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Original Paper

Protein Tyrosine Kinase 2 Circular RNA Promotes Proliferation and Invasion of Bladder Cancer

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Key Words
Bladder Cancer • CircRNAs • CircPTK2 • MiRNA

Abstract
Background/Aims: Bladder cancer is a type of malignant tumor that disrupts normal urinary function in patients, thereby significantly impacting their quality of life. This disease also imposes a heavy economic burden on both patients and public health agencies due to high medical costs. Current common therapies, such as surgical intervention, chemical treatment, and radiotherapy, are associated with serious adverse reactions and risks of metastasis recurrence. Effective attenuation of bladder cancer proliferation and invasion remains a significant challenge. Circular RNAs have shown promise in regulating proliferation and migration of cancer cells, thus making it a potential therapeutic target for bladder cancer treatment and prognosis. This study aims to evaluate the impact of regulating circPTK2 expression on progression of bladder cancer. ***Methods:*** This research established overexpression and knock down circPTK2 models of bladder cancer cells (SW780 and UM-UC-3) primarily. Then evaluate the effect by a series of cell function test (including RT-qPCR, MTT, EdU assay, cell clone, transwell, cell cycle and cell apoptosis). ***Results:*** The findings suggest that regulated expression of circPTK2 in bladder cancer cells correlated with the abundance of mir129-5p. Meanwhile, knock down circPTK2 expression in bladder cancer cells reduced their ability to proliferate and invade; but these processes were reversed when circPTK2 expression was increased. ***Conclusion:*** In conclusion, circPTK2 may play a vital role in regulating bladder cancer progression, thereby showing potential for treatment of bladder cancer and improvement of prognosis by modulating circPTK2.

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Introduction

Bladder cancer, a type of malignant tumor that affects normal urinary function of patients, caused 572, 000 new cases and over 212, 000 deaths worldwide in 2020[1]. The financial burden and medical costs associated with bladder cancer are significant for both patients and public health agencies. Currently, bladder cancer can be classified into two categories: nonmuscle-invasive bladder cancer and muscle-invasive bladder cancer [2]. The former has a low risk of progression and a relatively high survival rate. It can preserve the physiological function of the bladder through local resection but is prone to recurrence [3, 4]. On the hand, the latter usually invades the muscle layer and requires complete cystectomy to prevent further deterioration and metastasis of the tumor [5]. The muscle-invasive balder cancer has exceedingly low survival rate of five-year and poor prognosis because greater than half of the patients of the disease progresses to distant metastasis [6, 7]. However, present treatment strategies of bladder cancer are in quandary because the pathomechanism of the initiation and development disease is still not understood properly. To treat bladder cancer effectively, the need for understanding the disease pathomechanistically and discover novel signaling pathways as therapeutic targets is scientifically urgent.

The regulation of normal cell proliferation is controlled by organ differentiation, apoptosis, and signaling pathways. However, cancerous cells lack the ability to self-regulate, leading to uncontrolled expansion and invasion of cancerous tissues. Non-coding ribonucleic acid (RNA), such as circular RNAs(circRNAs), long non-coding RNA and microRNA are RNA transcripts that do not have the ability to translate into proteins. These non-coding RNAs play a significant role in regulating cell differentiation, proliferation, and cancer migration [8–10].

Among them, circRNAs is a class of covalently closed cyclic nucleotide sequences without a 5' cap and 3' poly A tail. It was first identified as an endogenous RNA splicing product of eukaryotic cells in 1979[11, 12]. Current studies have demonstrated that circRNAs plays a vital role in the occurrence and progression of cancer [13, 14]. For example, Yuan et al [15]. confirmed that circRNA_102231 is up-regulated in gastric tumor tissue. Thus, knocking down circRNA_102231 expression was found to have an inhibitory effect on the proliferation and migration of gastric cancer cells. The same observation was made in non-small cell lung cancer as reported by Li et al [16]. study.

Current researches had demonstrated that abnormal expression of circRNAs in bladder cancer tissues has a significant impact on the progression and potential of the disease [17–19]. Gone et al [20]. showed that the circular RNA circ_102336 was overexpressed in bladder cancer and could inhibit proliferation when circ_102336 was suppressed. Conversely, Yu et al [21]. discovered that overexpression of circRNA CRIM1 could inhibit bladder cancer migration. Xu and colleagues discovered that proliferation and migration of bladder cancer cells were promoted by circular RNA hsa_circ_0003221 (circPTK2), wherein its underlying mechanism is still not clearly understood [22]. Notwithstanding, the specific number and types of circRNAs that influence bladder cancer occurrence and progression remain unclear.

As family member of mir129 with its location in a chromosome 7q32 fragile site, mir129-5p has been associated with cancer, wherein it has demonstrated the potential to suppress various tumors, particularly rectal adenocarcinoma proliferation, migration and invasion [23]. In another work, it was discovered that sponged mir129-5p (by lncARSR) promoted bladder cancer cell proliferation and metastasis via increased expression of sex determining region Y-related high mobility group box transcription factor-4 (SOX4)[24]. Also, Cao and colleagues [25] observed that mir129-5p could inhibit resistance of gemcitabine and promoted bladder cancer cell apoptosis through targeting of Wnt5a. Recently, Kong et al [26]. have indicated promotion of bladder cancer progression by lncRNA XIST through modulation of mir129-5p/TNFSF10 axis. However, the regulatory effect of circPTK2 on mir129-5p during the pathological process of bladder cancer has not been explored.

