Knockdown of TRPM7 prevents tumor growth, migration, and invasion through the Src, Akt, and JNK pathway in bladder cancer

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Abstract

Background: Bladder cancer (BC) is one of the most common malignancies of the urinary tract. The role of transient receptor potential melastatin 7 (TRPM7) in BC remains unclear. The aim of this study was to investigate the function and signal transduction pathway of TRPM7 in BC.

Methods: T24 and UMUC3 cells were used to evaluate the molecular mechanism of TRPM7 by immunoblot analysis. Small interfering RNA was used to knockdown TRPM7, and the effect of silencing TRPM7 was studied by wound healing, migration, and invasion assays in T24 and UMUC3 cells. Xenograft model study was obtained to analyze the effect of TRPM7 inhibition in vivo.

Results: Silencing of TRPM7 decreased the migration and invasion ability of T24 and UMUC3 cells. The phosphorylation of Src, Akt, and JNK (c-Jun N-terminal kinase) was also suppressed by TRPM7 silencing. Src, Akt, and JNK inhibitors effectively inhibited the migration and invasion of T24 and UMUC3 cells. In addition, the TRPM7 inhibitor, carvacrol, limited the tumor size in a xenograft model.

Conclusion: Our data reveal that TRPM7 regulates the migration and invasion of T24 and UMUC3 cells via the Src, Akt, and JNK signaling pathway. Therefore, TRPM7 suppression could be a potential treatment for BC patients.

Keywords: Bladder cancer, TRPM7, Src, Akt, JNK

Background

Bladder cancer (BC) is a critical public health issue, and is known as the most common cancer of the urinary tract and the ninth most common cause of cancer related death worldwide [1]. There are approximately 400,000 cases of BC and 150,000 deaths per year [2]. The incidence of BC rises with age and the incidence is three times greater in men than women. The most common symptom of BC is hematuria without pain [3], and BC is commonly diagnosed by cystoscopy and cytology [4].

The pathologic type of BC can be divided into two groups: non-muscle invasive BC (NMIBC) and muscle invasive BC (MIBC) [5]. NMIBC includes Ta and T1 stages, which are a low grade non-invasive papillary tumors that penetrate the basement membrane without invading the muscle layer of the bladder wall.
MIBC includes T2–T4 stages which invade the muscle layer of bladder wall [6]. Around 70% of BC patients are diagnosed with NMIBC; however, these patients show a high reoccurrence rate with occasionally progressed stages with muscle layer invasion within 5 years [7]. The reason for recurrence with muscle invasion remains unclear [3]. Most deaths from BC result from metastasis to other organs due to chemotherapy resistance [8], and the majority of MIBC cases have metastasis, migration, and invasion [9, 10]. Indeed, approximately 25% of MIBC patients show metastasis, and patients with metastasis show poor prognosis [11]. Recently used pathological markers for prognosis require further study to clarify the most appropriate parameter [12]. Thus, there has been a sustained effort to understand the role of pathological markers in predicting the therapeutic response and prognosis in BC patients [13]. In the clinic, immune check point inhibitors, such as Atezolizumab, have been approved and used in BC. However, the effectiveness is still under investigation, and has followed cisplatin-based chemotherapy for the past decade [14].

TRPM7 is endogenously expressed in several human organs [15] and presents in a tetrameric form [16]. TRPM7 is a member of the transient receptor potential (TRP) channel family which is a non-selective cation channel family [17, 18]. Specifically, TRPM7 is a bifunctional protein that is well known as an essential regulator of Ca$^{2+}$ and Mg$^{2+}$ homeostasis [17]. TRPM7 is a protein kinase that has crucial roles in regulating diverse cellular processes including cell proliferation, adhesion, migration, and survival by phosphorylating itself [19, 20]. As TRPM7 is highly involved in many types of cancers, research on TRPM7 related mechanisms is actively underway. TRPM7 is highly expressed in both glioblastoma [21] and pancreatic adenocarcinoma [22], and has been reported to be associated with tumor migration and invasion [20, 23, 24]. Indeed, somatic mutation of TRPM7 has been found in breast carcinoma [25], colon carcinoma [26], ovarian cancer, and gastric carcinoma [25]. In previous studies, TRPM7 has been reported to regulate cell migration and invasion through the MAPK pathway, as well as the PI3K/Akt, ERK, and JNK pathways in cancer [27–29].

