FOLIA HISTOCHEMICA ET CYTOBIOLOGICA
Vol. 59, No. 1, 2021
pp. 40–48

ORIGINAL PAPER

VIA MEDICA

Keratin 17 knockdown suppressed malignancy and cisplatin tolerance of bladder cancer cells, as well as the activation of AKT and ERK pathway

Chen Li, HongWei Su, CongGang Ruan, Xiang Dong Li

Department of Urology, The First Affiliated Hospital of Hebei North University, Zhangjiakou City, Hebei Province, 075000, China

Abstract

Introduction. Bladder cancer (BCa) is one the most common urinary system malignancies and approximately one quarter of diagnosis is invasive muscle-invasive BCa. Accumulating evidence revealed that keratin 17 (KRT17) is closely related to the prognosis and progression of various tumors including a recent study also implying the potential role of KRT17 in the diagnosis of BCa. However, the specific role of KRT17 in BCa remains to be elucidated.

Material and methods. The expression of KRT17 in 5637 BCa cells and SV-HUC-1 normal human urothelial cells was detected using quantitative real-time PCR (qRT-PCR) and western blot. Short hairpin RNA targeting KRT17 was used to knockdown KRT17 in BCa cells. The colony formation was assessed and the proliferation of cells was studied by Cell Counting Kit-8 (CCK-8). Invasion and epithelial-mesenchymal transition (EMT) capacity of BCa cells were assessed using transwell assay and western blot, respectively. Cisplatin sensitivity of cancer cells was measured by evaluating the cell viability using CCK-8 assay. The downstream pathway of KRT17 was explored by western blot.

Results. The expression of KRT17 was elevated in BCa cells in comparison with the normal human urothelial cell at the mRNA and protein levels. The *in vitro* assays demonstrated that KRT17 interference affected the proliferation, colony formation and invasion capacity of BCa cells, as well as EMT. Furthermore, knockdown of KRT17 enhanced cisplatin sensitivity in BCa cells. Mechanically, KRT17 ablation led to the inactivation of both AKT and ERK pathways.

Conclusions. Our results elucidate the vital role of KRT17 in the development of malignancy of BCa cells, probably by the activation of AKT and ERK pathways and suggest that it may represent a novel therapeutic target for BCa. *(Folia Histochemica et Cytobiologica 2021, Vol. 59, No. 1, 40–48)*

Key words: bladder cancer; SV-HUC-1 cells; 5637 cells; keratin 17; shRNA; EMT; proliferation; invasion; cisplatin sensitivity; AKT; ERK

Introduction

Bladder cancer (BCa) is one of the most common malignancies of the urinary tract with approximately 440,000 newly diagnosed and 150,000 deaths every year [1]. Approximately 25–30% patients are diagnosed as muscle-invasive BCa [2]; the rest are diagnosed as non-muscle-invasive BCa. Muscle-invasive BCa is more prone to infiltrate other tissues and metastasize. Patients with metastatic BCa have poor prognosis with less than 10% of 5-year survival rate [3]. Surgical techniques, radiation therapy and chemotherapy are widely applied in BCa treatment; however, the high rate of recurrence and mortality are not well controlled. For example, cisplatin is a front-line chemotherapeutic agent for BCa treatment since most patients show a good initial response to cisplatin, unfortunately they will ultimately develop

Correspondence address: Chen Li,
The First Affiliated Hospital of Hebei North University,
12 Changqing Road, Qiaoxi District,
Zhangjiakou City, Hebei Province, 075000, China
phone: 0313-8046907, e-mail: ChenLifgh12@163.com

Folia Histochem Cytobiol. 2021
10.5603/FHC.a2021.0005
ISSN 0239-8508, e-ISSN 1897-5631

https://journals.viamedica.pl/folia_histochemica_cytobiologica

the cisplatin-resistance [4]. The patients with cisplatin resistance usually suffer a recurrence what limits the therapeutic potential of chemotherapy [5]. Recent advances in morphological investigation have indicated some ultrastructural changes in cisplatin-resistant (Cis-R) bladder cancer cells, *e.g.*, more double membrane vesicles and pinocytic vesicles [6]. Moreover, the Cis-R cells develop some strategies to reduce cisplatin uptake and accelerate cisplatin efflux [7]. The development of malignancy and chemoresistance of BCa are not well understood; thus, there is a great need to increase our knowledge about progression of BCa. Keratins are members of the intermediate filament family of proteins, and are widely expressed in epithelial cells. Currently, there are 54 known distinct keratins in mammals, 28 type I keratins and 26 type II keratins [8], according to their substructure and sequence homology. The keratins provide structural support and maintain cellular integrity of epithelial cells. Besides, accumulating evidence suggests the regulatory roles of keratins in multiple cellular functions, including proliferation and motility. The role of keratins as markers in tumor progression has been widely documented. For instance, the loss of keratin 8 and 18 in epithelial cancer cells increases their migration and invasion capacity, as well as cisplatin sensitivity [9]. It was reported that keratin 19-positive hepatocellular carcinoma cells display such properties like self-renewal capacity, higher proliferation rate and 5-fluorouracil resistance [10]. Besides, the high level of keratin 14 was noticed to be closely associated with the nodal metastasis of lung cancer, and the further research revealed the relationship between keratin 14 and gastrokine 1 expression in metastasis [11]. The pro-tumor role of keratin 23 was also observed in human colorectal cancer by promoting stem cell properties, proliferation and migration [8]. Keratin 17 (KRT17) belongs to the type I intermediate filaments family. The aberrant expression of KRT17 has been investigated in various carcinomas, and could serve as a potential diagnostic and prognostic marker, including cervical carcinoma, breast cancer and oral carcinoma [12–14]. A recent study based on RNA-seq analysis of urinary extracellular vesicle mRNA from patients with urological diseases identified the overexpression of KRT17 in high stage BCa [15]. Moreover, KRT17 has been recognized as a sensitive and specific biomarker in diagnosis of urothelial neoplasia, and muscle-invasive urothelial carcinoma had a higher proportion of positive KRT17 detection than the non-muscle-invasive type [16]. These clinical findings may imply the potential role of KRT17 in bladder tumorigenesis; however, its specific effect remains to be elucidated.

