($P < 0.05$). Furthermore, overexpression of S100A16 activated cell signaling proteins AKT and ERK and downregulated tumor suppressors p21 and p27. Specific inhibitors, LY294002 and PD98059, suppressed activation of AKT and ERK, which attenuated DU-145 cell clone formation and invasion induced by S100A16 overexpression. S100A16 may promote human prostate cancer progression via signaling pathways involving AKT, ERK, p21, and p27 downstream effectors. Our findings suggest that S100A16 may serve as a novel therapeutic or diagnostic target in human prostate cancer.

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Keywords Prostate cancer · S100A16 · Invasion · Migration · Proliferation · PI3K/AKT and MAPK/ERK · Signaling

Introduction

Prostate cancer is a heterogeneous disease. Despite the discovery of novel molecules targeted at more personalized therapies, the mechanisms underlying its progression from abnormal epithelial proliferation to invasive carcinoma and metastatic disease are poorly understood [1]. There is a lack of effective therapeutic and chemopreventive interventions for advanced prostate cancer; therefore, the identification of novel predictive biomarkers is still required to improve the clinical management of prostate cancer.

S100A16 is a member of the S100 family of proteins which are small, 10–12 kDa acidic proteins containing the EF-hand Ca^{2+} -binding motif [2]. S100 proteins perform multiple regulatory functions in cellular processes such as motility, differentiation, cell growth, contraction, transcription, cell cycle progression, and secretion [3]. S100 protein levels are found to be altered in many types of tumors and are therefore considered potential markers of cancerous tissues [4]. S100A4 has been reported to be overexpressed in prostate cancer and shown to accelerate prostate cancer metastasis [5]. S100A16 has been reported to be upregulated in breast cancer, ovarian cancer, and lung cancer [6]. In addition, we previously reported that S100A16 may be a novel adipogenesis-promoting factor which was associated with cell proliferation [7]. However, the detailed relationship between S100A16 and prostate cancer remains to be established.

Based on our hypothesis that S100A16 promotes proliferation and metastasis in prostate cancer, we employed prostate cancer tissue samples and cell lines with low, moderate and high metastatic potential (LNCaP, DU-145 and PC-3, respectively) in order to explore the role of S100A16 in human prostate cancer metastasis.

Materials and methods

Prostate cancer tissue samples, cell lines, and cell culture

In accordance with the Local Ethics Committees of the First Affiliated Hospital of Nanjing Medical University, China, eight matched pairs of human prostate cancer tissues and normal adjacent tissue samples were acquired during surgery, immediately frozen in liquid nitrogen and stored at $-80\text{ }^{\circ}\text{C}$ for further analysis.

The human prostate cancer cell lines PC3, LNCaP, and DU-145 were purchased from the American Type Culture Collection (ATCC; Rockville, MD, USA) and normal prostate epithelial cells PrECs was a gift from the Department of Gastroenterology, The First Affiliated Hospital of Nanjing Medical University.

Cells were cultured in RPMI 1640 (LNCaP, DU-145, and PrECs) and F12K (PC3) containing 10 % fetal bovine serum (FBS) supplemented with 100 U/ml penicillin and 0.1 mg/ml streptomycin, and incubated at $37\text{ }^{\circ}\text{C}$ in a humidified atmosphere of 5 % CO_2 .

Plasmids and S100A16 transfection

S100A16 RNA interference (RNAi) plasmids and S100A16 overexpression plasmids were obtained from an earlier study [7]. S100A16-shRNA1 (target sequence: CCATCGCCAAACTCATCCATGAGCAGGAG) and S100A16-shRNA2 (target sequence: TGTCAGACTGCTA

CACGGAGCTGGAGAAG) were generously provided by Dr. Wenbin Zhou (Department of Breast Surgery, The First Affiliated Hospital of Nanjing Medical University, Nanjing). DU-145 cells were transfected with S100A16 overexpression plasmids or vector controls; PC-3 cells were transfected with S100A16 shRNA or scrambled shRNA, as previously described [7]. The transfected cells were cultured as described above in the presence of 200 $\mu\text{g}/\text{ml}$ G418 (Sigma-Aldrich, St. Louis, MO, USA). For the experiments, the cells were treated with serum-free RPMI 1640 media overnight and then cultured in normal media for the specified times.