However, the function and the mechanism of TRPM7 in BC is not clearly understood and further studies are required to fully elucidate its role. In the current study, by exploring the role of TRPM7 and its underlying mechanisms using BC cell lines, we suggest TRPM7 as a beneficial treatment for BC patients. In addition, we evaluated whether the TRPM7 inhibitor, carvacrol, inhibited tumor size in a xenograft model.

**Methods**

**Reagents**

Anti-phospho-Src, anti-Src, anti-phospho-Akt, anti-Akt, anti-phospho-Erk, anti-Erk, anti-phospho-JNK, anti-JNK, anti-phospho-p38, anti-p38, and anti-TRPM7 antibodies were obtained from Cell Signaling Technology (Beverly, MA, USA). Carvacrol, dimethyl sulfoxide (DMSO), crystal violet, cell migration kit, and cell invasion kit were purchased from Sigma-Aldrich (St. Louis, MO, USA).

**Bladder cancer cell lines**

T24, J82, UMUC3, 5637, and HT1376 cell lines were purchased from American Type Culture Collection. Each cell line was cultured in RPMI 1640, DMEM high glucose, and MEM alpha media containing 10% FBS and 100 U/ml penicillin-streptomycin (Gibco, Waltham, MA, USA) and incubated at 37 °C in a humidified 5% CO$_2$ atmosphere.

**siRNA treatment**

Cells were plated at $2 \times 10^5$ cells per well. AccuTarget Predesigned siRNAs specific for human TRPM7, and scramble siRNAs purchased from Bioneer (Daejeon, Korea) were used to knockdown TRPM7 expression. The sequences for human TRPM7 siRNA were as follows: Fwd 5'-GUC UUG CCA UGA AAU ACU CUU-3' and Rev. 5'-GAG UAU UUC AUG GCA AGA CUU-3' (siRNA #1), and Fwd 5'-AGG AGA AGA UGC AUA UAA ATT-3' and Rev. 5'-UUU AUA UGC AUC UUC UCC UAG-3' (siRNA #2). The negative control (NC) group was treated with a non-targeted sequence, and the sequences for NC were as follows: Fwd 5'- UUC UCC GAA CGU GUC ACG UTU-3' and Rev. 5'-ACG UGA CAC GUU CGG AGA ATT-3'. For transient transfection, Lipofectamine® RNAiMAX (13778–150; Invitrogen, Carlsbad, CA, USA) was used with siRNA (2–200 pmol/μL) to treat the cells for 6 h. Following treatment, the media was changed to a transfection reagent free media containing 10% FBS, and incubated for 48 h at 37 °C in a humidified 5% CO$_2$ atmosphere.

**Immunoblot analysis**

Treated cells were lysed using RIPA buffer as described previously [30]. Protein samples were loaded into SDS-PAGE gels and transferred to nitrocellulose membrane. The primary antibodies were applied overnight at 4 °C at 1:1000 dilutions after blocking with 5% skim milk solution. The secondary antibodies were applied at 1:5000 for 2 h at room temperature. The bands were developed using ECL reagent (iBright 1500, Thermo...
Fisher Scientific), and the values were quantified with Image J software (Wayne Rasband, retired from NIH).

**Wound healing assay**
T24 and UMUC3 cells were plated in 6-well plates, and the cell layer was scratched with a pipette tip. Cells were washed with phosphate buffered saline to remove cell debris. The scratched area was monitored photographically and imaged at 0 and 24 h using an Olympus CKX41 inverted microscope coupled with a digital imaging system.

**Migration and invasion assay**
T24 and UMUC3 cells in serum free media were seeded in 8-μm pore size cell culture inserts (#353097, BD Falcon, Franklin Lakes, NJ, USA) and in Matrigel coated inserts (#354480, Corning, NY, USA) for migration and invasion, respectively. Media containing 10% FBS was added to the lower chamber. The cells were removed from the insert after 24 h incubation, and cells on the chamber were stained with crystal violet solution. The stained cells were dissolved in DMSO, and measured with a microplate spectrophotometer (wavelength 590 nm, BioTek Instruments, Winooski, VT, USA).

**Cell viability assay (MTT assay)**
T24 and UMUC3 cells were seeded in 96-well plates at a density of 1 × 10^4 per well and treated with different doses of carvacrol or siRNA. Cells were stained with filtered 1 mg/mL MTT solution for 4 h at 37 °C in a humidified 5% CO₂ atmosphere. Stained cells were dissolved in DMSO after removing media and MTT solution. Absorbance was measured with a microplate spectrophotometer at 570 nm wavelength.

