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Original Research

Inhibition of TRPM7 suppresses migration and invasion of prostate cancer cells via inactivation of ERK1/2, Src and Akt pathway signaling

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Abstract

Background: Prostate cancer is the second most common cause of cancer related death in males worldwide. Most patients show no response to androgen deprivation therapy in case of recurrence and proceed to advanced stage with metastasis. TRPM7 is reported to be upregulated in diverse types of tumors. **Methods**: We analyzed the expression of TRPM7 and related proteins by Western blotting analysis. We performed cell migration and invasion assay to analyze the relationship between tumor aggressiveness and TRPM7. In addition, we proceeded an animal study by using stable TRPM7 knockdown cell line in xenograft. **Results**: In our results, TRPM7 regulates prostate cancer cell biology including proliferation, migration and invasion through ERK1/2, PI3K/Akt and JNK signaling pathways. We produced stable TRPM7 knockdown prostate cancer cell line. To analyze the relationship between TRPM7 and tumorigenesis, we proceeded migration and invasion assay as well as xenograft model. TRPM7 down-regulated DU145 cells showed suppressed migratory and invasion ability, 0.65- and 0.05-fold, respectively. In addition, we confirmed that the anti-cancer effect of TRPM7 is mediated through inactivation of ERK1/2, Src and Akt signaling pathways by western blotting analysis. P-ERK1/2, p-Src, and p-Akt expressions were reduced to 0.66-, 0.68-, and 0.66-fold, respectively. Moreover, we treated ERK, Akt and Src inhibitors to clarify the involvement of related each protein in migration and invasion ability, and we could observe that inhibitor treated cells showed suppressed migration and invasion ability. *In vivo*, TRPM7 knockdown cells projected decreased cell proliferation rate. **Conclusions**: Taken these results together, out study suggested TRPM7 might be an essential gene for prostate cancer metastasis by regulating prostate cancer cell proliferation, migration and invasion ability.

Keywords: Prostate cancer therapy; Transient receptor potential cation channel-subfamily M member 7; Migration ability; Cell proliferation; Src signaling

1. Introduction

The transient receptor potential cation channel-subfamily M, member 7 (TRPM7) belongs to TRP channels, including transient receptor potential cation channel (TRPC), transient receptor potential cation channel-subfamily M (TRPM), transient receptor potential cation channel subfamily V member 1 (TRPV) and other subfamilies [1]. In normal condition, TRPM7 functions as non-selective Mg^{2+}/Ca^{2+} permeable channel and protein kinase that are widely expressed in normal human tissues [2]. TRPM7 is a tetrameric complex composed of four subunits [3]. TRPM7 may activate itself by phosphorylating and regulates various physiologic and pathologic conditions of cells, such as Mg^{2+} homeostasis, cell proliferation, survival, differentiation, adhesion, invasion and migration [4]. TRPM7 has been reported to be involved in several cancer types, including breast cancer, gastric cancer, head and neck cancer, pancreatic cancer and leukemia [5]. In previous studies, TRPM7 is reported to be overexpressed in various cancer tissues [6–8]. TRPM7 is highly involved in Mg^{2+}/Ca^{2+} channel and Mg^{2+}/Ca^{2+} are related to prostate cancer. Mg^{2+}/Ca^{2} signaling takes critical role in cell pro-

liferation and TRPM7 is reported to be regulated by intracellular Mg^{2+} level. In reported studies, serum Ca^{2+} is a possible biomarker of fatal prostate cancer. High level of serum Ca^{2+} predicts fatal prostate cancer [6,9,10].

The prostate is an accessory sex gland in male. The main function of the prostate is to produce an alkaline fluid during ejaculation, that helps the motility and nourishment of sperms. The urinary problems (increased frequency, painful urinating, hematuria), bowel problem (diarrhea, constipation) and/or sexual dysfunctions are the prediagnostic symptoms of prostate diseases [11]. Prostate cancer is the second leading causes of cancer related deaths in males worldwide and is a significant public health problem [12–14].

At present, androgen deprivation therapy (ADT) has become the standard treatment of prostate cancer as hormone therapy, due to the androgen depending characteristic of prostate cancer [15]. In case of cancer recurrence in few years after hormone therapy, most of the patients no longer show the responds to hormone therapy and progresses to castration-resistant prostate cancer (CRPC) stage, which at few drugs are effective with high mortality [16]. In advanced prostate cancer, cancer cells tend to metastasize to bones and lymph nodes. Then, patients undergo chemotherapeutic treatment such as mitoxantrone, estramustine, doxorubicin, etoposide, vinblastine, paclitaxel, carboplatin, vinorelbine [17]. However, these chemotherapeutic agents induce severe side effects and damage to not only prostate cancer cells but also normal organs in case of repeated administration [18]. Therefore, early detection of prostate cancer and development of effective and novel therapeutic agent is important to reduce prostate cancer deaths due to temporary effect of hormone therapy and drug administration. In addition, understanding of the molecular mechanism underlying the metastasis and invasion of prostate cancer cells.

In past study, TRPM7 is reported to be related in migration and invasion of prostate cancer via ERK1/2 and Akt mediated pathway [19], however, we improved the study by inhibitors, *in vivo* study, and human tissue sample analysis. The present study provided the evidence that TRPM7 knockdown prevents prostate cancer cell migration and invasion through inactivation of p-ERK1/2, p-Akt and p-Src *in vitro* by treating siRNA and producing stable knockout cell lines. Moreover, we confirmed the relationship of involved proteins by treating inhibitors. Furthermore, we explored the study of TRPM7 knockdown *in vivo* analysis as well as the relation of TRPM7 in human prostate samples.