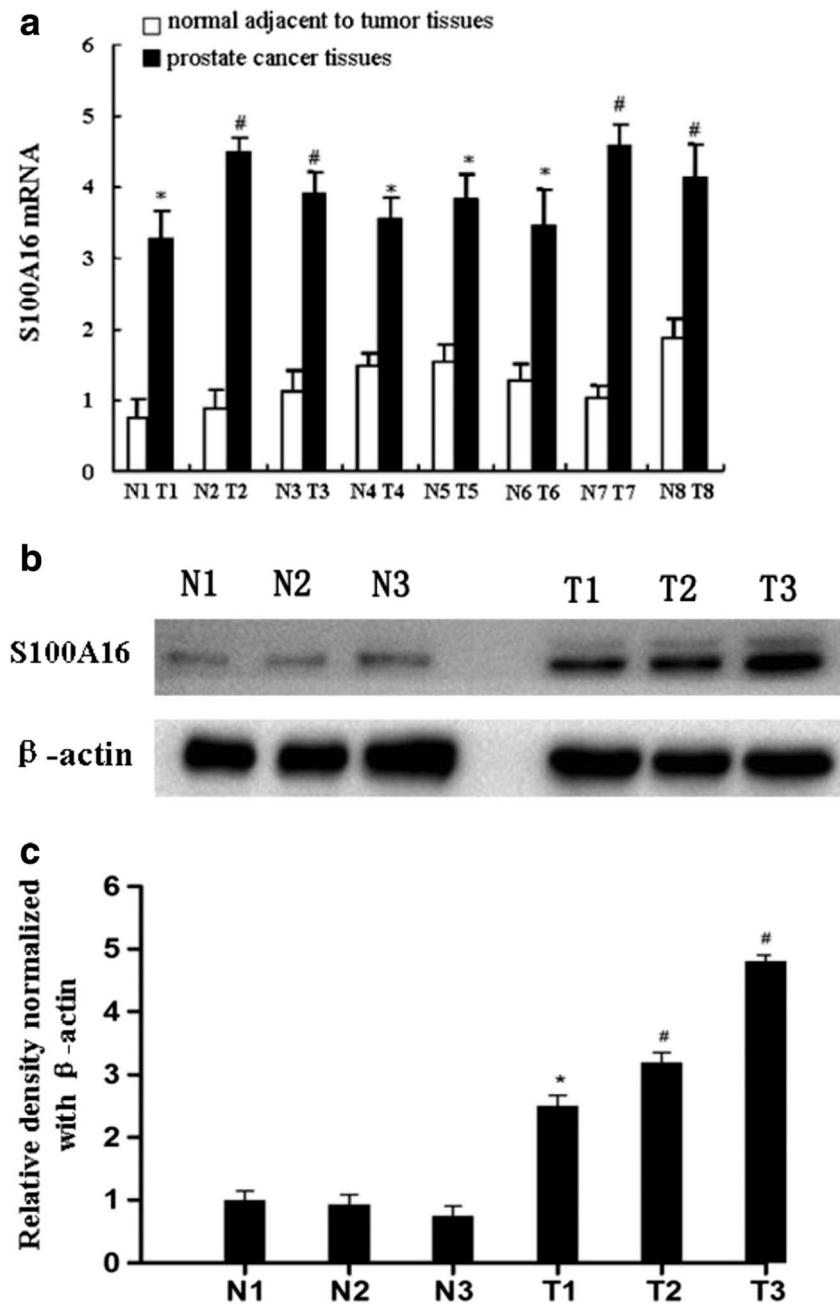
RNA extraction and real-time quantitative reverse transcription PCR (qRT-PCR)

Total RNA was extracted from prostate cancer tissues using TRIzol according to the manufacturer's instruction (Invitrogen). Reverse transcription was performed using a Revert Aid™ First Strand cDNA Synthesis Kit (MBI-Fermentas; Thermo Scientific, Rockford, IL, USA). Transcripts for S100A16 and β -actin (control) were used for real-time qRT-PCR. The primers were as follows: β -actin-F, 5'-TCACCAACTGGGACGACAT-3'; β -actin-R, 5'-GCACAGCCTGGATAGCAAC-3'; S100A16-F, 5'-ACCACATGCTGACGGACACA-3'; S100A16-R, 5'-GTCCAGTATTCGTCAAAGCAGATG-3'.

Western blot analysis

The cells were washed twice with ice-cold phosphate-buffered saline (PBS). They were harvested by scraping in lysis buffer [50 mM Tris-HCl (pH 7.4), 150 mM NaCl, 1 % (v/v) Nonidet-P40, 1 mM EDTA, 1 mM NaF, 10 $\mu\text{g}/\text{ml}$ aprotinin, 10 μM leupeptin, 1 mM phenylmethanesulfonyl fluoride] and allowed to stand on ice for 30 min. After centrifugation (12,000 rpm, 20 min) at $4\text{ }^{\circ}\text{C}$, the supernatant was collected and equal amounts (60 μg) of total protein were separated on 12 % SDS-PAGE gels before being electrophoretically transferred to Immobilon-P membranes (Millipore, Bedford, MA, USA). The membranes were probed overnight at $4\text{ }^{\circ}\text{C}$ with primary antibodies to S100A16, AKT, p-AKT, ERK, p-ERK, p21, p27, and monoclonal anti- β -actin in TBST (1:5000) containing 1 % BSA (w/v). The membranes were then incubated with anti-rabbit or anti-mouse secondary antibodies for 2 h. The immune complex was detected by electrochemical luminescence (ECL) using an ECL Plus detection kit (Pierce, Rockford, IL, USA) and analyzed using a scanning densitometer with molecular analysis software (Bio-Rad, Hercules, CA, USA).

Fig. 1 Analysis of S100A16 expression in human prostate cancer tissue. **a** Real-time qRT-PCR analysis of S100A16 mRNA transcripts, comparing S100A16 expression levels in eight matched prostate cancer tissue samples and adjacent normal tissue samples. **b** Western blot analysis of S100A16 protein expression in four of the six paired tissue samples. β -Actin is included as a loading control. **c** Densitometric quantification of the Western blot results showing relative expression of S100A16 protein normalized to β -actin. *T* prostate cancer tissue, *N* matched adjacent normal tissue; * $P < 0.05$; # $P < 0.01$, compared to control. The results represent at least three independent experiments