In an attempt to certify the implication of KRT17 in BCa, we knockdowned KRT17 in two different BCa cell lines, T24 and 5637, and our results demonstrated that knockdown of KRT17 leads to a decrease of proliferation and invasion, as well as epithelial-mesenchymal transition (EMT); furthermore, KRT17-deficient BCa cells were more sensitive to cisplatin. Mechanistically, silencing of KRT17 deactivated AKT and ERK pathway by suppressing phosphorylation. These data may suggest the critical role of KRT17 in the tumorigenesis and cisplatin resistance of BCa implicating KRT17 as a possible target for BCa therapy.

Materials and methods

Cell culture and lentiviral transfection. The normal human urothelial cells SV-HUC-1 and human BCa cell lines 5637 were obtained from American Type Culture Collection (Manassas, VA, USA), and the human BCa cell lines (T24, J82 and TCCSUP) were obtained from the Chinese Academy of Sciences (Shanghai, China). All the cells were cultured in DMEM medium (Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS). Lentiviral vectors expressing shRNA targeting KRT17 or negative control were generated from GenPharma (Shanghai, China). BCa cells were transfected with recombinant lentiviruses in the presence of 5 μg/mL polybrene according to the manufacturer's instruction. At 48 hours after infection, the efficiency of transfection was measured by western blot.

RNA isolation and quantitative RT-PCR (qRT-PCR). Total RNA was extracted using TRIzol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Complementary DNA (cDNA) was synthesized using Takara First Strand cDNA Synthesis Kit following the manufacturer's instructions. Real-time (RT)-PCR amplifications were performed with SYBR Green (TaKaRa, Dalian, Liaoning, China). The GAPDH was used as reference to normalize the KRT17 expression. The genes primers and accession numbers employed in this study were listed in Table 1. The relative expression of the KRT17 was calculated using $2^{-\Delta\Delta CT}$ method [17].

Western blot assay. Total proteins were extracted from cells using pre-cooling mammalian protein extraction reagent RIPA (Beyotime Bio, Shanghai, China) containing cocktail (SigmaAldrich, St. Louis, MO, USA). BCA Protein Assay kit (Beyotime Bio) was applied for protein concentration determination. An equal amount of protein was separated with 10% SDS-PAGE and transferred onto polyvinylidene fluoride (PVDF) membranes (SigmaAldrich). After blocked by 5% non-fat milk for 2 h, the PVDF membranes were incubated overnight at 4°C with specific primary antibodies

Table 1. Genes, primers sequences and accession numbers

Gene	Accession number	Forward primer (5'-3')	Reverse primer (5'-3')
KRT17	NM_000422	CTCAGTACAAGAAAGAACCGGTGA	CACAATGGTACGCACCTGAC
GAPDH	NM_002046	AATCCCATCACCATCTTCCAG	CACGATACCAAAGTTGTCATGG

against keratin 17 (ab109725, 1:2000, Abcam, Cambridge, MA, USA), GAPDH (ab8245, 1:5000, Abcam), E-cadherin (ab238099, 1:2000, Abcam), N-cadherin (ab98952, 1:5000, Abcam), vimentin (ab92547, 1:1000, Abcam), p-AKT (SC-1619, 1:2000, Santa Cruz Biotechnology, Santa Cruz, CA, USA), AKT (SC-1619, 1:2000, Santa Cruz Biotechnology), p-ERK (ab76299, 1:2000, Abcam), ERK (ab184699, 1:3000, Abcam). Protein signals were visualized using Bio-Rad imaging system (Hercules, CA, USA) and analyzed using Image J Software (NIH, Bethesda, MD, USA).

Cell viability assay and colony formation assay. Cell viability was detected using the Cell Counting Kit-8 (CCK-8; Diojindo Laboratories, Kumamoto, Japan) following manufacturer's instructions. The transfected cells were seeded on 96-well plates at 2000 cells/well. CC-K8 solution was added at indicated time and cells were incubated for 1 h in dark. The absorbance at a wavelength of 450 nm was measured using a microplate reader.

For colony formation assay, cells were plated into 6-well plates at 500 cells/well and incubated at 37°C. After two weeks of cultivation, the plates were fixed with methanol and stained with crystal violet, and the colonies were counted and photographed.

Transwell assay. Approximately 2×10^4 cells suspended in serum-free medium were seeded to the upper chambers (8 μm pore size, Costar, Cambridge, MA, USA). The lower chamber was filled with medium containing10% FBS. After incubation at 37°C for 24 h, non-invasion cells on the upper chamber were removed and the invasion cells on the lower surface were fixed with 4% formaldehyde and stained with crystal violet. Images of stained cells were counted in 5 randomly selected microscopic fields.

Cisplatin sensitivity determination. The transfected cells were seeded into 96-well plates at a density of 5000 cells/well and incubated with increasing concentrations of cisplatin (SigmaAldrich) for 24 h or 48 h. After incubation, the cell viability was measured using the CCK-8 method according to the manufacturer's instructions.

Statistical analysis. All results are present as mean ± standard deviation (SD) from at least three independent determinations. Student *t*-test was performed to compare the difference between two groups. $P < 0.05$ was considered statistically significant.

Results

Expression of KRT17 was upregulated in BCa cells

Given the KRT17 as an oncoprotein, we first determined whether the expression KRT17 varies between normal and malignant cells. Firstly, one normal human urothelial cell line (SV-HUC-1) and four BCa cell lines (5637, T24, J82 and TCCSUP) were employed in our experiment to detect the expression of KRT17. As indicated in Figure 1A and 1B, KRT17 mRNA and protein levels were significantly higher in four BCa cell lines compared to normal urothelial cells, and T24 and 5637 cells showed the highest expression. These results may imply the involvement of KRT17 in malignance of bladder cancer cells.