Thus, this experimental work sought to investigate the role and potential mechanism of circPTK2 in progression of bladder cancer. This aim was achieved by integrating a series of cell functional experiments. The findings from this study will offer novel insights and potential therapeutic options for the treatment and prognosis of bladder cancer.

Materials and Methods

Culturing of bladder cancer cell lines

Shanghai Institute for Biological Science supplied the bladder cancer cell lines, namely UM-UC-3 and SW780. Minimum Essential Medium (MEM, Gibco, China) supplemented with 10% fetal bovine serum (FBS, Gibco, USA) was used to maintain UM-UC-3 cells, whilst Dulbecco's modified Eagle's medium (DMEM, Gibco, China) containing 10% FBS was utilized to maintain SW780 cells. Incubation of all the cultures was performed in humidified atmosphere of 5% carbon dioxide at 37°C. After reaching 70%-80% confluence, cells were trypsinized with a trypsin-EDTA solution for subculturing.

Cell transfection

In this research, circPTK2 overexpressed model of bladder cancer cells were established using the Lentil-UBCIII-Puro system (Addgene, USA). CircPTK2 knock-down model of bladder cancer cells were also established with LentiCrisprV2 system (Addgene, USA). The empty vector was used as negative control. The cells were handled by adding puromycin to obtain drug-resistance cell strain. The cells were harvested for subsequent cell function experiments when after cultured two weeks. The target sequences for circPTK2-1 and circPTK2-2 were has_circ_0003221 and hsa_circ_0005982, respectively. The target sequences for si-circPTK2-1 and si-circPTK2-2 were 5'-GCCGTCTCTGTGTCAGAAAAG-3' and 5'-GCUGCAAUAAUAUGACAGATT-3', respectively. The negative control sequence was 5'-GGGCGAGGAGCTGTTCACCG-3'.

RT-qPCR

TRIzol agent (Invitrogen, USA) was utilized to harvest total RNA in the transfected bladder cells. One Step TB Green® PrimeScript™ RT-PCR Kit (Takara, Japan) and LightCycler® 96 Instrument (Roche, Switzerland) was used to assess expression level of RNAs. Specifical protocol according to the instruction. β-actin were used to normalize the RNA expression. The relative expression of each gene was assessed using the $2^{-\Delta\Delta Ct}$ method [27]. The sequences of primers were shown in Table 1.

Methylthiazolyldiphenyl-tetrazolium bromide (MTT) test

Normal and transfected bladder cells were digested and centrifuged, before they were resuspended with culture medium (without FBS). The 96-well plates (Labselect, China) were inoculated with 3000 cells in 100 μL medium/well, and each group was set with 5 multiple wells, prior to incubation for 24 h, 48 h, 72 h and 96 h. At the corresponding time, 20 μL MTT (Aladdin, China) solution (prepared by dissolving the MTT powder into phosphate buffered saline (PBS) solution, final concentration was 5 mg/mL)/well was added. After 4 h of incubation, aliquot (100 μL) of dimethylsulfoxide (DMSO, Solarbio, China) per well was added and gently shaken. Meanwhile, blank wells were set up to correct errors. Particularly, MTT solution was added to the blank wells which did not contain cells. After synchronized incubation for 4 hours, DMSO was added. BioTek Epoch plate reading system (Agilent, China) was used to detect the absorbance (Ab) at

Table 1. The sequences of primers

Name	Sequence (5'-3')	
	Forward	Reverse
CircPTK2	GGCGATCATACTGGGAGATG	TGTGATTCAAGTTGGGGTCA
mir129-5p	GTCGTATCCAGTGCAGGGTCCGAGG TATTCGCACTGGATACGACGCAAGC	CTTTTTGCGGTCTGGGCTTGC
PTK2	GCTTACCTTGACCCCAACTTG	ACGTTCCATACCAGTACCCAG
β-actin	CATGTACGTTGCTATCCAGGC	CTCCTTAATGTCACGCACGAT

570 nm. Cell viability is calculated using the following formula:

$$cell\ viablity = \frac{Ab\ of\ transfected\ cells\ well - Ab\ of\ blank\ well}{Ab\ of\ normal\ cells\ well - Ab\ of\ blank\ well} \times 100\% \quad (1)$$

5-Ethynyl-20-deoxyuridine (EdU) assay

BeyoClick™ EdU Cell Proliferation Kit (Beyotime, China) was used to the EdU assay. The Edu stock solution (20 μM) was prepared using a medium (with FBS). Inoculation of transfected cells with 5000 cells/well was performed into the 96 well plates. After 48 h of culturing, EdU stock solutions which had been incubated with 37°C water-bath was added per well, thereby resulting in a final concentration of EdU (10 μM). After 2 h of incubation, the medium was discarded before addition of cell fixative solution (100 μL, 4% PFA) into well and fixed for 15 min.

After the solution has been discarded, aliquot (100 μL) of BSA (3%) was added to well, before washing was performed for 5 min with shocking, amid 3 times repetition. Aliquot (100 μL) of transparent solution (0.3% TritionX-100) was added and incubated for 15 min at room temperature. Later, 3% BSA was used to wash the solution for 5 min, amid 3 time repeat 3 times.