Wound healing migration assay

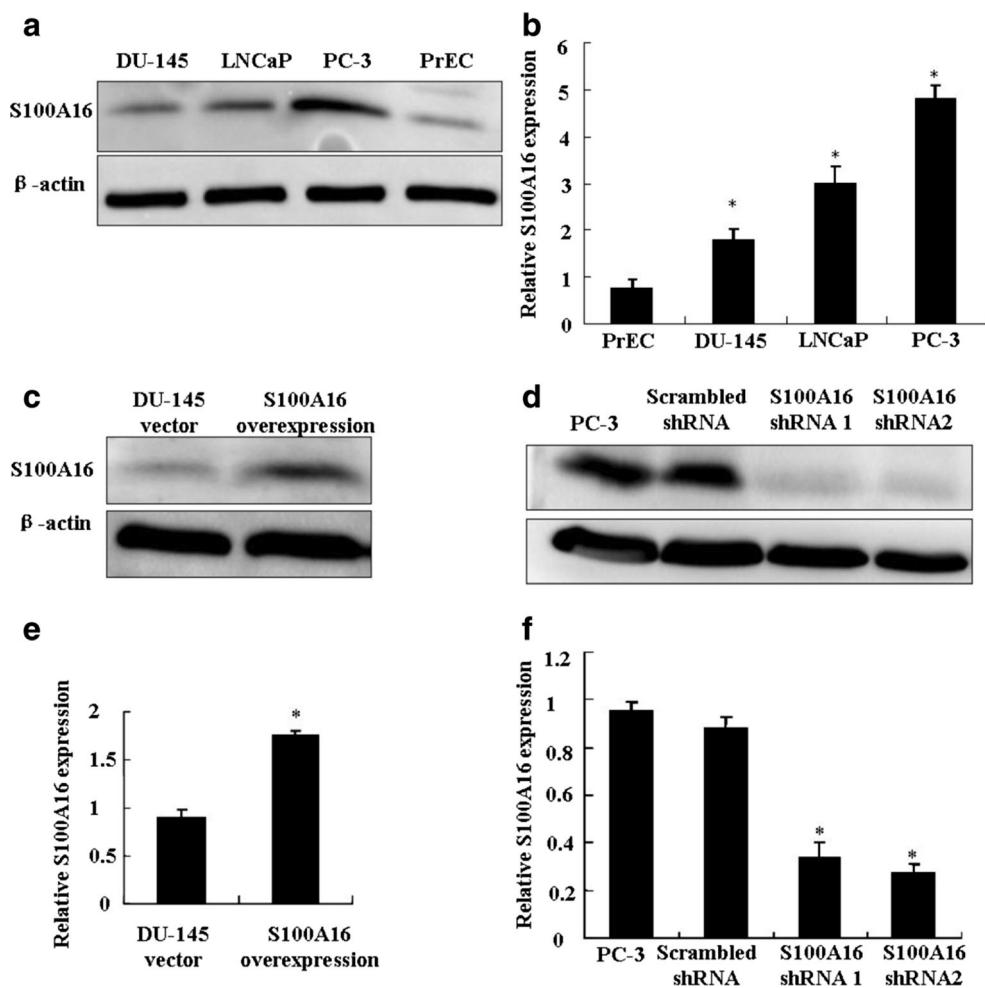
Cells (10×10^4) were seeded in a 12-well plate and cultured until they formed a confluent (80 %) monolayer. Scratches were made using a 100- μ l pipette tip, and the culture medium was replaced with fresh complete medium. The extent of closure at 12 and 24 h after the scratches had been formed were analyzed under an optical microscope.

Invasion assay

Harvested cells were washed twice in PBS, resuspended in serum-free culture medium with 0.2 % BSA, and adjusted to

a final concentration of 10^6 cells/ml. The invasion assay was performed in a 24-well transwell plate (Costar Corning, Schiphol-Rijk, Netherlands) with porous polycarbonate membrane inserts (8 μ m) precoated with 60 μ l ECM gel solution. RPMI 1640/10 % FBS (300 ml) media was added to the lower compartment of each well, and 200 μ l of the cell suspension was added to the precoated upper compartment. The inserts were incubated for 48 h at 37 $^\circ$ C, 5 % CO_2 . Cells remaining on top of the filter were removed by soft mechanical dislodging. The numbers of cells that had migrated to the bottom of the filter were counted using a trypan blue dye exclusion assay under an optical microscope. Six random fields from each chamber were counted for each condition.

Fig. 2 S100A16 expression in human prostate cancer cell lines, including S100A16-overexpressing and S100A16-silenced cells. **a, b** Western blotting and densitometric analysis of S100A16 expression in DU-145, LNCaP, and PC-3 human prostate cancer cell lines, compared to normal prostate epithelial cells PrEC. β -Actin is included as a loading control. **c** Western blot analysis of S100A16 protein expression in DU-145 cells transfected with S100A16 overexpression plasmid compared to vector control; **d** PC-3 cells transfected with S100A16 shRNAs compared to scrambled shRNA controls. β -Actin is included as a loading control. **e, f** Densitometric quantification of the Western blotting results showing relative expression of S100A16 protein in the transfected cells normalized to β -actin. $*P < 0.05$, compared to control. The results represent at least three independent experiments



Cell proliferation assay

The proliferation assay was performed using a WST-1 assay kit (Roche, Mannheim, Germany) according to the manufacturer's protocol. The cells were seeded in a 96-well plate in 100 μ l of culture medium at a density of 10^3 cells/well. After incubation for 0, 24, 48, and 72 h at 37 $^{\circ}$ C, 5 % CO_2 , 10 μ l of the reconstituted WST-1 mixture was added to each well and gently mixed. The cells were incubated for a further 4 h, and the absorbance of each sample was measured at a wavelength of 450 nm.

Flow cytometry

The cells were collected with trypsin/EDTA and washed with PBS, 2 mM EDTA, 0.5 % BSA. The cells were fixed in 10 % ice-cold ethanol and incubated with propidium iodide in the dark for 2 h. Flow cytometric analysis was performed on a total of 10^4 cells/sample using a FACS Calibur with CellQuest software (BD Biosciences, San Jose, CA, USA).