Knockdown of KRT17 suppressed BCa cells proliferation

To study the biological role of KRT17 in the regulation of cell proliferation, the BCa cells with the highest level of KRT17 were selected for lentiviral knockdown assay. After 48 h past transfection, the knockdown efficiency was evaluated using western blot (Fig. 2A). As shown in Figure 2B, depletion of KRT17 led to a significant decline of cell viability in two BCa cell lines. Furthermore, KRT17 knockdown also impaired the colony-forming capacity in both types of cancer cells (Fig. 2C). Taken together, these findings suggested the proliferation inhibitory effects of KRT17 knockdown in BCa cells.

Knockdown of KRT17 suppressed BCa cells invasion and EMT

It is well established that invasion is the first step of metastasis, translocation of the cells of the primary tumor to the distant organs [18]. As previous reported, higher expression of KRT17 in muscle-invasive urothelial carcinoma [16], it is necessary to elucidate the role of KRT17 in BCa cell invasion. As shown in Figure 3A, the invasion capacity assessed by transwell assay was remarkably suppressed by KRT17 ablation in both BCa cell lines. Since cells which undergo EMT lose some epithelial features and gain features much like mesenchymal cells, including migratory capacity [19], we decided to determine whether the knockdown of KRT17 affect EMT process in BCa. In accordance with our hypothesis, silencing of KRT17 significantly

Folia Histochem Cytobiol. 2021
10.5603/FHC.a2021.0005
ISSN 0239-8508, e-ISSN 1897-5631

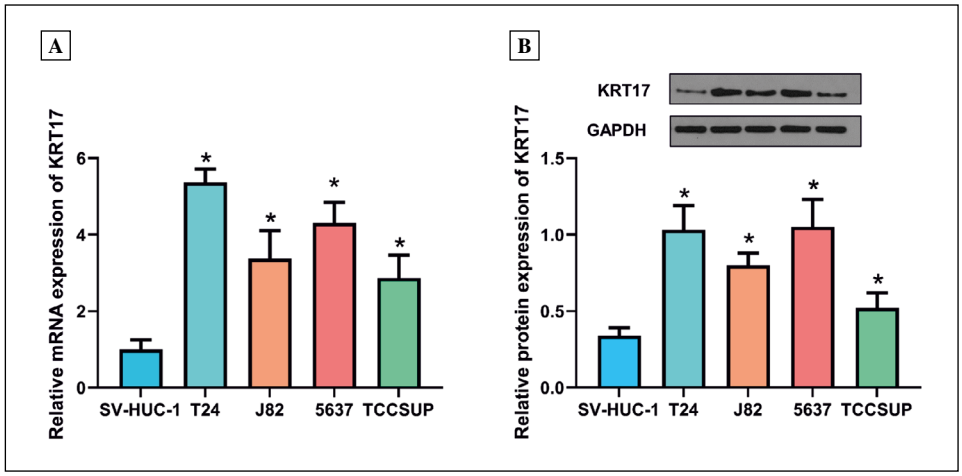

Figure 1. Expression of keratin 17 (KRT17) was increased in bladder cancer (BCa) cells. Relative KRT17 mRNA (**A**) and protein (**B**) expression in human BCa cells (T24, J82, 5637, TCCSUP cell lines) and normal human urothelial cells (SV-HUC-1), $^*p < 0.05$, Student's *t*-test.