According to manufacturer instruction, click additive solution was prepared and aliquot (100 μL) of the solution was added per well. After gentle shock, the sample was incubated for 30 min at room temperature in darkness. Regarding washing with 3% BSA for 5 min, the procedure repeated 3 times. Finally, DAPI (biosharp, China) staining solution was added and incubated for 5 min at room temperature. Afterward, PBS washing of the sample for 5 min with PBS was carried out, amid 3 times repetition before fluorescent microscopic imaging (Nikon eclipse Ti, Japan).

Cell cloning test

Inoculation of transfected cells was performed at 3000/well in 6 well plates (Labselect, China). After 2 weeks of culturing, the clone was formatted, fixed and stained with 0.1% Crystal violet solution (Beyotime, China) for 20 min. The cells were subsequently washed 3 times with PBS and microscopic imaging.

Transwell test

For the invasion test, Polycarbonate membrane transwell chamber (8 μm, Labselect, China) was placed at the top chamber of 24-plates (Labselect, China) before cold Matrigel (CORNING, USA) was added to the transwell chamber, and subsequently incubated 2 h at 37°C. The transfected cells were resuspended using the medium (without FBS) and inoculated in transwell chamber, while the medium (with FBS) was added to 24-well plates chamber. After gentle shock, static culture was conducted for 24 h before collection of the transwell chamber. Fixed and stained the cells that were retained in transwell chamber with crystal violet solution were performed prior to microscopic imaging.

Cell cycle analysis

Transfected cells were digested and rinsed thrice with cold Dulbecco's PBS, before they were fixed with 75% ethanol for 24 h at -20°C. Subsequently, centrifugation of the sample was performed before supernatant was discarded and rinsed with DPBS. Later, prepared the propidium iodide (PI) staining solution according to the instruction of Cell Cycle and Apoptosis Analysis Kit (Beyotime, China) for resuspended cell and incubated for 30min in darkness. Ultimately, the cell cycle was detected by BD Accuri™ C6 Plus Flow Cytometry (BD Biosciences, USA). The results were processed using FlowJo software (BD Biosciences, USA).

Cell apoptosis assay

Based on the instruction of the Annexin V-FITC Apoptosis Detection Kit (Beyotime, China) manufacturer, the transfected cells were digested and rinsed with cold DPBS before resuspension with binding buffer. Subsequently, PI and annexin V-FITC solution were added prior to incubation for 30 min at 37°C. The apoptosis of the cells was accordingly detected via flow cytometry. The results were processed using FlowJo software.

Statistical analysis

Data are presented as means ± standard error and analyzed using GraphPad Prism 9.5 software. One-way ANOVA analysis of variance with Tukey`s post hoc analysis was used as the statistical comparison. P-values (<0.05) were deemed that the comparison was statistically significant.

Results

CircPTK2 regulation accompanied with mir129-5p abundance change

In this research, Lentil-UBCⅢ-Puro system and LentiCrisprV2 system were used to respectively establish circPTK2 overexpression model (LUP-circPTK2) and knock-down model (LCV-si-circPTK2).

From the results (Fig. 1), it was obvious that an increase in circPTK2 expression in the overexpression models (LUP-circPTK2-1 and LUP-circPTK2-2) of the two categories bladder cancer cell, namely SW780 and UM-UC-3. On the contrary, it was evidently revealed that circPTK2 expression was decreased in the knock-down models (LCV-si-circPTK2-1 and LCV-si-circPTK2-2) of two categories cells. Furthermore, consistent changes in cellular PTK2 expression levels were observed following modulation of circPTK2 expression (Fig. 2). These observations indicate that the overexpression and knock-down models of circPTK2 were successfully established. Furthermore, a significant decrease or increase in mir129-5p abundance was accordingly observed in LUP-circPTK2 group or LCV-si-circPTK2 group. This phenomenon may be due to circPTK2 acting as miRNA sponge. A more detailed discussion will be presented in the discussion section.

Regulation of CircPTK2 effect in bladder cancer cell proliferation

From EdU staining results (Fig. 3), it was evident that proliferation of circPTK2 overexpression models (LUP-circPTK2-1 and LUP-circPTK2-2) increased in the two class bladder cancer cells (SW780 and UM-UC-3). Instead, the proliferation of knock-down model (LCV-si-circPTK2-1 and LCV-si-circPTK2-2) was obviously decreased in these cells.

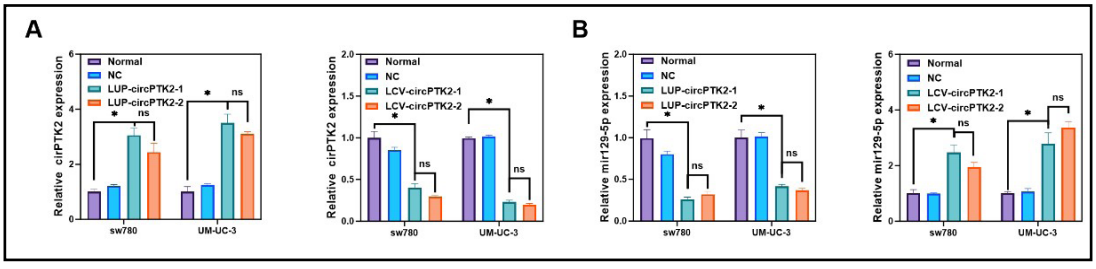

Fig. 1. The abundance of circPTK2 and mir129-5p in the transfected bladder cancer cells. (A) Relative circPTK2 expression of the circPTK2 overexpression models(left) and knock down models (right) of SW780 and UM-UC-3 cells. (B) Relative mir129-5p abundance of the circPTK2 overexpression models(left) and knock down models(right). * P<0.05.