Colony formation assay

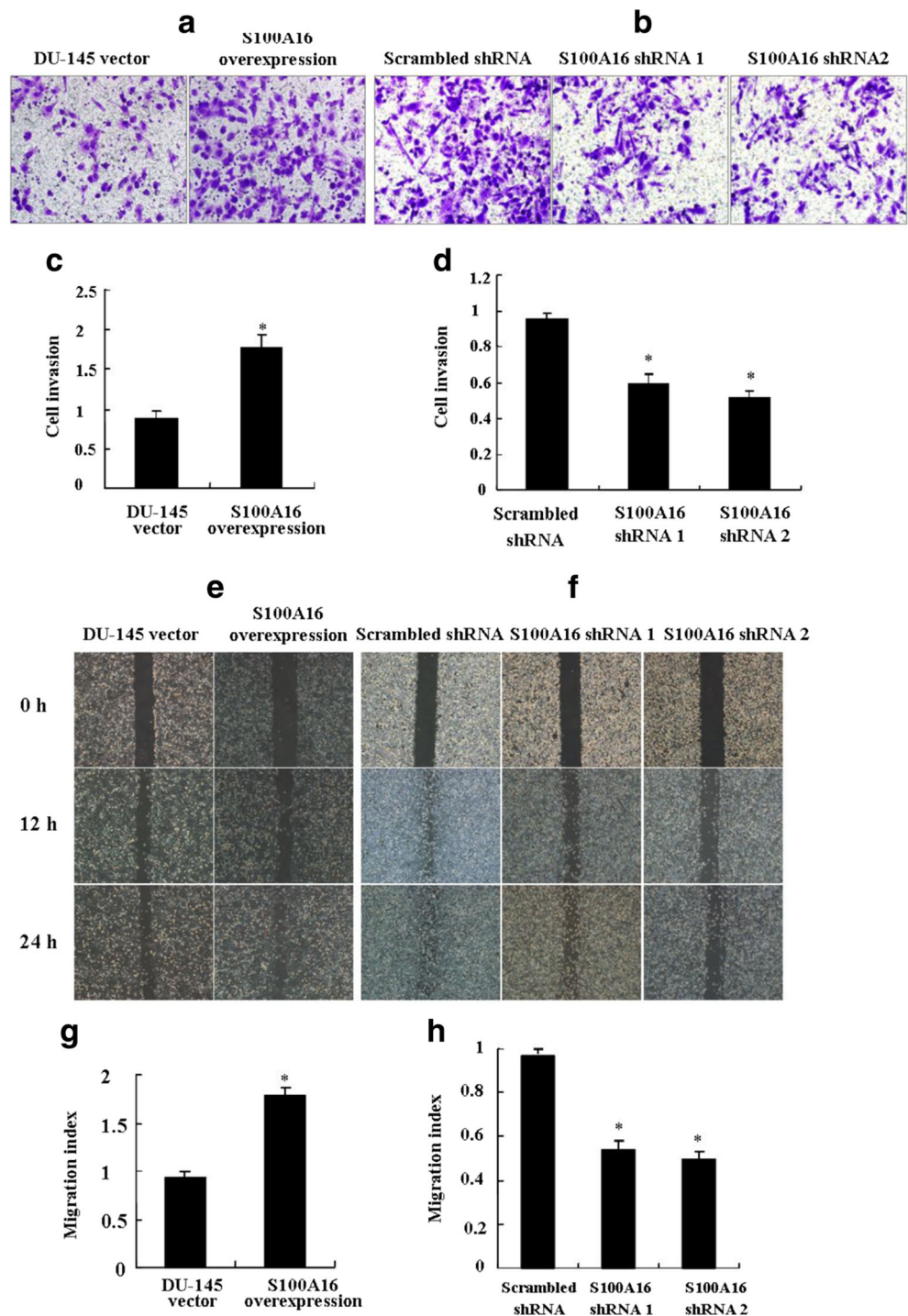
Transfected DU-145 and PC-3 cells, as described above, were used for the colony formation assays. Stably expressing cells were harvested and mixed with RPMI 1640 media containing 10 % FBS and 0.5 % agar (bottom layer). Cells (5×10^3 cells/well) in 20 % FCS/0.7 % agarose (top layer) were plated and incubated at 37 $^{\circ}$ C for 14 days. After incubation, the plates were stained with 0.005 % crystal violet for >2 h. The colonies were counted (two colony grids/well) under an optical microscope [6].

Specific inhibitors LY294002 (L9908, SIGMA) and PD98059 (P215, SIGMA) (100 mM) were added to DU-145 stably overexpressing S100A16 cells to observe the effects of PI3K and ERK inhibition, respectively.

Statistical analysis

Data were analyzed by two-tailed Student's *t* tests to compare pairs of data and by one-way analysis of variance (ANOVA) for comparisons between multiple groups. Differences were considered significant at $P < 0.05$ (*) and highly significant $P < 0.01$ (#).

Fig. 3 S100A16 promotes invasion and migration in human prostate cancer cells in vitro. **a–d** Invasion assays in matrigel-coated chambers after incubation for 48 h. **a** DU-145 cells overexpressing S100A16 compared to vector control; **b** PC-3 cells with S100A16 knockdown compared to scrambled shRNA controls. Magnification $\times 200$. **c, d** Quantification of the invasion assays based on six random fields from each chamber for each condition. **e–h** Wound healing assays of cell migration showing closure at 0, 12, and 24 h after the scratches had been formed. **e** DU-145 cells overexpressing S100A16 compared to vector control; **f** PC-3 cells with S100A16 knockdown compared to scrambled shRNA controls. Magnification $\times 200$. **g, h** Quantification of the wound healing assays representing the distances between four random points within the wound edge, in three replicate experiments. $*P < 0.05$, compared to control



Results

S100A16 was upregulated in prostate cancer tissue samples

We evaluated S100A16 mRNA and protein expression in eight matched human prostate cancer tissue samples and normal adjacent tissues by real-time qRT-PCR and Western

blotting, respectively. The data showed that both S100A16 mRNA and protein expression were significantly higher in prostate cancer tissue compared to hyperplastic prostate tissue ($P < 0.05$; Fig. 1). The average fold change in the tumor tissue samples was approximately 3.5 relative to nontumor tissue. These results demonstrated that S100A16, which is associated with cell migration, invasion, and metastasis, was upregulated in prostate cancer tissue.

S100A16 protein expression in prostate cancer cell lines

We also examined S100A16 protein expression in three human prostate cancer cell lines, LNCaP, PC-3, and DU-145, and compared these to a normal prostate epithelial cell line PrEC. Western blotting revealed that S100A16 was upregulated in all three prostate cancer cell lines ($P < 0.05$); however, the highest levels were observed in PC-3 cells and the lowest levels in DU-145 cells, compared to normal PrEC controls (Fig. 2a, b). Therefore, we selected DU-145 and PC-3 cells to investigate the effects of forced overexpression and S100A16 knockdown, respectively. Western blotting confirmed that S100A16 was upregulated in DU-145 cells transfected with S100A16 overexpression plasmid ($P < 0.05$; Fig. 2c, e), whereas it was silenced in PC3 cells transfected with S100A16 shRNAs ($P < 0.05$; Fig. 2d, f).