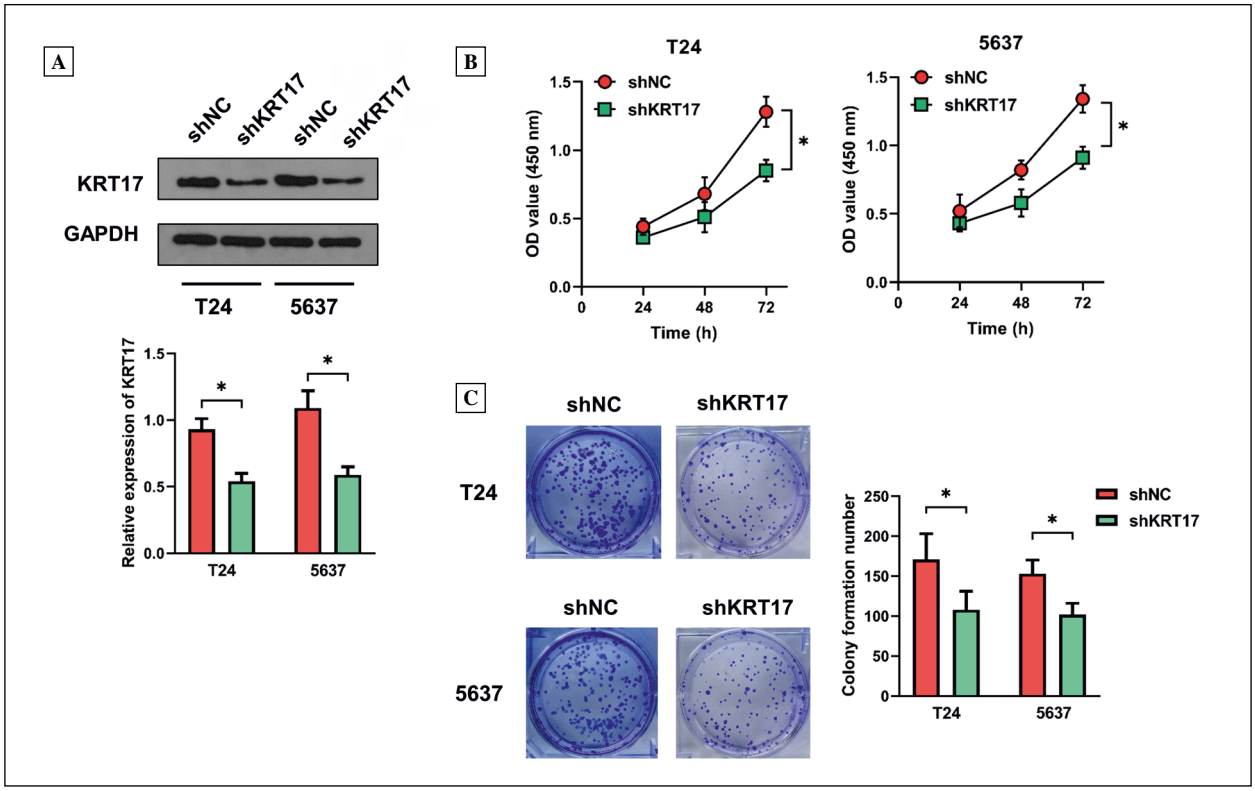

Figure 2. KRT17 depletion suppressed proliferation and colony formation of BCa cells. T24 and 5637 cells were transfected with KRT17 shRNA or scramble shRNA as described in Methods. **A.** The KRT17 knockdown efficiency in T24 BCa cells and normal urothelial 5637 cells was evaluated by western blot. **B.** and **C.** Viability and colony formation capacity of BCa cells was detected using CCK-8 (**B**) and colony formation assays (**C**), respectively ($^*p < 0.05$, Student's *t*-test).

changed EMT-related markers, such as the increase of E-cadherin, and decrease of N-cadherin and vimentin expression (Fig. 3B). These data indicated that KRT17 may have an important role in BCa cells' invasion and EMT.

Knockdown of KRT17 enhanced the sensitivity to cisplatin of BCa cells

Previous studies have linked KRT17 expression with paclitaxel resistance in cervical cancer [20]. To determine whether depletion of KRT17 could influence the

Folia Histochem Cytobiol. 2021
10.5603/FHC.a2021.0005
ISSN 0239-8508, e-ISSN 1897-5631

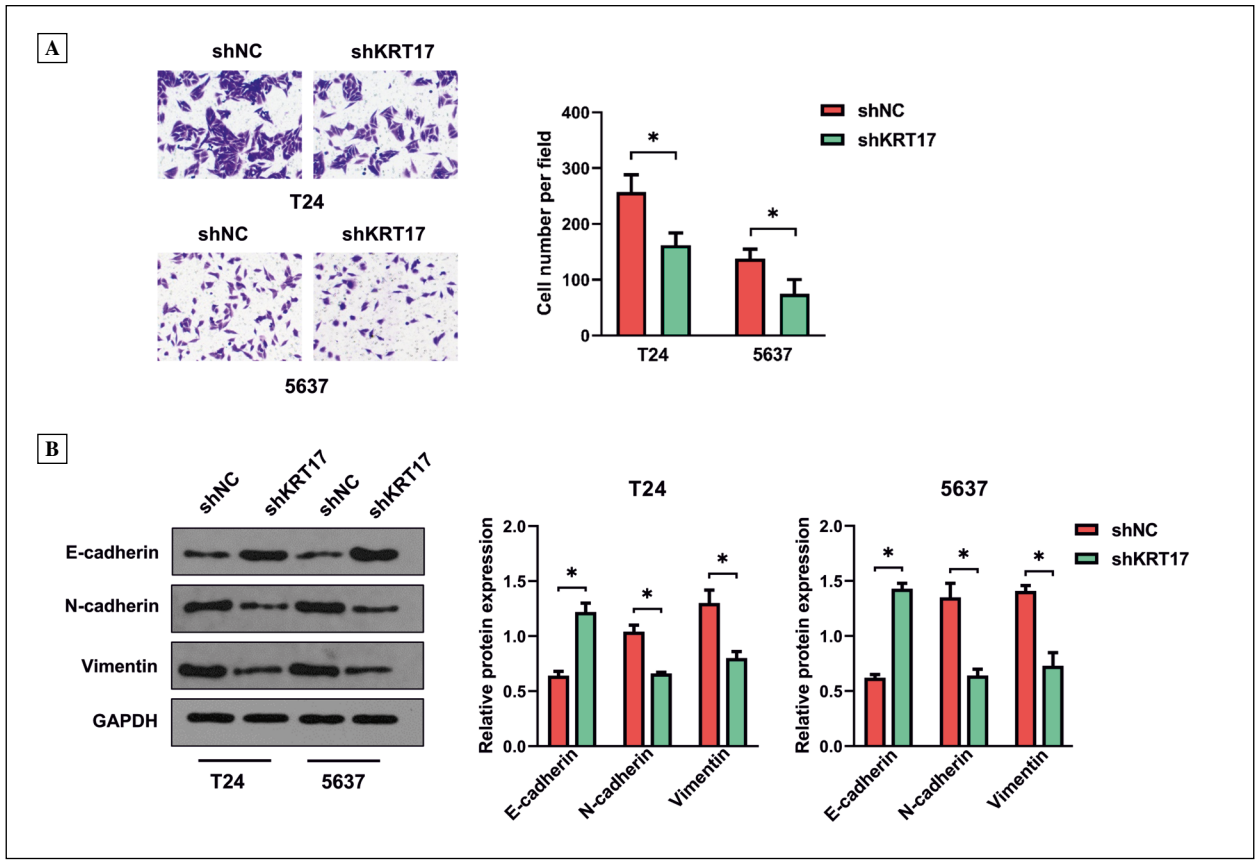

Figure 3. KRT17 depletion suppressed invasion capacity and epithelial-mesenchymal transition in BCa cells. **A.** Transwell assay was conducted to evaluate the invasion capacity of T24 BCa cells and normal urothelial 5637 cells after shKRT17 transfection. **B.** EMT-related expression of proteins (epithelial marker: E-cadherin; mesenchymal markers: N-cadherin and vimentin) was evaluated using western blot after transfection of T24 and 5637 cells with shKRT17 or its negative control (Y axis represent the relative protein abundance normalized to the GAPDH), $^*p < 0.05$, Student's *t*-test.

cisplatin tolerance, BCa cell lines T24 and 5637, transfected with shKRT17 were incubated with indicated concentration of cisplatin for 24 h and 48 h. As shown in Figure 4A and 4B, survival T24 cells with KRT17 interference was dramatically reduced compared to the counterpart transfected with negative control, after incubation for 24 h and 48 h. A similar result was also investigated in 5637 cells (Fig. 4C and 4D), suggesting the implication of KRT17 in the cisplatin tolerance of BCa.