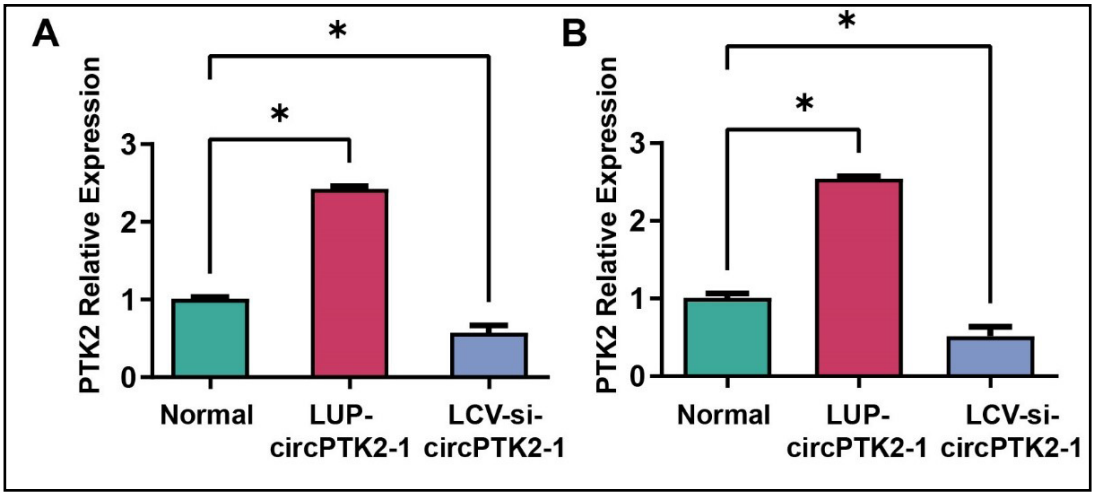

Fig. 2. Relative PTK2 expression levels in SW780 (A) and UM-UC-3 (B). * P<0.05.

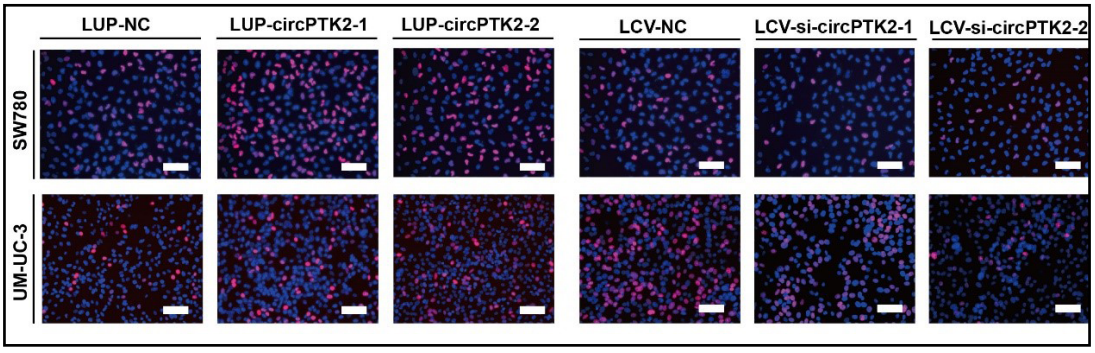

Fig. 3. The EdU stain results of circPTK2 overexpression (LUP-circPTK2-1 and LUP-circPTK2-2) and knock down (LCV-si-circPTK2-1 and LCV-si-circPTK2-2) models of SW780 and UM-UC-3. Blue fluorescence (DAPI) represents cell nuclei, while red fluorescence (EdU) represents proliferating cells. Scare bar = 50 μm.