S100A16 promoted cell invasion and migration in human prostate cancer cell in vitro

To determine whether S100A16 played a role in prostate cancer cell metastasis in vitro, we investigated whether upregulation of S100A16 could induce prostate cancer cell invasion and migration. The results showed that a significantly higher proportion of S100A16-overexpressing DU-145 cells migrated through matrigel-coated chambers compared to vector

control cells ($P < 0.05$; Fig. 3a, c). In contrast, knockdown of S100A16 with shRNA1 or shRNA2 resulted in a significantly lower number of invading PC-3 cells, compared to scrambled controls ($P < 0.05$; Fig. 3b, d).

These effects were confirmed by wound healing assays, which showed that overexpression of S100A16 induced migration of DU-145 cells (Fig. 3e, g), whereas knockdown of S100A16 inhibited migration of PC-3 cells, compared to control cells (Fig. 3f, h). The effects were significant at 24 h ($P < 0.05$).

S100A16 regulated cell proliferation in human prostate cancer cell in vitro

We investigated whether S100A16 might play a role in cell proliferation and cell cycle progression in prostate cancer cells. Silencing of S100A16 with shRNA1 and shRNA2 inhibited colony formation in PC-3 cells compared to control cells ($P < 0.05$; Fig. 4b, d), whereas forced overexpression of S100A16 promoted colony formation in DU-145 cells ($P < 0.05$; Fig. 4a, c).

Flow cytometric analysis showed that the ratio of G1 cells was significantly lower in S100A16-overexpressing DU-145 cells than in control cells ($P < 0.05$; Fig. 4e, g), suggesting that upregulation of S100A16 promoted cell proliferation in prostate cancer. In contrast, downregulation of S100A16 in PC-3 cells showed the converse effect ($P < 0.05$; Fig. 4f, g). Furthermore, the percentage of S100A16-silenced PC-3 cells

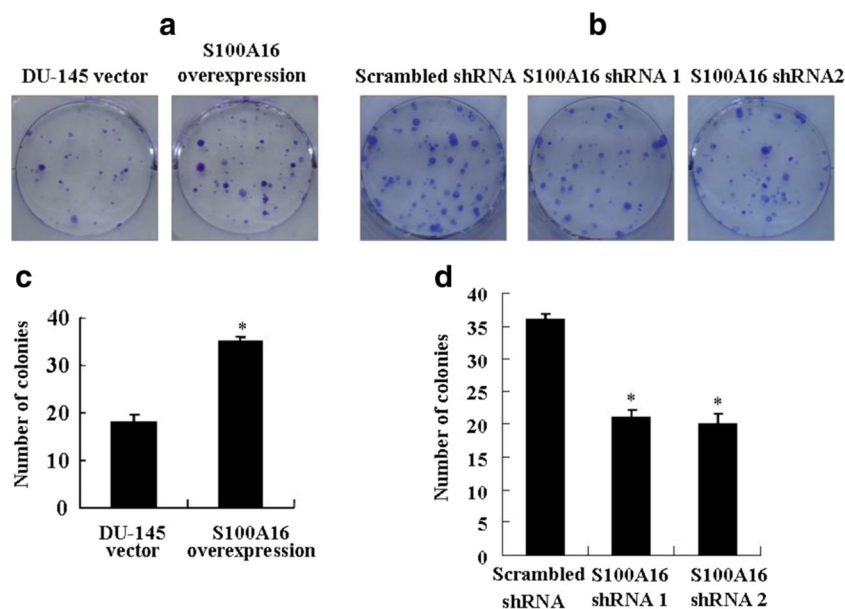


Fig. 4 S100A16 promotes proliferation in human prostate cancer cells in vitro. **a–d** Soft agar colonies reflecting cell growth. **a** DU-145 cells overexpressing S100A16 compared to vector control; **b** PC-3 cells with S100A16 knockdown compared to scrambled shRNA controls. Magnification $\times 200$. **c**, **d** Quantification of the colony formation assays based on two colony grids/well. **e–h** Flow cytometric analysis showing the proportion of prostate cancer cells at each phase of the cell cycle. **e** DU-145 cells overexpressing S100A16 compared to vector control; **f** PC-

3 cells with S100A16 knockdown compared to scrambled shRNA controls. **g**, **h** Quantification giving the percentage of cells in each phase of the cell cycle, for each transfected population. **i**, **j** Proliferation assays showing cell growth after 0, 24, 48, and 72-h incubation. **i** DU-145 cells overexpressing S100A16 compared to vector control; **j** PC-3 cells with S100A16 knockdown compared to scrambled shRNA controls. The results represent at least three independent experiments. * $P < 0.05$, compared to control