KRT17 regulated AKT and ERK pathway

It has been reported previously that KRT17 silencing inactivates ERK1/2 but not AKT pathway in pancreatic cancer cells [21]. Therefore, we tested whether the same mechanism of KRT17 action is involved in BCa. The activation of ERK and AKT pathway was evaluated by western blot as shown in Figure 5. Our results suggest that phosphorylated-ERK1/2 was decreased in KRT17-knockdown cells while the content of the total ERK1/2 remained unchanged. Besides, the activation of AKT was also reduced after KRT17 ablation in both cell types when compared to the control, as assessed by the reduction of phosphorylated-AKT. Thereby, these results indicate that targeting KRT17 may exert antitumor function through inactivation of both ERK and AKT pathways.

Discussion

BCa is still a major cause of mortality of human urinary tract malignancies due to the distant metastasis and high recurrence rates. Metastasis is an important feature of malignancy and EMT is a critical process for metastasis of cancer cells. Cells which undergo EMT lose some epithelial features and gain features much like mesenchymal cells, with more migratory capacity, escaping from the primary tumor and disseminating to other organs *via* continuity or by the blood or lymphatic vessels [22]. Since the gain of invasive capacity increases aggressiveness and mortality of BCa, a better understanding of the pathogenesis

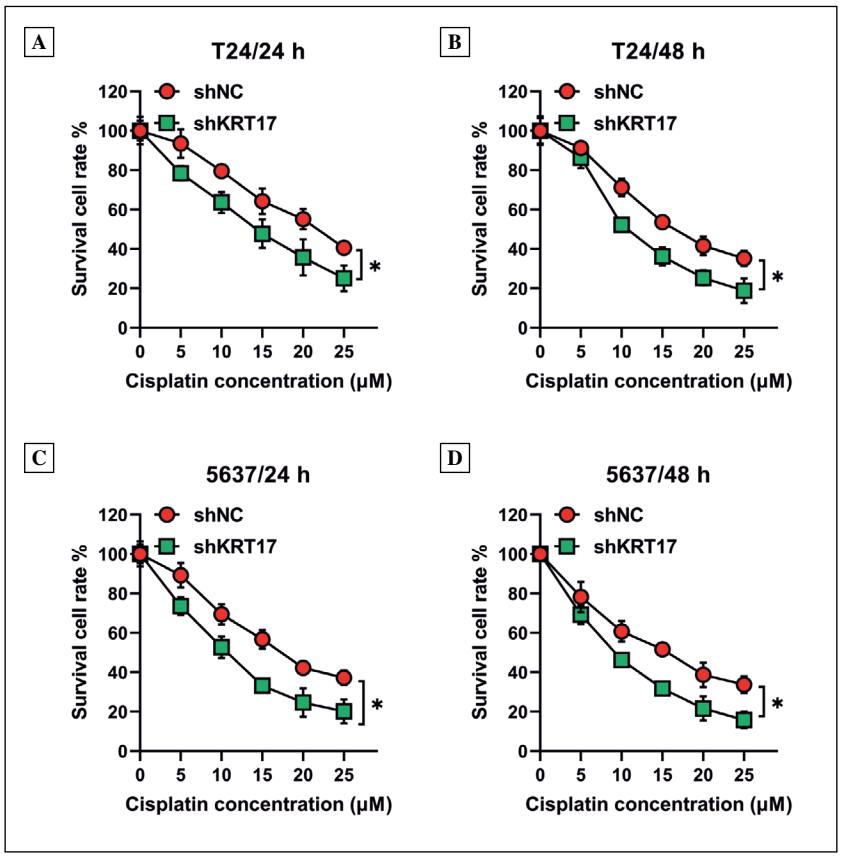

Figure 4. KRT17 depletion increased the cisplatin sensitivity of BCa cells. Cisplatin tolerance of T24 (A &B) and 5637 (C & D) cells was assessed after shNC or shKRT17 transfection after 24 h and 48 h of incubation with the indicated concentration of cisplatin, respectively. *p <0.05, Student's t-test.