**In vivo analysis**
All animal study protocols were approved by the Institutional Animal Ethics Committee of Yeungnam University, College of Medicine (YUMC-2017-024). All animals were kept in a controlled specific pathogen free environment under a 12 h light/dark cycle at a temperature of 25.0 °C ± 0.2 °C and humidity of 45% ± 2%. All mice were freely provided with food and water. UMUC3 cells were injected into Balb/c nude mice (n = 6, 6-week-old, 18-20 g, female). According to 3R (reduction, replacement, refinement) of Institutional Animal Care and Use Committee (IACUC), we chose the minimal number of animals used. For animal welfare, in case of movement disorder, eating disorder and weight loss (20% of normal

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Fig. 1 Protein expression of TRPM7 in bladder cancer cell lines by immunoblot analysis. **a** Protein expression of TRPM7 in 5637, T24, HT1376, J82, and UMUC3 cells. **b** Downregulation of TRPM7 in T24 and UMUC3 with different doses of siRNA. β-actin served as a loading control. M: Mock, N: Negative.
weight) we euthanized the animals in CO₂ gas. UMUC3 cells (5 × 10⁵) were subcutaneously injected in 200 μL PBS-Matrigel (1:1, Matrigel: PBS). When the tumor reached 4–6 mm in diameter, carvacrol was intraperitoneally injected at a 50 mg/mL concentration once a week for 4 weeks. The tumor size was measured twice a week, for 4 weeks. After 4 weeks from starting day of size measurement, mice were anesthetized with Zoletil 0.006 cc/10 g (30 mg/kg) and Rompun 0.004 cc/10 g (10 mg/kg) and euthanized in CO₂ gas.

**Source of animals**
Balb/c nude mice were purchased from Orient (Seoul, Korea).

**Immunohistochemistry stain**
The tumor tissues were fixed in 10% formalin solution to produce paraffin blocks. The samples were sectioned at a 4 μm thickness and deparaffinized. Slides were heated in citrate buffer and 0.2% triton x solution for 30 mins and 10 mins, respectively, and 5% BSA solution was used as a blocking reagent. The primary antibody was incubated overnight at 4 °C, and the secondary antibody (Alexa Fluor 594 Goat anti-rabbit IgG, 1:1000 dilution, Abcam, Cambridge, MA, USA) was incubated for 1 h at room temperature. The slides were mounted with DAPI containing mounting solution (Veetashield, Vector Laboratories, Burlingame, CA, USA).

**Statistical analysis**
One-way analysis of variance was used to assess differences among the treatment groups. The criterion for statistical significance was set at \( p < 0.05 \) or \( p < 0.01 \).

**Result**
**TRPM7 knockdown suppresses bladder cancer cell viability**
In order to select the most suitable cell line, we checked the protein expression of TRPM7 in 5637, T24, HT1376, J82, and UMUC3 BC cell lines by immunoblot (Fig. 1a). Among these five cell lines, T24 and UMUC3 showed higher expression than HT1376. T24 and UMUC3 cell lines belong to transitional cell carcinoma, which is the most common type of bladder cancer, and T24 and UMUC3 showed high siRNA transfection efficacy. Next, we treated T24 and

![Fig. 2](image_url) Effect of TRPM7 knockdown in bladder cancer cell proliferation. (a) T24 cells and (b) UMUC3 cells were treated with different doses of siRNA and incubated for 24 h. All data represent the means ± SD of three independent experiments (*\( p < 0.05 \) and **\( p < 0.01 \) between M and siRNA treated groups). M: Mock, N: Negative.
UMUC3 cells with two different sequences of siRNA in doses of 50 and 100 pmol/μL. The #2 sequence at dose of 100 pmol/μL siRNA significantly suppressed the expression of TRPM7 in T24 and UMUC3 cell lines, compared to mock and NC groups (Fig. 1b). Furthermore, the proliferation of T24 (Fig. 2a) and UMUC3 (Fig. 2b) cells was significantly inhibited by TRPM7 siRNA treatment, even at 2 pmol/μL, compared to the mock and NC groups.

TRPM7 knockdown reduces the motility of bladder cancer cells

A wound healing assay, migration assay, and invasion assay performed in order to analyze the effect of TRPM7 knockdown on cell motility. In the wound healing assay, siRNA treated (100 and 50 pmol/μL, non-lethal dose for each cell line) T24 and UMUC3 cells showed relatively wider wound gaps than non-treated groups following 24 h treatment (Fig. 3a and b). In the migration assay, siRNA treated T24 and UMUC3 cells showed significantly inhibited migration ability compared to non-treated groups (Fig. 4a and b). The invasion assay also showed significantly inhibited invasion in siRNA treated T24 and UMUC3 cells (Fig. 5a and b).