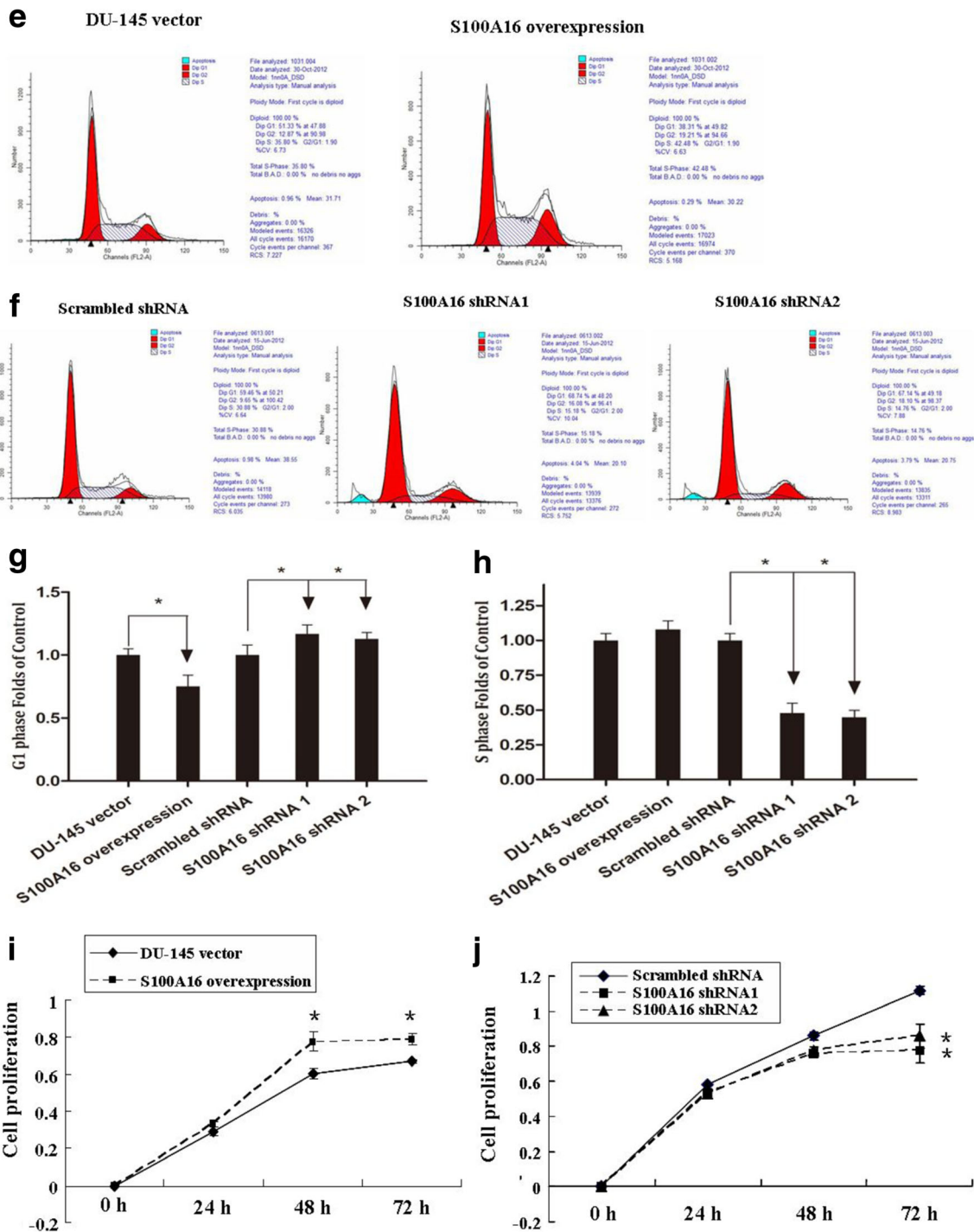


Fig. 4 (continued)

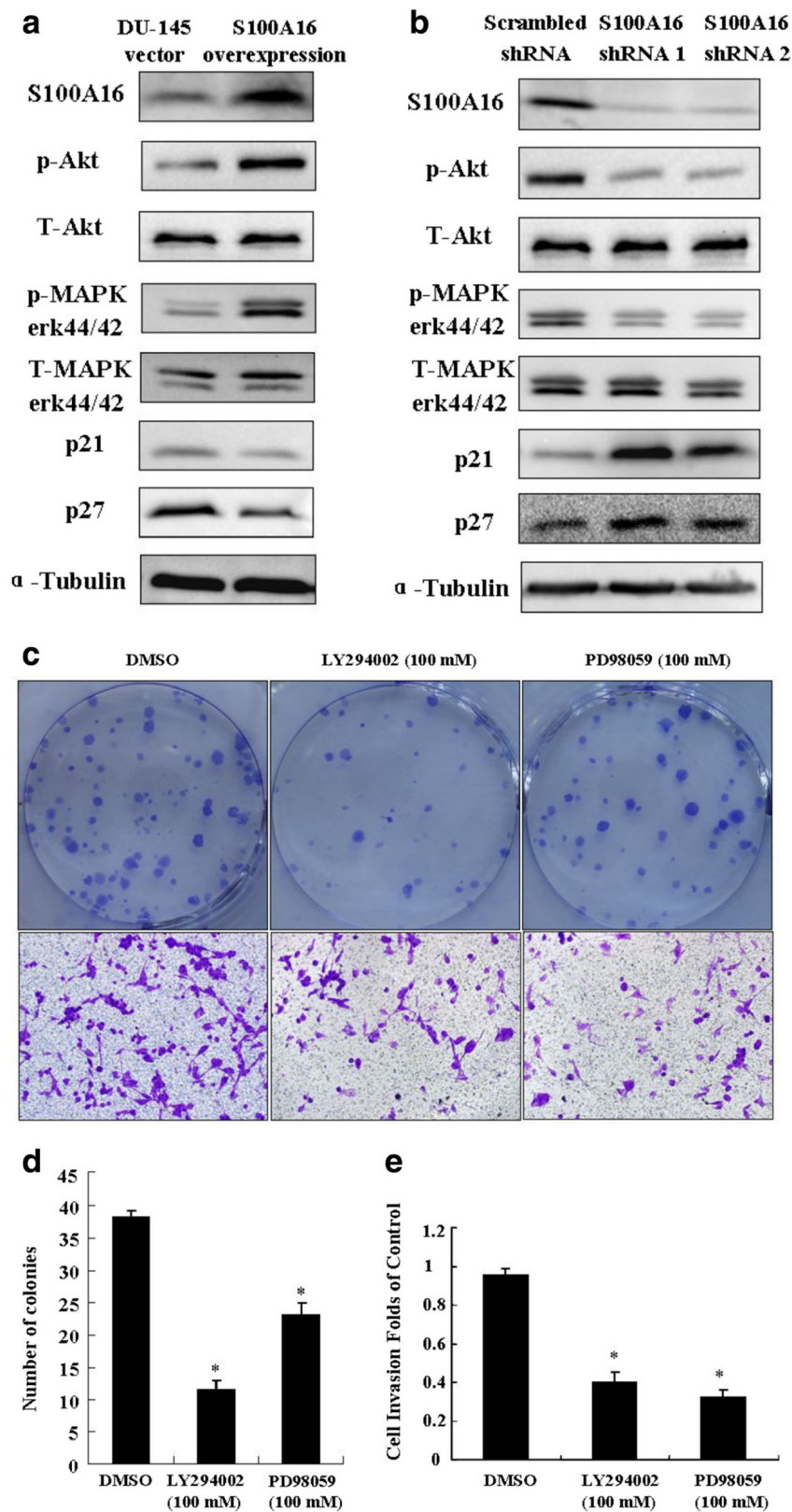
at S phase was significantly lower than that of control cells ($P < 0.05$); however, the difference in S phase was not significant between S100A16-overexpressing DU-145 cells and control cells (Fig. 4h).