2. Materials and methods

2.1 Patients sample

From January 2013 to April 2016, 95 patients participated in this study. The biospecimens and data used for this study were provided by the National Biobank of Korea-Kyungpook National University Hospital, a member of the Korea Biobank Network-KNUH, and were obtained (with informed consent) under Institutional Review Board-approved protocols (approval number: KNUMC 2016-05-021). All samples were obtained from 95 patients treated at the Kyungpook National University Chilgok Hospital. All tumor samples were acquired after acquiring patient consent for tissue sample donation and an examination was performed. The diagnosis of PCa was verified based on the outcomes of pathological analyses. For each patient, both normal and tumor tissues were collected and used in these studies. Both the normal tissues and prostate cancer tissues (separated into T2 and T3 stages) were stored at –80 °C before use. The baseline characteristics of the 95 patients enrolled in the study are listed in Table 1.

Table 1. Patients' demographics.

Parameters	Mean or N (%)
Mean Age (yrs) ± *SD*	65.43 ± 6.20
Median PSA (ng/mL) ± *SD*	8.90 ± 1.783
Body mass index (kg/m^2) ± *SD*	24.49 ± 2.77
Pathologic stage (n_%)	
pT2	41 (43.2)
pT3a	35 (36.8)
pT3b	19 (20.0)
Gleason score (n_%)	19 (20.0)
6–7	66 (69.5)
8–10	29 (30.5)

2.2 Reagents

Anti-TRPM7 antibody was purchased from Abcam (Cambridge, MA, USA). Anti-ERK1/2, anti-phospho-ERK1/2, anti-Akt, anti-phospho-Akt, anti-Src, anti-phospho-Src, anti-JNK, anti-phospho-JNK, and anti-beta actin anti bodies were purchased from Cell Signaling Technology (Danvers, MA, USA). Goat-anti-rabbit IgG HRP-conjugated and rabbit-anti-mouse IgG HRP-conjugated secondary antibodies were purchased from Invitrogen (Carlsbad, CA, USA). PD98059 (ERK inhibitor), LY-294002 (Akt inhibitor), bosutinib (Src inhibitor), dimethyl sulfoxide (DMSO), Alexa-FluorTM 594 antibodies and crystal violet solution were obtained from Sigma-Aldrich (St Louis, MO, USA).

2.3 Cell culture

LNcap, DU145m (vector only treated) and DU145sh (shRNA treated) cell lines were purchased from American Type Culture Collection (ATCC; Manassas, VA, USA). DU145 cells (human prostate cancer cell line) were maintained Dulbecco's modified Eagle's medium liquid (DMEM) high glucose (Hyclone Laboratories, Inc., Logan, UT, USA) with 10% FBS (Hyclone Laboratories, Inc., Logan, UT, USA) and 50 units/mL penicillin and 50

μg/mL streptomycin (Hyclone Laboratories, Inc., Logan, UT, USA) at 37 °C in a humidified 5% CO_2 atmosphere.

2.4 siRNA treatment

Cells were seeded into 6 well plate in number of 3×10^5 and grown to 80% of confluency. Cells were treated with 25 μmol of Dharma FECT™ Transfection reagent 1 (Dharmacon, Colorado, USA) and 10 μmol of ON-TARGETplus Human TRPM7 siRNA (4 siRNA sets in single mixture, Dharmacon, Colorado, USA) in serum free media according to its manufacturer's protocol. After 6 h of incubation, serum free media were changed to serum containing growth media. Cells were harvested after 48 h and 72 h for mRNA and protein, respectively.

2.5 Vectors, transfection and transduction

To produce TRPM7 knockdown cell line, pLKO.1-scramble and pLKO.1-shTRPM7 vectors were purchased from Sigma-Aldrich (St Louis, MO, USA). The number of 5×10^6 293T cells were plated in 100 mm culture plate in 10% FBS, 50 units/mL penicillin and 50 μg/mL streptomycin (Hyclone Laboratories, Inc., Logan, UT, USA) containing DMEM high glucose media. After 24 h, packaging plasmid (pMDLg/pRRE, pMD2.G, pRSV-Rev) and Fugene HD reagents were treated to 293T cells with pLKO.1-scramble vector and pLKO.1-shTRPM7 vector for transfection. Followed by 24 h, media was changed to fresh media and incubated for 48 h before harvest. Harvested supernatant of cell culture was filtered with 0.45 μm pore size filter to collect Lentivirus. For infecting Lentivirus, DU145 cell lines were grown in 60 mm culture plate up to 70% of confluency. The equal volume of filtered supernatant was added to DU145 cell culture media and 8 μg/mL of polybrene (Sigma-Aldrich) were treated, and cells were incubated for 48 h. After 48 h of incubation, 1.5 μg/mL of puromycin was used to select uninfected cells. Followed by 5 days of incubation, scrambled and TRPM7 knockdown DU145 cell lines were obtained.

2.6 Migration and invasion assay

For migration and invasion assay, 1×10^5 number of cells were seeded in cell culture insert with 8 μm pore size (#353097, BD falcon, Franklin Lakes, NJ, USA) and in matrigel coated insert (#354480, Corning, NY, USA) with 300 μL of serum free media, respectively. In 12-well-plate, 700 μL of 10% FBS containing media was added. After 24 h of incubation at 37 °C in a humidified 5% CO_2 atmosphere, cells on top of the insert were removed and cells, moved to bottom, were stained with crystal violet solution. The stained cells in insert membrane were dissolved in DMSO, and measured with microplate spectrophotometer (wavelength 590 nm, BioTek Instruments, Winooski, VT, USA).