TRPM7 regulates cell proliferation via Src, Akt, and JNK pathways

The protein expression of Src, Akt and JNK (frequently related to cancer cell metastasis and invasion) was examined by immunoblot (Fig. 6). The results demonstrated that siRNA treated cells showed significantly reduced expression of p-Src, p-Akt, and p-JNK protein, while the total forms of Src, Akt, and JNK protein were not significantly changed by siRNA.
The association of these proteins with TRPM7 regulated cell proliferation was confirmed by treatment with the inhibitor of each protein (Fig. 6b). Both cell lines showed significantly decreased cell proliferation compared to the non-treated groups. Full-length blots that presented in Fig. 6 are presented in Suppl. Fig. 5.

**In vitro responses to TRPM7 inhibitor**

To analysis cell responses to the TRPM7 inhibitor, the cell lines were treated with 100–500 μM of carvacrol. In cell proliferation analysis, the proliferation of T24 cells was significantly decreased in a dose dependent manner (from 300 μM) (Suppl. Fig. 1a) compared to UMUC3 (Suppl. Fig. 1b). In the cell migration (Suppl. Fig. 2) and invasion (Suppl. Fig. 3) assays, UMUC3 cells were significantly more sensitive to carvacrol (from 200 μM) compared to T24 cells.

**In vivo responses to TRPM7 inhibitor**

Based on in vitro results, the in vivo condition was established using UMUC3 cells treated with 50 mg/mL carvacrol. At Day 24 after cell injection, the tumor volume was significantly reduced in the carvacrol treated group (468.45 ± 291.04 mm³) compared to the ctrl (4361.14 ± 2817.94 mm³) and vehicle (5176.45 ± 1603.25 mm³) groups (Fig. 7a). TRPM7 tumor expression was analyzed by IHC stain, wherein the carvacrol treated group showed significantly down-regulated expression of TRPM7 compared to the other groups (Fig. 7b).

**Discussion**

Recently, TRPM7 has been reported to be implicated in carcinogenesis and has been considered as a potential target for diverse cancer treatment [27, 31]. TRPM7, one of the TRP member channels, is permeable to Ca²⁺ and Mg²⁺. Since Ca²⁺ is an important regulator of cell cycle and proliferation, TRPM7 regulation is considered to be critical to the biological function of cancer cells [31]. TRPM7 is reported to be overexpressed in human pulmonary adenocarcinoma, and pancreatic and prostate adenocarcinoma [18, 32]. Furthermore, downregulation of TRPM7 is
known to inhibit the migration and invasion of breast cancer cells [27], while up-regulation has been shown to promote migration of lung cancer cells and vascular smooth muscle cells [23, 33].

Although numerous studies support that TRPM7 is involved in cancer cell migration and invasion [34–36], the precise understanding of the TRPM7 signaling pathway that modulates the molecular process of cancer cells remains unclear. According to previous studies, Src, Akt, and JNK are involved in cancer cell metastasis and invasion [37–40]. Indeed, Src was the first discovered oncogene, and it is able to activate caspase-8 by phosphorylation. Phosphorylated caspase-8 switches its function from pro-apoptotic to pro-migratory [41]. It has been reported that Src modulates the invasiveness of prostate cancer cells via regulating the E-cadherin/β-catenin complex [42]. Moreover, phosphorylated Akt serves as a cell proliferation boosting agent [43], while Akt has also been reported to promote invasion in fibrosarcoma and thyroid cancer cells [44, 45]. JNK is well known cancer regulating protein due to its action on oncogenes such as, Ras, c-fos, Met, and Bcr-Abl [46]. Furthermore, activated JNK induces the activation of apoptosis related Bcl-2 family proteins; thus, phosphorylated JNK acts as a pro-apoptotic signal in cancer cells [47]. In this study, we evaluated the TRPM7 mediated signaling pathway involved in BC cell lines, and demonstrated that silenced TRPM7 suppressed the protein expression of phosphorylation of Src, Akt, and JNK by immunoblot analysis. Based on this result, we can deduce TRPM7 regulates the Src, Akt, and JNK signaling pathway as a complex.