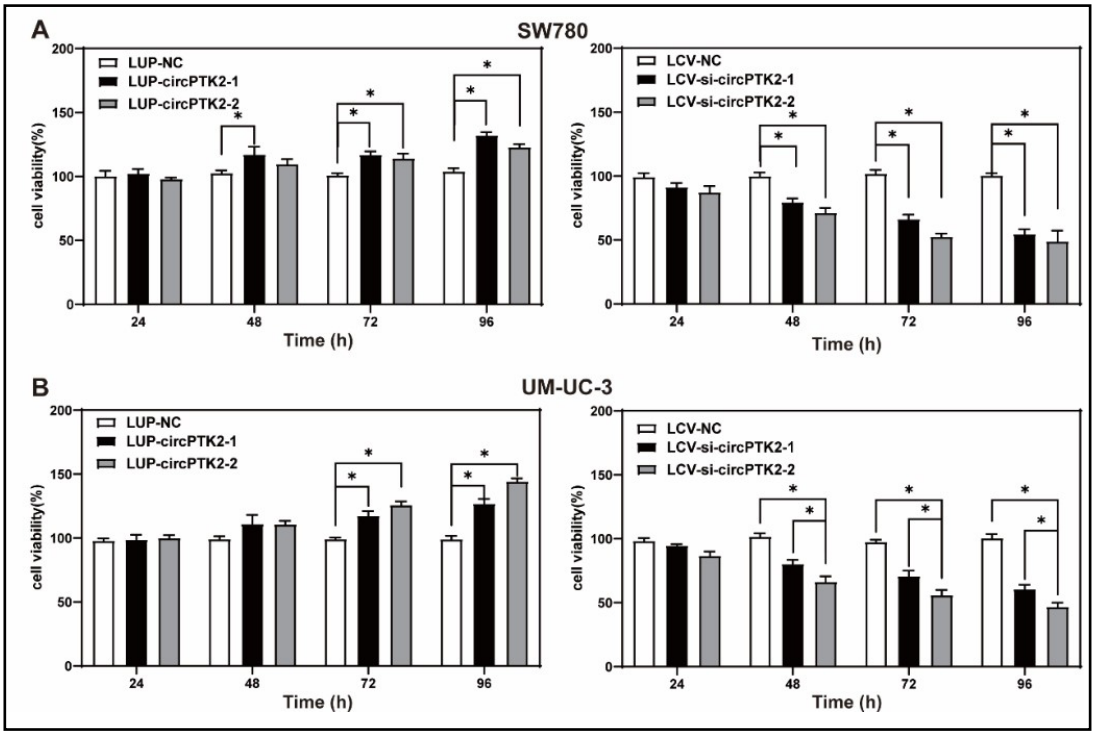

Fig. 4. The proliferation of circPTK2 regulative expression models of blader cancer cells. (A). The cell viability of SW780 cells in circPTK2 overexpression models (left, LUP-circPTK2-1 and LUP-circPTK2-2) and knock down models (right, LCV-si-circPTK2-1 and LCV-si-circPTK2-2). (B). The cell viability of UM-UC-3 in overexpression models(left) and knock down models(right). * P<0.05.

The regulatory effect of circPTK2 expression on proliferation of bladder cancer cells was assessed using MTT assay (Fig. 4). For the proliferation of the transfected cancer cells (SW780 and UM-UC-3), LUP-circPTK2 group showed significant increase at 72 h and 96 h. By contrast, LCV-si-circPTK2 group displayed substantial reduction at 48 h, 72 h and 96 h.

Additionally, cell cloning assay showed that regulated circPTK2 expression had effect on clone formation (Fig. 5). It was discovered that LUP-circPTK2 group formed cell clusters that were obviously more than negative control group. On the contrary, cell clusters with no significant difference were observed between LCV-si-circPTK2 group and negative control group.

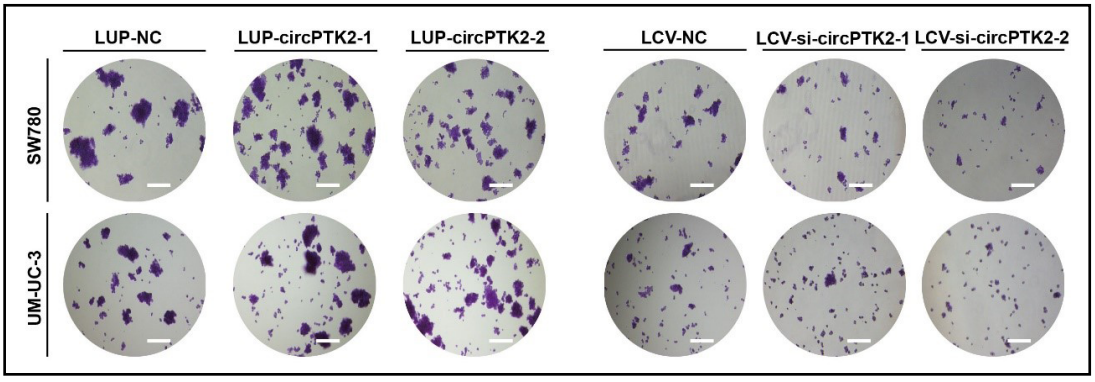

Fig. 5. The cell clone formation ability of circPTK2 overexpression (LUP-circPTK2-1 and LUP-circPTK2-2) and knock down (LCV-si-circPTK2-1 and LCV-si-circPTK2-2) models of SW780 and UM-UC-3 cells. Purple clumps indicate cell clusters from cell cloning. Scare bar = 100 μm.

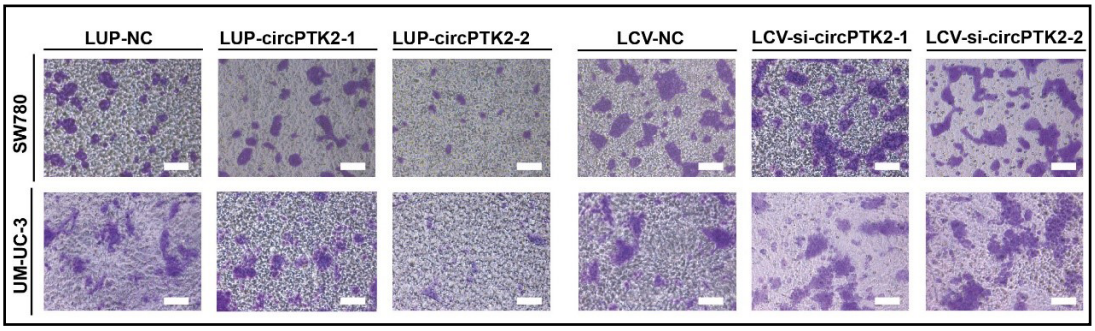

Fig. 6. The invasive ability of circPTK2 overexpression (LUP-circPTK2-1 and LUP-circPTK2-2) and knock down (LCV-si-circPTK2-1 and LCV-si-circPTK2-2) models of SW780 and UM-UC-3 cells. Purple clumps indicate cells retained in the chamber. Scare bar = 50 μm.