2.7 Real time polymerase chain reaction (real time PCR)

The number of 5×10^5 DU145m and DU145sh cells were prepared to extract mRNA. RNA extraction was performed by Maxwell® RSC simply RNA cell kit using MaxwellTM 16 instrument (Promega Corporation, Madison, WI, USA). One μg of the extracted RNA was used to synthesize cDNA using Cell Script All-in-One cDNA Master Mix (CellSafe, Yongin, Korea) according to manufacturer's protocol. PCR was performed in StepOnePlus™ Real-Time PCR System (Applied Biosystems® Inc., Foster City, CA, USA). A pair of primers (left primer: 5′ TGGAAAGTATGGGGCAGAAG 3′, right primer: 5′ ATCAAAGCCACCACAGGAAC 3′) was designed to obtain real time PCR result of TRPM7 expression.

2.8 Western blot analysis

Treated cells were lysed in RIPA buffer for 1 h of incubation at 4 °C and centrifuged at 15,000 r.p.m, 30 min, 4 °C. The supernatant was collected, and protein was quantified by BCA protein assay kit (Thermo Fisher Scientific, Rockford, IL, USA). 10–20 μg of proteins were loaded onto SDS-PAGE gels and transferred to nitrocellulose membrane. The primary antibodies were applied at dilution of 1:1000 for overnight at 4 °C after blocking procedure. After washing with tris-buffered saline (pH 7.5; 100 mM NaCl, 50 mM Tris, and 0.1% Tween-20) solution for 3 times, the second antibodies were applied at dilution of 1:5000 for 2 h at room temperature. The desired bands were developed using ECL reagent (Advensta, Menlo Park, CA, USA), images were captured with a chemi-doc image analyzer (iBright 1500, Thermo Fisher Scientific), and the values were quantified with Image J software v.1.8.0 (Wayne Rasband, GitHub, San Francisco, CA, USA).

2.9 Immunohistochemistry

The tissue samples were fixed in 10% formalin and produced in paraffin block by passing dehydration, clearing and embedding processes. Paraffin blocks were sectioned in 4 μm thickness and stained with different antibodies. The slides were cleared and hydrated in xylene and distilled water, and antigen was retrieved in pH 6.0 citrate buffer solution. Triton-x solution was used for permeabilization and first antibodies were applied for overnight at 4 °C. FITC labelled secondary antibodies were applied for 2 h at room temperature and the slides were mounted by using mounting solution with DAPI (Vector Laboratories, Burlingame, CA, USA).

2.10 In vivo assay

All animal study protocols were approved by the institutional animal ethics committee of Yeungnam University, College of Medicine (YUMC-AEC2019-040). All animals (n = 5) were kept under a 12 h light/dark cycle, temperature of 25.0 $\pm$ 0.2 °C and humidity of 45 $\pm$ 2% controlled specific pathogen free environment. DU145 cells (2×10^6)

were subcutaneously injected to Balb/c nude mice (6-week-old, Orient, Seoul, Korea). When tumor grows to 4–6 mm in diameter, tumor size was measured twice in a week for four weeks. All animals were sacrificed after sample collection by using CO_2 gas chamber according to IACUC guideline.

2.11 Statistical analysis

One-way analysis of variance procedures was used to assess significant differences among treatment groups. To normalize the highly skewed distribution of TRPM7 mRNA expression, the data were examined as natural logs and subsequently back transformed to generate the final results [18]. The results were expressed as the geometric mean (anti-log 95% confidence interval). The ratio of TRPM7 expression between the normal and tumor tissue was determined (the expression level of normal tissues was divided by that of tumor tissue [N/T ratio]) [19]. To compare the N/T ratio between groups, a 2-sample *t*-test was performed. Using this value, the patients were classified into high or low N/T ratio groups. Statistical analysis was performed using SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA), and a p value of <0.05 was considered statistically significant.

3. Results

3.1 Up-regulated expression of TRPM7 in human prostate cancer sample and the effect of TRPM7 knockdown in DU145 cell line

To prove the relationship between TRPM7 and prostate cancer, we evaluated mRNA expression of TRPM7 in prostate cancer patient tissues. In prostate cancer patient tissues, mRNA expression of TRPM7 was significantly higher in T3 patients than T2 (Fig. 1A). Moreover, the ratio (N/T ratio) of TRPM7 in tumor tissue to normal adjacent tissue increased in T3 patient samples compared to T2 patient samples (Fig. 1A). At first, to examine the effect of TRPM7 in prostate cancer, we treated human TRPM7 siRNA to DU145 and LNcap cell lines. Then, we confirmed decreased expression of TRPM7 protein by siRNA treatment in DU145 (**Supplementary Fig. 1A**) and LNcap (**Supplementary Fig. 2A**) cells. As next, we observed phosphorylated forms of Src and ERK in DU145 (**Supplementary Fig. 1B**) and LNcap (**Supplementary Fig. 2B**) cells which are reported to be related in cancer cell migration and invasion. In addition, siRNA treated group showed narrow wound gap in wound healing assay in both DU145 (**Supplementary Fig. 1C**) and LNcap (**Supplementary Fig. 2C**) cells.