Cell proliferation assays showed that S100A16 overexpression significantly increased cell growth in DU-145 cells at 48 and 72 h compared to vector controls ($P < 0.05$; Fig. 4i). In contrast, S100A16 knockdown significantly decreased cell

growth in PC-3 cells at 72 h compared to scrambled controls ($P < 0.05$; Fig. 4j).

The effect of S100A16 on cell signaling proteins and tumor suppressors

To investigate the molecular mechanisms and pathways by which S100A16 may influence cellular functions in human



prostate cancer, we examined the expressions of AKT, ERK, p-AKT, p-ERK, and cyclin-dependent kinase inhibitors p21 and p27. Western blotting revealed a significant increase in

phosphorylated AKT and ERK, indicating activation of their associated pathways, and downregulation of tumor suppressors p21 and p27 in S100A16-overexpressing DU-145 cells

◀ **Fig. 5** Cell signaling proteins targeted by S100A16 in prostate cancer cells. **a, b** Western blot analysis of total ERK, phospho-ERK, total AKT, phospho-AKT, p21, and p27 protein expression: **a** DU-145 cells overexpressing S100A16; **b** PC-3 cells with knockdown of S100A16 compared to scrambled shRNA controls. **c** Cell invasion assay of DU-145 prostate cancer cells overexpressing S100A16 following inhibition of ERK and P13K with PD98059 and LY294002, respectively. Magnification $\times 200$. **d** Quantification of the colony formation assay, based on two colony grids/well. **e** Quantification of the cell invasion assay based on six random fields from each chamber for each condition. $*P < 0.05$, compared to control. The data represent three independent experiments

compared to vector controls ($P < 0.05$; Fig. 5a). Conversely, S100A16 knockdown significantly inhibited activation of AKT and ERK, and upregulated p21 and p27 protein expression compared to scrambled controls ($P < 0.05$; Fig. 5b).

To establish whether the mitogen-activated protein kinases/extracellular signal-regulated kinases (MAPK/ERK) and phosphoinositide3-kinase/protein kinase B (PI3K/AKT) pathways played a role in S100A16-induced prostate cancer invasion and proliferation, we used LY294002 and PD98059 to inhibit AKT and ERK, respectively. The results showed a significant reduction in the invasive capacity and number of colonies formed in DU-145 cells stably overexpressing S100A16 in the presence of these inhibitors ($P < 0.05$; Fig. 5c–e).

Discussion

The S100 family of proteins includes at least 21 identified Ca^{2+} -binding proteins, many of which have been found to be upregulated and to play roles in different tumor types [2, 4, 6]. These include S100A7, which was found to enhance invasion in human breast cancer [8]. S100A4 was seen to accelerate tumorigenesis and invasion in human prostate cancer and is employed as a biomarker in chemoprevention regimens [7, 9]. Several S100 gene family members have been reported as overexpressed in human bladder cancers, including S100A2, S100A3, S100A5, S100A7, S100A8, S100A9, S100A14, S100A15, S100A16, and S100P [10, 11]; enhanced expressions of S100A8 and S100A9 were identified as early events in prostate tumorigenesis and may contribute to development, progression, and invasion in prostate carcinomas [12]. In addition, S100A4 has been shown enhance tumorigenesis through activation of nuclear factor-kappaB (NF- κ B) and matrix metalloproteinase 9 (MMP-9) pathways, and through suppression of tumor suppressor gene p53 [7, 14–17]. However, the role of S100A16 in human prostate cancer remains to be established. Therefore, our objective was to determine whether S100A16 has a direct functional link to human prostate cancer progression.

To test this hypothesis, we examined S100A16 expression in prostate cancer tissue samples and adjacent normal tissues, and in three prostate cancer cell lines with different metastatic

potentials, DU-145, LNCaP, and PC-3, compared to a normal prostate epithelial cell line PrEC. DU-145 cells are less invasive in vitro and exhibit relatively low potential for metastasis in vivo compared to more malignant PC-3 cells; LNCaP cells grow readily in tissue culture as well as in nude mice following inoculation [13]. Western blot analysis confirmed that S100A16 was expressed in both normal and prostate cancer tissues and cell lines; however, its expression was significantly higher in the tumorous tissues and cell lines compared to the normal samples ($P < 0.05$). The data on Fig. 2a may suggest that S100A16 protein levels show correlations with the metastatic potential, increasing from DU-145 cells to LNCaP and PC-3 cells.

Along with several other members of the S100 protein family, S100A16 is considered to be involved in progression of cancer and a potential marker of tumor cells [4]. In this study, overexpression of S100A16 was found to promote invasion, migration, and proliferation in DU-145 cells, a human prostate cancer cell line with low metastatic potential, compared to normal prostate cells. In contrast, these actions were suppressed in S100A16-silenced PC-3 cells, which have high metastatic potential. Taken together, S100A16 may function to support and enhance factors that promote progression and metastasis in human prostate cancer.