Regulated circPTK2 expression influences invasion of bladder cancer cell

Regulatory effect of circPTK2 on capability of cancer cell invasion was evaluated using transwell invasion test. The results (Fig. 6) depict that up-regulation of circPTK2 expression reduced the retentive cell clusters. This observation indicates that overexpression of circPTK2 could promote cells migration. Conversely, there was a slight increase in cell clusters of LCV-si-circPTK2 group compared with negative control group.

CircPTK2 regulatory effect on bladder cancer cell cycle

In this experiment, the influence of circPTK2 regulatory effect on cell cycle was investigated with flow cytometer. According to the results (Fig. 7), compared to LUP-NC group, the cell cycle of LUP-circPTK2 and LCV-si-circPTK2 groups differed significantly.

Specifically, comparison of LUP-circPTK2 overexpression models of two categories bladder cancer cells with NC group exhibited a decreased proportion of G1 phase cells, and increased proportion of S phase cells. Meanwhile, changes in cell cycle distribution in the LCV-si-circPTK2 knock-down models constructed using two kinds of cells were opposite with the above findings. Although not all the differences in the aforementioned results were significant, collectively, this suggests that the abnormal expression of circPTK2 in bladder cancer cells affected their normal cell cycle.

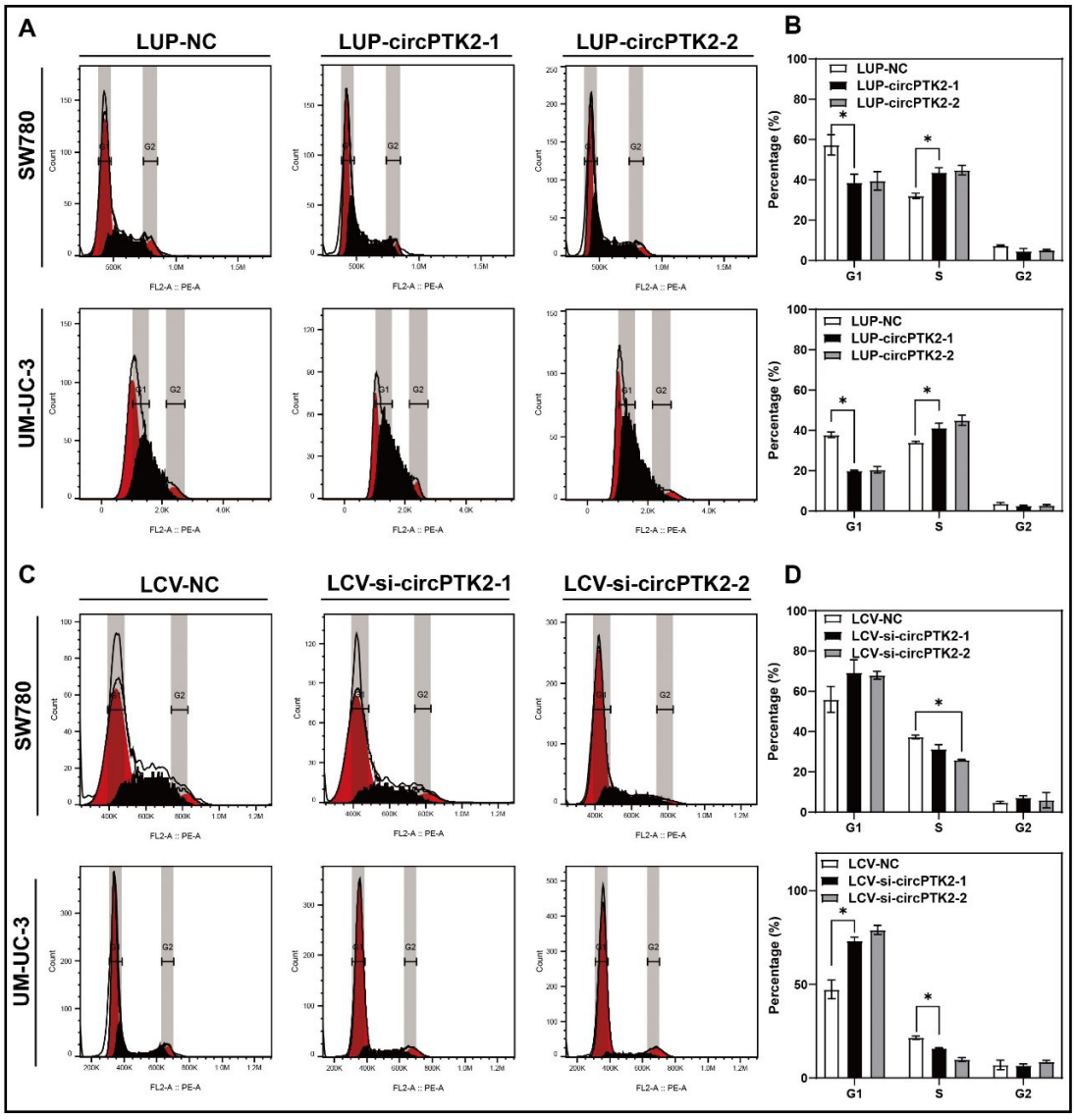

Fig. 7. The impact of regulated circPTK2 expression on cell cycle of bladder cancer cells. (A and C). The flow cytometry results of circPTK2 overexpression models and knock down models respectively. (B and D). The statistical results of the flow cytometry data. * $p<0.05$.