3.2 Production of stable TRPM7 knockdown in DU145 cells

Based on TRPM7 silencing by siRNA, we proceeded to produce stable TRPM7 knockdown cell line (DU145sh) with shRNA. The transfection was very effective as around

A

TRPM7 Expression (10^3 Copies/L)				
	N	Mean	95% CI	p
T2	41	11.3	6.9-18.5	0.046
T3	54	20.0	14.1-29.9	

TRPM7 Expression (10^3 Copies/L)				
	N	Mean	95% CI	p
T2	41	3.03	2.91-3.27	0.035
T3	54	2.75	2.63-2.88	

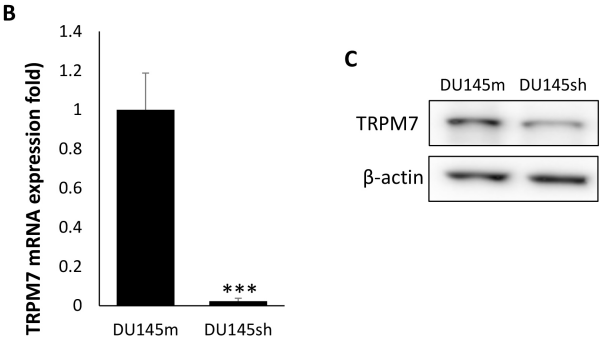

Fig. 1. Expression of TRPM7 related to prostate cancer and downregulation of TRPM7 in prostate cancer cell. The expression of TRPM7 in human prostate cancer tissue sample (A). Knockdown efficacy of TRPM7 in mRNA (B) and protein level (C) in DU145 cell line. β-actin served as the loading control. All data are expressed as the mean $\pm$ standard deviation from three independent experiments. $^*p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.005$ versus DU145m.

90% of TRPM7 mRNA expression (Fig. 1B) was suppressed as well as TRPM7 protein expression (Fig. 1C). Therefore, we chose DU145m and DU145sh cell line and used in the further experiments.

3.3 TRPM7 knockdown showed anti-cancer effect in prostate cancer

To evaluate the role of TRPM7 in tumor growth, we performed proliferation assay, migration and invasion assay by using DU145m and DU145sh cell lines. As we expected, TRPM7 knockdown resulted suppressed proliferation of DU145sh cells. Moreover, DU145sh cell line showed weak migratory (Fig. 2B) and invasion (Fig. 2C) ability. To quantify the ratio of migrated and invaded cells, we stained the cells with crystal violet and dissolved in DMSO to measure the relative absorbance. In relative absorbance of migration assay, DU145sh showed significantly decreased migration index (Fig. 2B) and invasion index (Fig. 2D) to 0.65- and 0.5- fold of DU145m, respectively. As supportive data, we performed migration and invasion assay by using siRNA in DU145 (**Supplementary Fig. 3A** and **B**) and LNcap (**Supplementary Fig. 4A** and **B**) cell lines, we observed wider gap in siRNA of TRPM7 treated group compared to mock group in both cell lines.