The molecular signaling pathways that were implicated in these S100A16-promoted functions remained to be established. Therefore, we examined the relationship between S100A16 protein expression in prostate cancer and two of the principal cell signaling pathways associated with carcinogenesis, MAPK/ERK and PI3K/AKT. MAPKs are serine/threonine kinases that mediate intracellular signaling associated with a variety of cellular activities, including proliferation, differentiation, survival, death, and transformation. ERK is one of the three key members of the MAPK family and plays a central role in normal prostatic epithelial proliferation. In addition, ERK is associated with the proliferation of cancer cells and tumor invasion via degradation of extracellular matrix proteins [18, 19, 21]. Activation of AKT through phosphorylation has been implicated in the initiation and development of prostate cancer and has been correlated with poor prognosis [20, 22]. As AKT phosphorylation can be antagonized by phosphatase and tensin homolog (PTEN), which is not expressed in PC-3 cells [23], high levels of p-Akt can be observed in PC-3 cells. This was confirmed by Western blot analysis. However, S100A16 knockdown in PC-3 cells was found to reduce p-AKT levels, suggesting that depletion of S100A16 might block activation of AKT and ERK. Conversely, Western blotting revealed an increase in the levels of p-AKT and p-ERK in DU-145 cells overexpressing S100A16. Taken together, these observations suggested that ERK and AKT were downstream effectors of S100A16.

A previous study reported that S100A16 might promote metastasis through suppression of p53 expression [15]. Therefore, we investigated whether S100A16 facilitated metastasis in

human prostate cancer via p53 target molecules, p21 and p27. Our results showed that expression of these two putative tumor suppressors was reduced by overexpression of S100A16, whereas silencing of S100A16 had the converse effect.

In conclusion, we demonstrated that overexpression of S100A16 promoted invasion, migration, and proliferation in human prostate cancer cells *in vitro*, whereas silencing S100A16 inhibited these effects. Furthermore, our results suggested that S100A16 may act via cell signaling molecules ERK and AKT and tumor suppressors p21 and p27. These results suggest that S100A16 may be a novel therapeutic target in human prostate cancer.

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Compliance with ethical standards

Conflicts of interest None

References

- Kiess AP, Cho SY, Pomper MG. Translational molecular imaging of prostate cancer. *Curr Radiol Rep*. 2013;1(3):216–26.
- Sturchler E, Cox JA, Durussel I, Weibel M, Heizmann CW. S100A16, a novel calcium-binding protein of the EF-hand superfamily. *J Biol Chem*. 2006;281:38905–17.
- Donato R. Functional roles of S100 proteins, calcium-binding proteins of the EF-hand type. *Biochim Biophys Acta*. 1999;1450(3):191–231.
- Heizmann CW. The multifunctional S100 protein family. *Methods Mol Biol*. 2002;172:69–80.
- Saleem M, Kweon MH, Johnson JJ, Adhami VM, Elcheva I, Khan N, et al. S100A4 accelerates tumorigenesis and invasion of human prostate cancer through the transcriptional regulation of matrix metalloproteinase 9. *PNAS*. 2006;103(40):14825–30.
- Marenholz I, Heizmann CW. S100A16, a ubiquitously expressed EF-hand protein which is up-regulated in tumors. *Biochem Biophys Res Commun*. 2004;313:237–44.
- Liu Y, Zhang R, Xin J, Sun Y, Li J, Wei D, et al. Identification of S100A16 as a novel adipogenesis promoting factor in 3T3-L1 cells. *Endocrinology*. 2011;152:903–11.
- Liu H, Wang L, Wang X, Cao Z, Yang Q, Zhang K. S100A7 enhances invasion of human breast cancer MDA-MB-468 cells through activation of nuclear factor- κ B signaling. *World J Surg Oncol*. 2013;11(93):1–8.
- Saleem M, Adhami VM, Ahmad N, Gupta S, Mukhtar H. Prognostic significance of metastasis-associated protein S100A4 (Mts1) in prostate cancer progression and chemoprevention regimens in an autochthonous mouse model. *Clin Cancer Res*. 2005;11:147–53.
- Yao R, Lopez-Beltran A, MacLennan GT, Montironi R, Eble JN, Cheng L. Expression of S100 protein family members in the pathogenesis of bladder tumors. *Anticancer Res*. 2007;27:3051–8.
- Salama I, Malone PS, Mihaimed F, Jones JL. A review of the S100 proteins in cancer. *EJSO*. 2008;34:357–64.
- Hermani A, Hess J, De Servi B, Medunjanin S, Grobholz R, Trojan L, et al. Calcium-binding proteins S100A8 and S100A9 as novel diagnostic markers in human prostate cancer. *Clin Cancer Res*. 2011;17(14):5146–52.
- Aalinkel R, Nair MP, Suffrin G, Mahajan SD, Chadha KC, Chawda RP, et al. Gene expression of angiogenic factors correlates with metastatic potential of prostate cancer cells. *Cancer Res*. 2004;64:5311–21.
- Grotterød I, Maelandsmo GM, Boye K. Signal transduction mechanisms involved in S100A4-induced activation of the transcription factor NF- κ B. *BMC Cancer*. 2010;10:241.
- Grigorian M, Andresen S, Tulchinsky E, et al. Tumor suppressor p53 protein is a new target for the metastasis-associated Mts1/S100A4 protein: functional consequences of their interaction. *J Biol Chem*. 2001;276:22699–708.
- Ginzburg S, Golovine KV, Makhov PB, Uzzo RG, Kutikov A, Kolenko VM. Piperlongumine inhibits NF- κ B activity and attenuates aggressive growth characteristics of prostate cancer cells. *Prostate*. 2013. doi:10.1002/pros.22739.
- Stearns M, Stearns ME. Evidence for increased activated metalloproteinase 2 (MMP-2a) expression associated with human prostate cancer progression. *Oncol Res*. 1996;8:69–75.
- Price DJ, Avraham S, Feuerstein J, Fu Y, Avraham HK. The invasive phenotype in HMT-3522 cells requires increased EGF receptor signaling through both PI 3-kinase and ERK 1,2 pathways. *Cell Commun Adhes*. 2002;9(2):87–102.
- Peng S, Zhang Y, Zhang J, Wang H, Ren B. ERK in learning and memory: a review of recent research. *Int J Mol Sci*. 2010;11(1):222–32.
- Junttila MR, Li SP, Westermarck J. Phosphatase-mediated crosstalk between MAPK signaling pathways in the regulation of cell survival. *FASEB J*. 2008;22(4):954–65.
- Kim EK, Choi EJ. Pathological roles of MAPK signaling pathways in human diseases. *Biochim Biophys Acta*. 2010;1802:396–405.