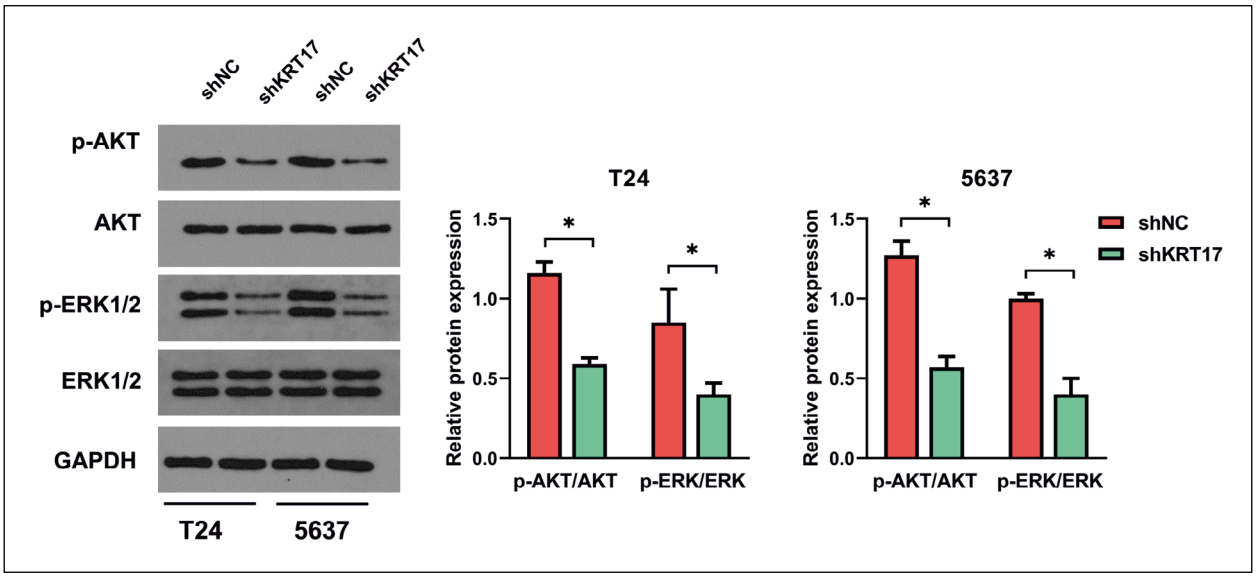

Figure 5. KRT17 depletion led to the inactivation of AKT and ERK pathways. Western blot was conducted to detect The effects of KRT17 knockdown on AKT and ERK pathways activation were assessed by western blot, p-AKT and p-ERK1/2 represent phospho-AKT and phospho-ERK1/2, AKT and ERK1/2 represent total AKT and phospho-ERK1/2. Y axis represents the relative protein abundance normalized to the GAPDH. *p < 0.05, Student's t-test.

Folia Histochem Cytobiol. 2021
10.5603/FHC.a2021.0005
ISSN 0239-8508, e-ISSN 1897-5631

of BCa is of great importance for anticancer therapy. In this study, we reported that KRT17 expression was associated with BCa malignant characteristics, such as proliferation, invasion, EMT and chemotherapy resistance. In addition, we also revealed that the silencing of KRT17 leads to the inactivation of both AKT and ERK pathway.

Recent studies have established that the aberrant expression of KRT17 has been observed in various types of malignancies. For instance, a study on pancreatic cancer suggests that high expression of KRT17 is closely related with short survival and implies aggressive form of pancreatic ductal adenocarcinoma [23]. Moreover, KRT17 expression is correlated with triple-negative breast cancer and predicts poor prognosis [14]. In our present study, higher KRT17 expression was found in BCa cells than normal human urothelial cell, similarly as shown by Murakami *et al.* [15] and Babu *et al.* [16]. Together, these findings may imply the potential role of KRT17 in the bladder tumorigenesis.

The relationship between KRT17 and cancer growth and metastasis has been documented in several solid tumors. For instance, KRT17 ablation suppresses the proliferation of oral squamous cell carcinoma *in vitro* and *in vivo* in nude mice [24]. Similarly, the pro-proliferation function of KRT17 was also observed in pancreatic cancer cell line [21]. It was demonstrated that pancreatic cancer cells transfected with KRT17 siRNA showed lower Reactive-Oxygen-Species and mTOR/S6K1 phosphorylation levels, as well as reduced proliferation, migration and invasion [25]. In lung adenocarcinoma, overexpression of KRT17 is closely associated with advanced TNM stage and poor overall survival, and KRT17 depletion remarkably suppresses cancer cell proliferation and invasion *in vitro* [26]. Besides, the high expression of KRT17 also predicts the poor prognosis in cervical cancer, and further investigations revealed that KRT17 functions as a negative regulator of $p27^{KIP1}$, which prevents cell G_0-G_1 to S-phase transitions [27]. KRT17 loss-of-function inhibits cervical cancer cells proliferation by modulating $p27^{KIP1}$ subcellular localization and degradation [27]. In the present study, we noticed that BCa cells with KRT17 depletion showed decreased viability, colony formation capacity and invasion, consistent with the previous observations in other cancer cell lines.

It is widely accepted that EMT plays critical roles in cancer progressions, including initiation, proliferation, dissemination, metastasis and chemotherapy resistance [28]. During the process of EMT, tumor cells lose their epithelial characteristics and acquire a mesenchymal phenotype, supporting cell survival and metastasis [29]. The involvement of KRT17 in EMT progression is also reported by some recent studies. Chiang and colleagues demonstrates the potential role of KRT17 in EMT process in areca nut-induced cancer [30]. Recent studies conducted on cervical cancer cells [20] and non-small cell lung cancer cells [31] also confirm the contribution of KRT17 in the EMT process. We noticed that KRT17 knockdown impaired EMT in BCa cell lines, in accordance with these previous reports.

It is well established that cancer cells would acquire some features similar to cancer stem cells, which confers the capacity to drug efflux or other mechanisms to resist chemotherapy cytotoxicity [31]. Data from studies of cervical cancer has proven that KRT17 depletion leads to drug sensitivity [20, 27]. In our study, the interference of KRT17 indeed increased cisplatin sensitivity, and this sensitivity to cisplatin may result from the EMT process reversal.

Intriguingly, when we analyzed the downstream pathways of KRT17, we noticed that the ablation of KRT17 also leads to the deactivation of both AKT and ERK pathway. It was reported that KRT17 deficiency impairs the activation of ERK, while makes little difference on AKT activation in pancreatic cancer cells [21], which disagrees with our present results. However, a recent *in vitro* and *in vivo* study on osteosarcoma cells have shown that KRT17 participates in the regulation of the AKT/mTOR/HIFα pathway [32]. Besides, the pro-tumor effect of KRT17 was found to be AKT-dependent in gastric cancer [33], as well as in esophageal squamous cell carcinoma [34]. The inconsistences between the reported studies may be caused by the underlying cell type specificity in various types of tumor.