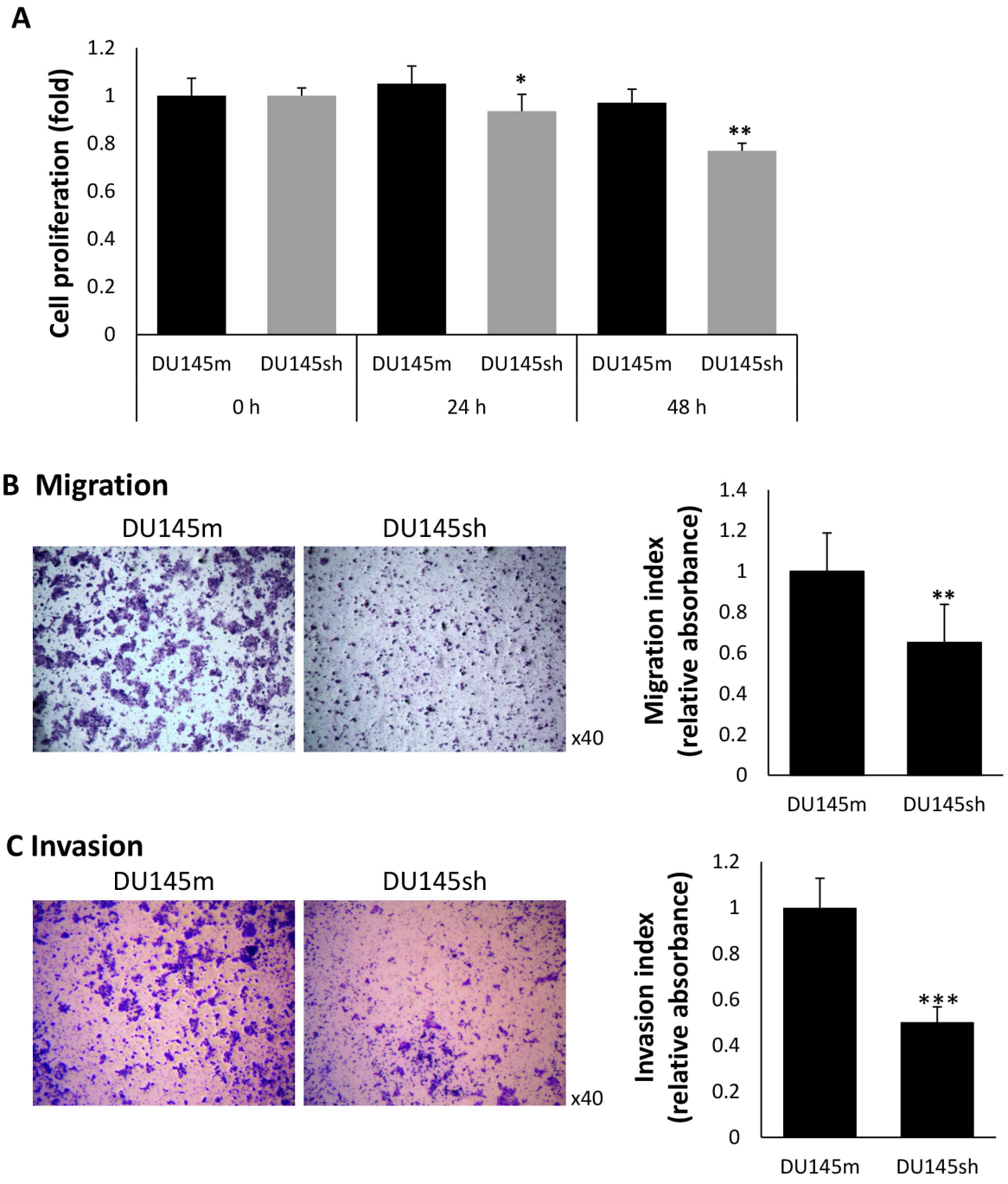

Fig. 2. Downregulation of TRPM7 inhibited cell proliferation, migration and invasion of prostate cancer cell. (A) Cell proliferation assay. DU145m and DU145sh cells were seeded in plate and cell viability was measured for indicated time period (0 h, 24 h, 48 h). (B) Migration assay. Cell migration was performed by transwell and cells were stained with crystal violet. (C) Invasion assay. All data are expressed as the mean ± standard deviation from three independent experiments. *$p < 0.05$; **$p < 0.01$; ***$p < 0.005$ versus DU145m. Magnification: ×40.

3.4 TRPM7 mediates ERK, Src, Akt pathway signaling

Next, to verify the underlying mechanism of TRPM7, we proceeded western blot analysis. We detected the change in protein expression in p-ERK1/2, p-Akt, p-Src and p-JNK. As shown in Fig. 3A, DU145sh exhibited decreased protein expression of p-ERK1/2, p-Akt, p-Src and p-JNK compared to DU145m while there is no protein expression change in total forms of ERK1/2, Akt, Src and JNK. The expression in transcriptional level of p-ERK1/2 (Fig. 3B), p-Akt (Fig. 3C), p-Src (Fig. 3D) and p-JNK (Fig. 3E) in DU145sh were 0.66-, 0.68-, 0.66-, 0.69-fold of DU145m, respectively.