CircPTK2 regulatory effect on apoptosis of bladder cancer cell

This experiment was conducted to evaluate the regulatory effect of circPTK2 on cell apoptosis (Fig. 8).

It can be seen that for the two high expression models of LUP-circPTK2-1 and LUP-circPTK2-2 of SW780 and UM-UC-3 cells, the cell proportion of early apoptosis(Q3) and late apoptosis(Q2) did not change significantly. In the LCV-si-circPTK2-1 low expression models of SW780 and UM-UC-3 cells, an increased SW780 late apoptosis(Q2) proportion was observed. In the LCV-si-circPTK2-2 low expression model, a significantly increased proportion of early apoptosis(Q3) and late apoptosis(Q2) of SW780 and UM-UC-3 cells was discovered. Overall, these results indicate that inhibition of circPTK2 expression promoted apoptosis of bladder cancer cells.

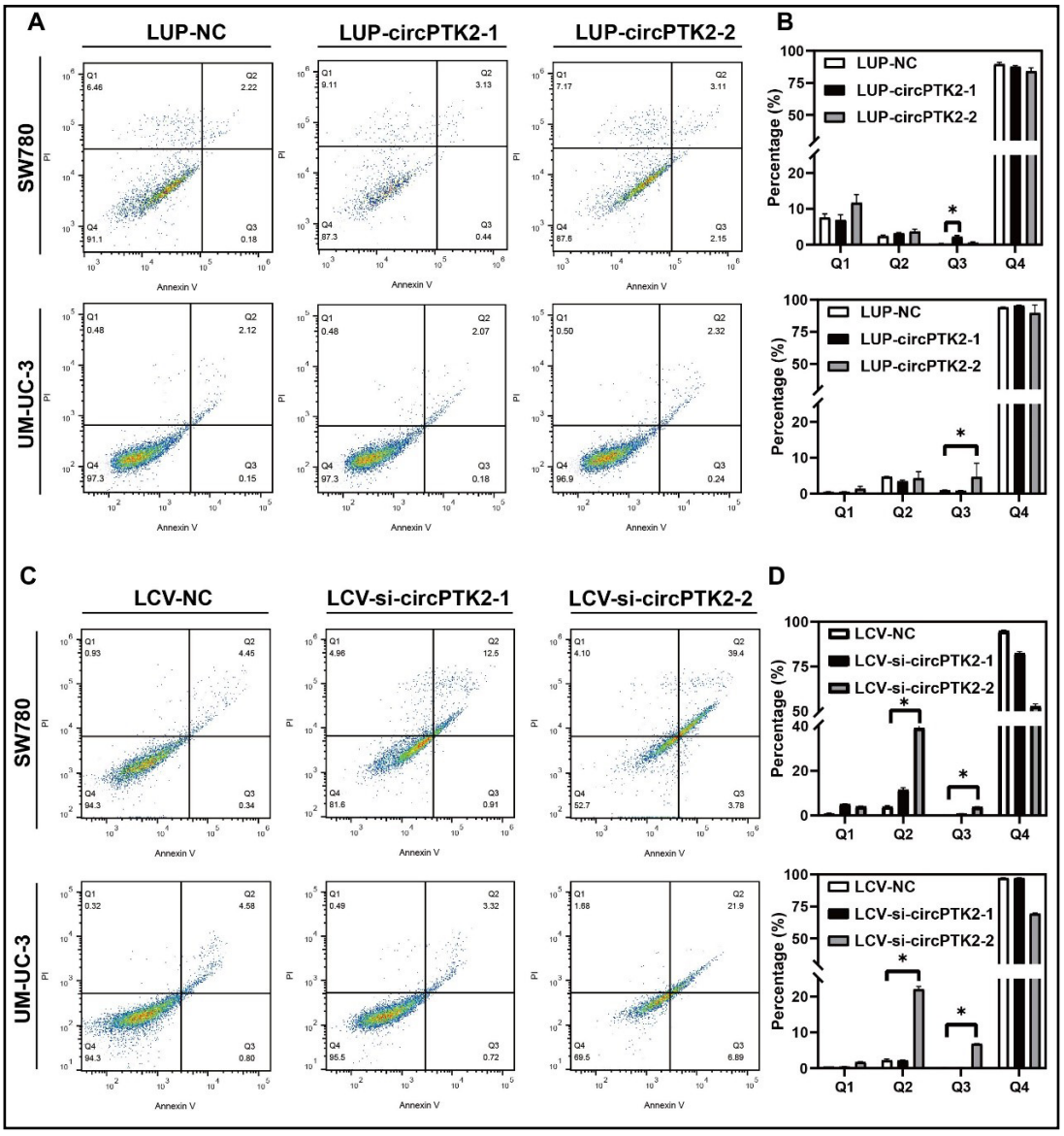

Fig. 8. The effect of regulated circPTK2 expression on cell apoptosis of bladder cancer cells. (A and C). The flow cytometry results of circPTK2 overexpression models and knock down models respectively. (B and D). The statistical results of the flow cytometry data. * $p<0.05$.