Our results also demonstrated that knockdown of TRPM7 by siRNA led to a decrease in proliferation of T24 and UMUC cells compared to the non-treated group, indicating that TRPM7 plays a key role in BC cell growth. In order to determine the effect of silenced TRPM7 on cell motility, we used T24 and UMUC3 cells in wound healing, migration, and invasion assays. Our results demonstrated that the TRPM7 silenced group showed a narrower area gap compared to the non-treated group in the wound healing assay, while the TRPM7 silenced group significantly suppressed the migration and invasion ability of BC cells. Thus, we were able to confirm that
TRPM7 plays a crucial role in cell metastasis and invasion of BC cells. To verify the effect of each protein (Src, Akt, and JNK) in cancer cell proliferation, we treated T24 and UMUC3 cells with inhibitors of Src, Akt, and JNK. As we expected, inhibition of Src, Akt, and JNK restricted cell proliferation. Based on the suppression of cell viability by Src, Akt, and JNK inhibition, we were able to derive that Src, Akt, and JNK play important roles in BC cell proliferation. In addition, we investigated the importance of TRPM7 in cancer growth by treatment with the TRPM7 inhibitor, carvacrol. In vitro, treatment of carvacrol showed decreased cell proliferation in a dose dependent manner compared to the non-treated group. In migration and invasion assays, as we expected, the migration and invasion capacity was lowest in carvacrol treated cells.

We evaluated the effect of TRPM7 inhibition in vivo by using carvacrol as a TRPM7 inhibitor in a xenograft model. Carvacrol injection reduced tumor size in nude mice, while the vehicle group showed similar tumor sizes to the control group. Furthermore, the carvacrol treated group had low TRPM7 protein expression by IHC. These in vivo results indicate that TRPM7 has a crucial role in tumor growth.

In previous studies regarding the function of TRPM7 in BC, the effect of TRPM7 downregulation induced BC cell apoptosis via the ERK1/2 pathway, while overexpressed TRPM7 promoted proliferation, migration, invasion, and tumor growth of BC [28]. Previous studies have demonstrated that TRPM7 downregulation increases reactive oxygen species, cell proliferation, migration, and invasion via elevated p-ERK1/2 and decreased PI3K/Akt protein expression [28]. According to other reports, overexpressed TRPM7 led to enhanced cell proliferation, migration, and invasion ability of BC cells [48]. In our study, we proved that TRPM7 mediates tumor growth, which is consistent with the findings of previously reports. As
our limits, we proceeded cell cycle analysis; however, we excluded the results due to complicated understanding of the results. In addition, we were unable to investigate the expression of proteins related to proliferation, although we did examine the effect of TRPM7 knockdown in vivo, in order to extend the findings of our study.

Conclusion
Taken together, this study provides the function and molecular mechanism of TRPM7 in BC. We demonstrated that knockdown of TRPM7 inhibits the migration and invasion of human BC cells via the Src, Akt, and JNK signaling pathway. Moreover, we demonstrated that suppression of TRPM7 inhibits tumor growth in a xenograft model. These results indicate that TRPM7 plays a crucial role in BC, and regulation of TRPM7 could be a potential treatment of BC.

Supplementary information
Supplementary information accompanies this paper at https://doi.org/10.1186/s12894-020-00714-2.
The authors declare that there are no conflicts of interest.

Consent for publication
– National University Chilgok Hospital (approval number: KNUMC 2016-05-021).

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate
This study was approved by the Institutional Review Board of Kyungpook National University Chilgok Hospital (approval number: KNUMC 2016-05-021).

Consent for publication
Not applicable.

Competing interests
The authors declare that there are no conflicts of interest.