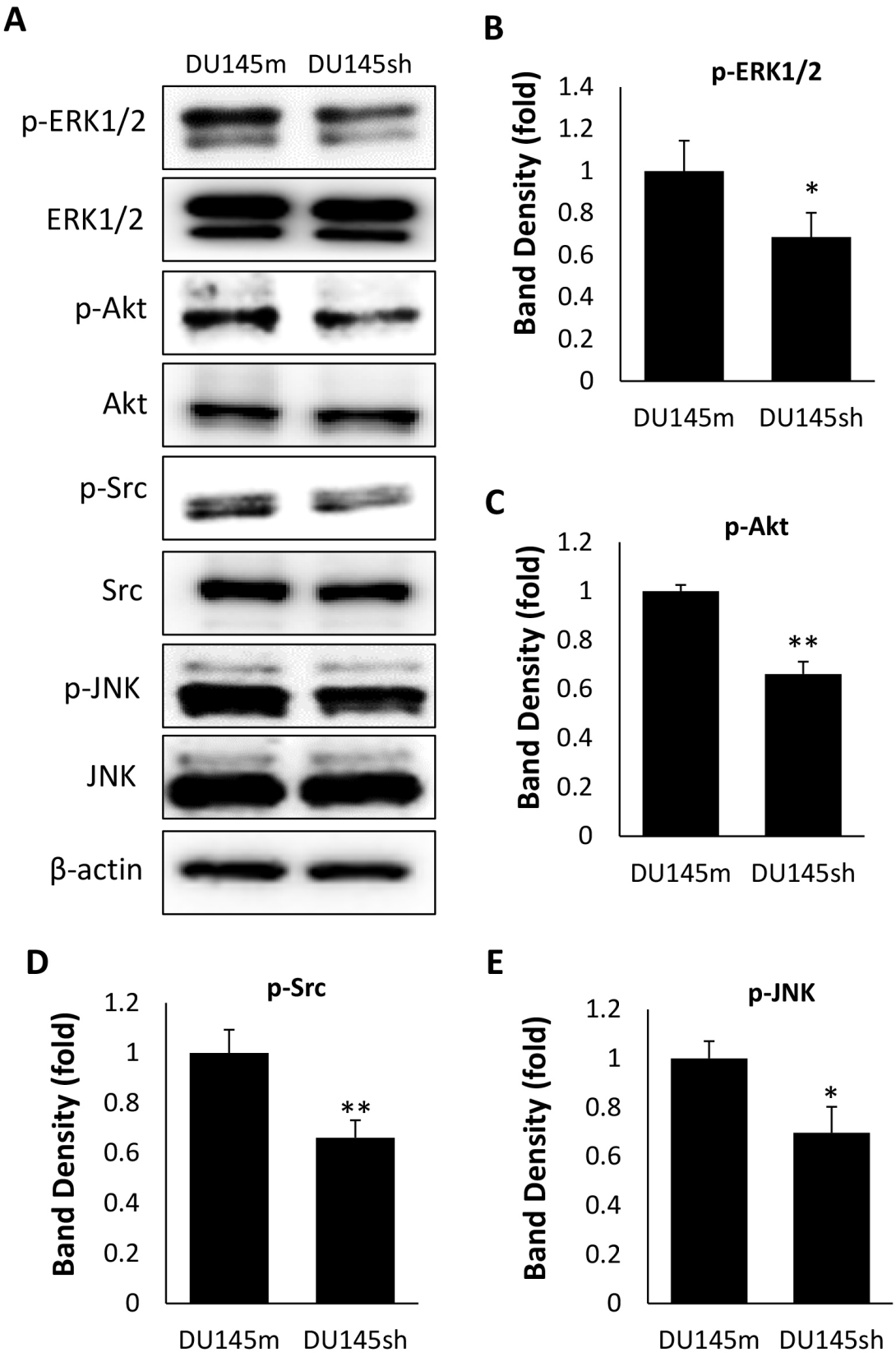

Fig. 3. Knockdown of TRPM7 inactivates ERK1/2, Akt, Src and JNK in DU145 cell line. (A) Representative image of western blot of phospho- and total ERK1/2, Akt, Src and JNK. β-actin served as the loading control. Band intensity of p-ERK1/2 (B), p-Akt (C), p-Src (D) and p-JNK (E) were quantified. All data are expressed as the mean $\pm$ standard deviation from three independent experiments. *$p < 0.05$; **$p < 0.01$ versus DU145m.

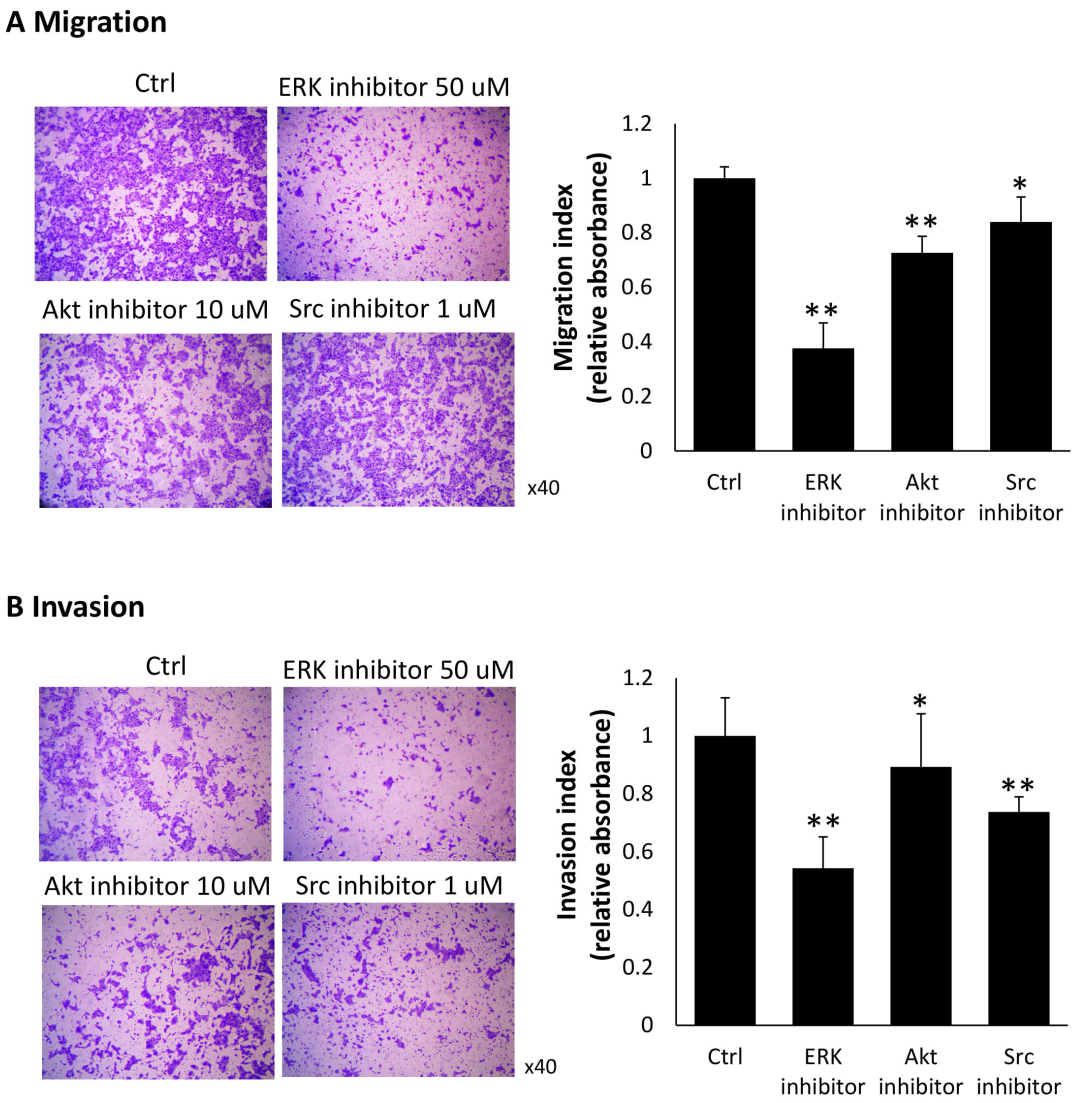

Fig. 4. Inhibitors of ERK, Akt and Src suppressed migration and invasion ability of DU145 cell line. DU145 cells were treated with ERK inhibitor (PD98059, 50 μM), Akt (LY-294002, 10 μM) and Src inhibitor (Bosutinib, 1 μM) for 24 hours. (A) Migration assay. (B) Invasion assay. All data are expressed as the mean $\pm$ standard deviation from three independent experiments. *$p < 0.05$; **$p < 0.01$ versus Ctrl. Magnification: $\times$40.