In the present work, we confirmed for the first time that KRT17 may promote BCa progression by means of various processes including proliferation, migration, invasion, EMT and drug resistance. The silencing of KRT17 impairs both ERK and AKT phosphorylation activation in BCa cells, suggesting ERK and AKT as downstream effectors of KRT17.

We are aware that our study has some weak points. The main drawback of this study is that although we revealed the role of KRT17 in BCa malignancy and identified ERK and AKT pathways as the downstream of KRT17, the correlation between BCa malignancy and pathways are not well illuminated. In other words, whether KRT17 affects BCa malignancy *via* modulation of ERK and AKT pathways is still elusive. In conclusion, our study is the first to suggest the vital role of KRT17 in the progression of bladder cancer, and may imply the potential therapeutic value of KRT17 targeting in BCa treatment.

Folia Histochem Cytobiol. 2021
10.5603/FHC.a2021.0005
ISSN 0239-8508, e-ISSN 1897-5631

Conflict of interests

All authors declare no conflicts of interest in this work.

Acknowledgement

Not applicable.

Funding

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

CL designed the study, supervised the data collection, analyzed the data, HWS and CGR interpreted the data and prepare the manuscript for publication, XDL supervised the data collection, analyzed the data and reviewed the draft of the manuscript.

Ethics approval and consent to participate

Not applicable.

References

1. Lv M, Zhong Z, Huang M, et al. lncRNA H19 regulates epithelial-mesenchymal transition and metastasis of bladder cancer by miR-29b-3p as competing endogenous RNA. Biochim Biophys Acta Mol Cell Res. 2017; 1864(10): 1887–1899, doi: 10.1016/j.bbamcr.2017.08.001, indexed in Pubmed: 28779971.
2. Mao W, Huang X, Wang L, et al. Circular RNA hsa_circ_0068871 regulates FGFR3 expression and activates STAT3 by targeting miR-181a-5p to promote bladder cancer progression. J Exp Clin Cancer Res. 2019; 38(1): 169, doi: 10.1186/s13046-019-1136-9, indexed in Pubmed: 30999937.
3. Kamat A, Hahn N, Efstathiou J, et al. Bladder cancer. The Lancet. 2016; 388(10061): 2796–2810, doi: 10.1016/s0140-6736(16)30512-8, indexed in Pubmed: PMID: 27345655.
4. Drayton RM, Dudziec E, Peter S, et al. Reduced expression of miRNA-27a modulates cisplatin resistance in bladder cancer by targeting the cystine/glutamate exchanger SLC7A11. Clin Cancer Res. 2014; 20(7): 1990–2000, doi: 10.1158/1078-0432.CCR-13-2805, indexed in Pubmed: 24516043.
5. Choi W, Porten S, Kim S, et al. Identification of distinct basal and luminal subtypes of muscle-invasive bladder cancer with different sensitivities to frontline chemotherapy. Cancer Cell. 2014; 25(2): 152–165, doi: 10.1016/j.ccr.2014.01.009, indexed in Pubmed: 24525232.
6. Yoon SJ, Park I, Kwak C, et al. Ultrastructural change due to acquired cisplatin resistance in human bladder cancer cells. Oncol Rep. 2003; 10(5): 1363–1367, doi: doi: 10.3892/or.10.5.1363, indexed in Pubmed: 12883708.
7. Drayton RM, Catto JWF. Molecular mechanisms of cisplatin resistance in bladder cancer. Expert Rev Anticancer Ther. 2012; 12(2): 271–281, doi: 10.1586/era.11.201, indexed in Pubmed: 22316374.
8. Zhang N, Zhang R, Zou K, et al. Keratin 23 promotes telomerase reverse transcriptase expression and human colorectal cancer growth. Cell Death Dis. 2017; 8(7): e2961, doi: 10.1038/cddis.2017.339, indexed in Pubmed: 28749462.
9. Fortier AM, Asselin E, Cadrin M. Keratin 8 and 18 loss in epithelial cancer cells increases collective cell migration and cisplatin sensitivity through claudin1 up-regulation. J Biol Chem. 2013; 288(16): 11555–11571, doi: 10.1074/jbc.M112.428920, indexed in Pubmed: 23449973.
10. Kawai T, Yasuchika K, Ishii T, et al. Keratin 19, a cancer stem cell marker in human hepatocellular carcinoma. Clin Cancer Res. 2015; 21(13): 3081–3091, doi: 10.1158/1078-0432.CCR-14-1936, indexed in Pubmed: 25820415.
11. Yao S, Huang HY, Han X, et al. Keratin 14-high subpopulation mediates lung cancer metastasis potentially through Gkn1 upregulation. Oncogene. 2019; 38(36): 6354–6369, doi: 10.1038/s41388-019-0889-0, indexed in Pubmed: 31320708.
12. Hobbs RP, Batazzi AS, Han MC, et al. Loss of Keratin 17 induces tissue-specific cytokine polarization and cellular differentiation in HPV16-driven cervical tumorigenesis in vivo. Oncogene. 2016; 35(43): 5653–5662, doi: 10.1038/onc.2016.102, indexed in Pubmed: 27065324.
13. Toyoshima T, Vairaktaris E, Nkenke E, et al. Cytokeratin 17 mRNA expression has potential for diagnostic marker of oral squamous cell carcinoma. J Cancer Res Clin Oncol. 2008; 134(4): 515–521, doi: 10.1007/s00432-007-0308-8, indexed in Pubmed: 17786476.
14. Merkin RD, Vanner EA, Romeiser JL, et al. Keratin 17 is overexpressed and predicts poor survival in estrogen receptor-negative/human epidermal growth factor receptor-2-negative breast cancer. Hum Pathol. 2017; 62: 23–32, doi: 10.1016/j.humpath.2016.10.006, indexed in Pubmed: 27816721.
15. Murakami T, Yamamoto CM, Akino T, et al. Bladder cancer detection by urinary extracellular vesicle mRNA analysis. Oncotarget. 2018; 9(67): 32810–32821, doi: 10.18632/oncotarget.25998, indexed in Pubmed: 30214686.
16. Babu S, Mockler DC, Roa-Peña L, et al. Keratin 17 is a sensitive and specific biomarker of urothelial neoplasia. Mod Pathol. 2019; 32(5): 717–724, doi: 10.1038/s41379-018-0177-5, indexed in Pubmed: 30443013.
17. VanGuilder HD, Vrana KE, Freeman WM. Twenty-five years of quantitative PCR for gene expression analysis. Biotechniques. 2008; 44(5): 619–626, doi: 10.2144/000112776, indexed in Pubmed: 18474036.
18. Clark AG, Vignjevic DM. Modes of cancer cell invasion and the role of the microenvironment. Curr Opin Cell Biol. 2015; 36: 13–22, doi: 10.1016/j.ceb.2015.06.004, indexed in Pubmed: 26183445.
19. Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. J Clin Invest. 2009; 119(6): 1420–1428, doi: 10.1172/JCI39104, indexed in Pubmed: 19487818.
20. Li J, Chen Q, Deng Z, et al. KRT17 confers paclitaxel-induced resistance and migration to cervical cancer cells. Life Sci. 2019; 224: 255–262, doi: 10.1016/j.lfs.2019.03.065, indexed in Pubmed: 30928404.
21. Chen P, Shen Z, Fang X, et al. Silencing of keratin 17 by lentivirus-mediated short hairpin RNA inhibits the proliferation of PANC-1 human pancreatic cancer cells. Oncol Lett. 2020; 19(5): 3531–3541, doi: 10.3892/ol.2020.11469, indexed in Pubmed: 32269627.
22. Yamashita N, Tokunaga E, Iimori M, et al. Epithelial paradox: clinical significance of coexpression of E-cadherin and vimentin with regard to invasion and metastasis of breast