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References
1. Yonemori M, Seki N, Yoshino H, Matsushita R, Miyamoto K, Nakagawa M, Enokida H. Dual tumor-suppressors mir-139-5p and mir-139-3p targeting matrix metalloproteinase 11 in bladder cancer. Cancer Sci. 2016;107(9):1333–42.
2. King MA, Ganley IG, Fleming V. Inhibition of cholesterol metabolism underlies synergy between mTOR pathway inhibition and chloroquine in bladder cancer cells. Oncogene. 2016;35(34):4518–28.
3. Kaufman DS, Shipley WU, Feldman AS. Bladder cancer. Lancet. 2009;374(9685):239–49.
4. Tilk D, Burger M, Daibagri G, Grossman HB, Hakenberg OW, Palou J, Reich O, Roupert M, Shariat SF, Zlotta AR. Urine markers for detection and surveillance of non-muscle-invasive bladder cancer. Eur Urol. 2011;60(3):484–92.
5. Enokida H, Yoshino H, Matsushita R, Nakagawa M. The role of microRNAs in bladder cancer. Investig Clin Urol. 2016;57(Suppl 1):S80–76.
6. Bursaci S, Xu SQ, Stanello M, Moskalev I, Morcavallo A, Genova M, Tanimoto R, Birbe R, Peiper SC, Gomella LG, et al. Suppression of progranulin expression inhibits bladder cancer growth and sensitizes cancer cells to cisplatin. Oncotarget. 2016;7(26):39980–95.
7. Kawahara T, Inoue S, Ide H, Kashiwagi E, Ohtake S, Matsushita T, Li P, Li, Zheng Y, Uemura H, et al. ZKSCAN3 promotes bladder cancer cell proliferation, migration, and invasion. Oncotarget. 2016;7(33):53599–610.
8. Kim EY, Seo JM, Kim C, Lee JE, Lee KM, Kim JH. BLT2 promotes the invasion and metastasis of aggressive bladder cancer cells through a reactive oxygen species-linked pathway. Free Radiat Biol Med. 2010;49(6):1072–81.
9. Kong DB, Chen F, Sima N. Focal adhesion kinases crucially regulate TGFBeta-induced migration and invasion of bladder cancer cells via Src kinase and E-cadherin. Onco Targets Ther. 2017;10:1783–92.
10. Park SL, Lee EJ, Kim WJ, Moon SK, p27KIP1 is involved in ERK1/2-mediated MWF-9 expression via the activation of NF-kappaB binding in the IL-7-induced migration and invasion of 5637 cells. Int J Oncol. 2014;44(4):1349–56.
11. Robertson AG, Kim J, Al-Ahmied M, Bellmunt J, Guo G, Cherniack AD, Hinoue T, Laird PW, Hoadley KA, Akbani R, et al. Comprehensive molecular characterization of muscle-invasive bladder cancer. Cell. 2018;174(4):1033.
12. van Rhijn BW, Catto JW, Goebell PJ, Knuchel R, Shariat SF, van der Poel HG, Sanchez-Carbayo M, Thalmann GN, Schmitz-Dagner EJ, Kemeny LA. Molecular markers for urothelial bladder cancer prognosis: toward implementation in clinical practice. Urol Oncol. 2014;32(7):1078–87.
13. Friedlander TW, Welty C, Anantharaman A, Schonhoft JD, Lee J, Li P, Hough J, Stromlund B, Edwards M, et al. Identification and characterization of circulating tumor cells in men who have undergone prostatectomy for clinically localized High Risk Prostate Cancer J Urol. 2019;202(4):732–41.
14. Patel R, Bock M, Polotsi CF, Elsamar S. Pharmacokinetic drug evaluation of atezolizumab for the treatment of locally advanced or metastatic urothelial carcinoma. Expert Opin Drug Metab Toxicol. 2017;13(2):225–32.
15. Kim BJ, Park EI, Lee EJ, Jeon JH, Kim SJ, So I. Suppression of transient receptor potential melastin 7 channel induces cell death in gastric cancer. Cancer Sci. 2008;99(12):2502–9.
16. Zou ZG, Rios FJ, Montezano AC, Touyz RM. TRPM7, Magnesium, and Signaling. Int J Mol Sci. 2019;20(8):1877.
17. Ha YS, Kim YY, Yu NH, Chun SY, Choi SH, Lee JN, Kim BS, Yoo ES, Kwon TG. Down-regulation of transient receptor potential melastin 7 prevents migration and invasion of renal cell carcinoma cells via inactivation of the Src and Akt pathway. Investig Clin Urol. 2018;59(4):263–74.
18. Dhennin-Duthille E, Gautier M, Koricheva I, Quaidid-Ahidouch H. TRPM7 involvement in cancer: a potential prognostic factor. Magnes Res. 2014;27(3):103–12.
42. Wei J, Xu G, Wu M, Zhang Y, Li Q, Liu P, Zhu T, Song A, Zhao L, Han Z, et al. Src signaling in cancer invasion. J Cell Physiol. 2010;223(1):14–53.
41. Guarino M. TRPM7 in cancer: Potential as Molecular Biomarker and Therapeutic Target. Pharmaceuticals (Basel). 2017;10(2):39.
40. Cheng HL, Lin CW, Yang JS, Hsieh MJ, Yang SF, Lu KH. Zoledronate blocks tumor metastasis via src regulation. Anticancer Res. 2008;28(1A):327–34.