3.5 Inhibitor confirms the mediation of each proteins in migration and invasion

To elucidate the involvement of p-ERK1/2, p-Akt and p-Src in cancer cell migration and invasion, we treated inhibitors of ERK1/2 (PD98059), Akt (LY-294002) and Src (bosutinib) in DU145 cells. We performed observation under light microscope and measured the relative absorbance of stained cells. By treating the inhibitors, the number of migrated (Fig. 4A) and invaded cancer cells (Fig. 4B) were significantly decreased under light microscope examination as well as in relative absorbance measurement. In migration assay, migration ability of DU145sh was significantly suppressed to 0.37-, 0.72-, 0.83-fold of DU145m by PD98059, LY-294002 and bosutinib. In invasion assay, invasion ability of DU145sh was significantly suppressed to 0.54-, 0.89-, 0.73-fold of DU145m by PD98059, LY-294002 and bosutinib.

3.6 TRPM7 knockdown inhibits tumor formation in xenograft model

To further investigate the underlying mechanism of TRPM7 in cancer, we assessed xenograft model using nude mice, *in vivo*. As shown in Fig. 5A, the size of tumor from DU145m cell injection is clearly larger than DU145sh cell injection. In IHC staining, we could observe decreased expression of TRPM7 in DU145sh injected tumor samples (Fig. 5B). In addition, to demonstrate the correlation of TRPM7 and p-ERK1/2, p-Akt and p-Src, we proceeded IHC staining on tumor tissues. As the result, a weak expression of p-ERK1/2 (Fig. 5C), p-Akt (Fig. 5D) and p-Src

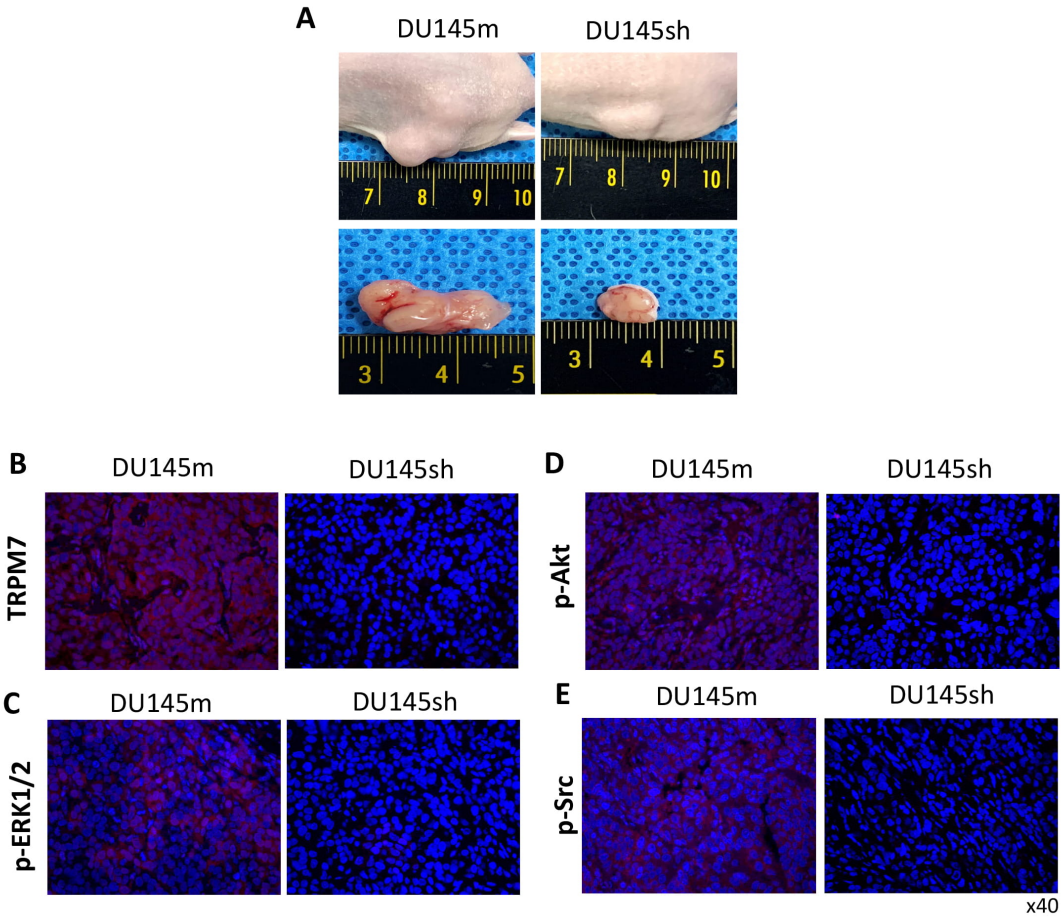

Fig. 5. Knockdown of TRPM7 suppressed prostate cancer cell growth in xenograft model. (A) Representative image of mice and tumor gross. Representative images of TRPM7 (B), p-ERK1/2 (C), p-Akt (D) and p-Src (E) of DU145m and DU145sh injected tumor (n = 4). Magnification: ×40.

(Fig. 5E) was observed in DU145sh injected tumor samples, compared to DU145m.