cancer. Clin Breast Cancer. 2018; 18(5): e1003–e1009, doi: 10.1016/j.clbc.2018.02.002, indexed in Pubmed: 29526677.
23. Roa-Peña L, Leiton CV, Babu S, et al. Keratin 17 identifies the most lethal molecular subtype of pancreatic cancer. Sci Rep. 2019; 9(1): 11239, doi: 10.1038/s41598-019-47519-4, indexed in Pubmed: 31375762.
24. Khanom R, Nguyen CT, Kayamori K, et al. Keratin 17 Is Induced in Oral Cancer and Facilitates Tumor Growth. PLoS One. 2016; 11(8): e0161163, doi: 10.1371/journal.pone.0161163, indexed in Pubmed: 27512993.
25. Li D, Ni XF, Tang H, et al. KRT17 functions as a tumor promoter and regulates proliferation, migration and invasion in pancreatic cancer via mTOR/S6k1 pathway. Cancer Manag Res. 2020; 12: 2087–2095, doi: 10.2147/CMAR.S243129, indexed in Pubmed: 32256116.
26. Liu J, Liu L, Cao L, et al. Keratin 17 promotes lung adenocarcinoma progression by enhancing cell proliferation and invasion. Med Sci Monit. 2018; 24: 4782–4790, doi: 10.12659/MSM.909350, indexed in Pubmed: 29991674.
27. Escobar-Hoyos LF, Shah R, Roa-Peña L, et al. Keratin-17 promotes p27KIP1 nuclear export and degradation and offers potential prognostic utility. Cancer Res. 2015; 75(17): 3650–3662, doi: 10.1158/0008-5472.CAN-15-0293, indexed in Pubmed: 26109559.
28. Brabletz T, Kalluri R, Nieto MA, et al. EMT in cancer. Nat Rev Cancer. 2018; 18(2): 128–134, doi: 10.1038/nrc.2017.118, indexed in Pubmed: 29326430.
29. Bill R, Christofori G. The relevance of EMT in breast cancer metastasis: Correlation or causality? FEBS Lett. 2015; 589(14): 1577–1587, doi: 10.1016/j.febslet.2015.05.002, indexed in Pubmed: 25979173.
30. Chiang CH, Wu CC, Lee LY, et al. Proteomics analysis reveals involvement of Krt17 in areca nut-induced oral carcinogenesis. J Proteome Res. 2016; 15(9): 2981–2997, doi: 10.1021/acs.jproteome.6b00138, indexed in Pubmed: 27432155.
31. Du B, Shim JS. Targeting epithelial-mesenchymal transition (EMT) to overcome drug resistance in cancer. Molecules. 2016; 21(7), doi: 10.3390/molecules21070965, indexed in Pubmed: 27455225.
32. Yan X, Yang C, Hu W, et al. Knockdown of KRT17 decreases osteosarcoma cell proliferation and the Warburg effect via the AKT/mTOR/HIF1α pathway. Oncol Rep. 2020; 44(1): 103–114, doi: 10.3892/or.2020.7611, indexed in Pubmed: 32627037.
33. Chivu-Economescu M, Dragu DL, Necula LG, et al. Knockdown of KRT17 by siRNA induces antitumoral effects on gastric cancer cells. Gastric Cancer. 2017; 20(6): 948–959, doi: 10.1007/s10120-017-0712-y, indexed in Pubmed: 28299464.
34. Liu Z, Yu S, Ye S, et al. Keratin 17 activates AKT signalling and induces epithelial-mesenchymal transition in oesophageal squamous cell carcinoma. J Proteomics. 2020; 211: 103557, doi: 10.1016/j.jprot.2019.103557, indexed in Pubmed: 31669361.

Submitted: 2 November, 2020
Accepted after reviews: 29 January, 2021
Available as AoP: 12 February, 2021