4. Discussion

Prostate cancer is closely related with aged men. As aged, the prostate shows high incidence of diseases, both benign and malignant. Many patients experience local recurrence and metastasis after resistance to ADT [20,21]. Metastasis, one of main characteristics in malignant tumor progression, is considered as a major lethal factor of prostate cancer [22]. The occurrence of metastasis depends on migration and invasion of the primary cancer cells to surrounding tissue [23]. In this study, we explored that TRPM7 knockdown suppressed migration and invasion ability of prostate cancer cells. TRPM7 is reported to be related in migration, invasion, and proliferation of various cancer cells. Moreover, TRPM7 expression was discovered to be upregulated in numerous human cancers tissues [24,25].

Based on human prostate tissue samples, we observed increased expression of TRPM7 in prostate tumor tissue (Fig. 1A), then, we treated siRNA to DU145 and LNcap cells to confirm the effect of TRPM7 gene silencing and to discover TRPM7 related proteins. The siRNA treated cells showed decreased expression of TRPM7 (**Supplementary Fig. 1A** and **2A**), p-Src and p-ERK1/2, (**Supplementary Fig. 1B** and **2B**) as well as narrow wound gap (**Supplementary Fig. 1C**, **Supplementary Fig. 2C**). Moreover, siRNA treated group showed suppressed migration (**Supplementary Fig. 3A**, **Supplementary Fig. 4A**) and invasion (**Supplementary Fig. 3B**, **Supplementary Fig. 4B**) ability of DU145 and LNcap cells. Therefore, we designed the study by producing stable TRPM7 knockdown prostate cancer cell line with DU145 cells which was more effectively produced compared to LNcap cells. Followed by transfection, we confirmed the downregulated mRNA (Fig. 1B) and protein expression (Fig. 1C) by quantitative PCR and immuno blot. Then, TRPM7 knockdown significantly reduced migration (Fig. 2A,B) and invasion (Fig. 2C,D) ability of DU145 cells. As next, to study the underlying mechanism of TRPM7 knockdown, we examined the p-ERK1/2, p-Src, p-Akt and p-JNK expression. ERK

is known to play crucial role in cell proliferation [26]. In previous studies, knockout of ERK during murine development leads to embryonic lethality [27]. ERK is reported to regulate cancer cell proliferation and tumorigenesis and frequently to be dysregulated in human cancers [28]. Phosphorylated ERK protein has been upregulated in patient tumor samples [29]. Similarly, increased Src protein kinase activity has been observed in several human cancer cell lines [30]. Src is the first discovered oncogene and oncogenically activated Src may induce cancer cell growth and survival, resulting tumor mass formation [31].

We observed downregulated expression of p-ERK1/2, p-Src, p-Akt and p-JNK with no change in total form of each protein (Fig. 3A). In previous study, it is reported that TRPM7 regulates migration and invasion of prostate cancer [19], however, to improve the study about the function and mechanism of TRPM7 in prostate cancer, we treated inhibitors of ERK, Akt and Src proteins to original DU145 cells. As we expected, inhibition of p-ERK1/2, p-Src and p-Akt suppressed prostate cancer cell migration (Fig. 4A), as well as invasion ability (Fig. 4B). Therefore, we found out that migration and invasion ability of DU145 cells may be regulated by the activation of ERK1/2, Src and Akt. As extra enhancement research, we demonstrated *in vivo* analysis of TRPM7 knockdown by xenograft model. We observed suppressed tumor growth in TRPM7 knockdown cells (Fig. 5A) as well as downregulated expression of TRPM7, p-ERK1/2, p-Src and p-Akt (Fig. 5B).

Previously researchers reported studies about the relationship of TRPM7 and prostate cancer [19], however, our study has improved experiments from past papers. In present study, we examined the expression of TRPM7 in human prostate tissue samples, as well as the anti-cancer effect in xenograft model. In addition, we proceeded the confirmation of signaling pathway through treating inhibitors.

5. Conclusions

Taken these results together, we could conclude that TRPM7 knockdown inhibited ERK1/2, Akt, and Src related migration and invasion potential of prostate cancer cells in the study. We examined that TRPM7 gene silencing suppressed tumor growth in xenograft model as well as change in tumor growth related protein expression. These outcomes indicate that TRPM7 regulates the cell proliferation, migration and invasion of prostate cancer cells and as well as underlying mechanism via both of *in vivo* and *in vitro*. Moreover, our data provide the evidence that TRPM7 would act as biomarker in detecting prognosis of metastatic prostate cancer.

Author contributions

TGK and YSH designed the study. BHY and SP collected data. HTK and SYC analyzed the data. EHL, SL and JNL interpretated the results. JWC, SHC, BSK, THK and JYC provide help in experiments. EHL, JNL, ESY and YSH prepared the manuscript. All authors reviewed the results and approved the final version of the manuscript.

Ethics approval and consent to participate

For human samples, the study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Institutional Review Board (or Ethics Committee) of the National Biobank of Korea-Kyungpook National University Hospital, a member of the Korea Biobank Network-KNUH (approval number: KNUMC 2016-05-021-006, date of approval: 28th Jun. 2017).

For animal samples, *in vivo* study protocols were approved by the institutional animal ethics committee of Yeungnam University, College of Medicine (approval number: YUMC-AEC 2019-040, date of approval: 21st Feb.2020).

Informed consent was obtained from all subjects involved in the study.

Acknowledgment

Not applicable.

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Conflict of interest

The authors declare no conflict of interest.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at http://doi.org/10.31083/j.jomh1807144.

Availability of data and materials

Data available on request due to privacy/ethical restrictions.

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