Discussion

Currently, bladder cancer as a type of malignant tumor still has high fatality rate [28–32]. Available literature has reported high mortality of invasive muscle bladder cancer and increase recurrence rate and progression risk of invasive non-muscle bladder cancer [33]. This makes the disease particularly important to explore the progression mechanism of bladder cancer and provide targets for its diagnosis, treatment and prognosis.

At present, a series of study had shown that circPTK2 affect cancer progression of gastric carcinoma [34–36], colorectal cancer [37, 38], squamous-cell carcinoma [39], non-small cell lung cancer [40] and hepatic carcinoma [41]. This study also suggests that regulated circPTK2 expression influenced progression of blader cancer. Through the cell function experiment, it was observed that regulation of circPTK2 expression could affect proliferation and invasion of bladder cancer cells (SW780 and UM-UC-3). Thus, regulation of circPTK2 expression in

bladder cancer cells was accompanied with the interference on the cell cycle. In addition, knock-down circPTK2 promoted cell apoptosis. These results suggest that knock down circPTK2 may play a potential role in inhibiting progression of bladder cancer.

Usually, circPTK2 demonstrates sponging effect of negative regulation of miRNA in the tumor or cells abundance [34], and then intervene in tumor cell proliferation and migration capability. In 2018, Xu et al [22]. first pointed out the role of silencing the circPTK2 in inhibiting the bladder cancer cells' proliferation, migration and invasion. Subsequently, Meng et al [42]. further showed that circPTK2 could bind to PABPC1 (poly A-binging protein cytoplasmic 1) via RNA-binding proteins, stable SETDB1(SET domain bifurcated histone lysine methyltransferase 1) mRNA function, so as to promote bladder cancer cell migration ability and resistance.

In this investigation, it was evidently discovered that regulation of circPTK2 expression was accompanied with a decrease in mir129-5p abundance. Based on these findings, it appears that circPTK2 may function as a miRNA sponge for mir129-5p. The observed correlation supports the previously mentioned role of circPTK2 as a miRNA sponge absorber, although further research is needed to confirm this relationship.

Notwithstanding, this study has some shortfalls which may limit the generalization of the findings. In this regard, the *in vitro* conditions are not able to mimic biological conditions coupled with the complex nature of physiological processes of tumors, it is possible that *in vivo* may differ significantly from that of *in vitro*. In not too-distant future, this research team will comprehensively investigate the expression of circPTK2 and its interaction with mir129-5p in bladder cancer animals to understand the mechanistic role of the circular RNA in the tumor. Besides, although knock down of circPTK2 has been discovered to promote apoptosis in the bladder cancer cells, how the circular RNA affects apoptosis related proteins like Bcl-2, Bax-1 and caspase-3 in bladder cancer cells and the actual mechanism involves are not clear. Hence, further investigations are urgently needed in this regard.

Conclusion

In conclusion, the potential effect of circPTK2 on bladder cancer progression was investigated. This study reveals that circPTK2 is crucial in regulating bladder cancer progression. Also, modulating circPTK2 expression is linked to changes in mir129-5p levels, indicating a potential link between them. The results indicate that knock down circPTK2 had an inhibitory effect on bladder cancer proliferation and invasion, influences the cell cycle of bladder cancer cells, wherein it promoted apoptosis of bladder cancer cells. This study explores the mechanism of the occurrence and progression of bladder cancer, and provides a potential option for the diagnosis, treatment and prognosis of bladder cancer.

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Author contributions

Designed and performed the experiment - J.Y.C., G.Y.W., Y.Z. and G.H.Z.; analyzed the data -Q.W., H.P. and J.L.; wrote the paper - J.Z., Y.N.D. and S.S.L.; revised the manuscript - K.J. and C.S.P.; concept and management - Q.C.W.

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Statement of Ethics
The authors have no ethical conflicts to disclose.

Disclosure Statement

The authors have declared that they have no potential competing interest.

References

1 Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209–49.
2 Zengzhao W, Xuan L, Xiaohan M, Encun H, Jibing C, Hongjun G. Molecular mechanism of microRNAs, long noncoding RNAs, and circular RNAs regulating lymphatic metastasis of bladder cancer. Urologic Oncology: Seminars and Original Investigations. 2024;42(1):3–17.
3 Tyson MD, Lee D, Clark P. New developments in the management of non-muscle invasive bladder cancer. Curr Opin Oncol. 2017;29(3):179–83.
4 Rayn KN, Hale GR, Grave GP-L, Agarwal PK. New therapies in nonmuscle invasive bladder cancer treatment. Indian J Urol. 2018;34(1):11–9.
5 Su H, Jiang H, Tao T, Kang X, Zhang X, Kang D, et al. Hope and challenge: Precision medicine in bladder cancer. Cancer Med. 2019;8(4):1806–16.
6 Witjes JA, Bruins HM, Cathomas R, Compérat EM, Cowan NC, Gakis G, et al. European Association of Urology Guidelines on Muscle-invasive and Metastatic Bladder Cancer: Summary of the 2020 Guidelines. Eur Urol. 2021;79(1):82–104.
7 van Rhijn BWG, Burger M, Lotan Y, Solsona E, Stief CG, Sylvester RJ, et al. Recurrence and progression of disease in non-muscle-invasive bladder cancer: from epidemiology to treatment strategy. Eur Urol. 2009;56(3):430–42.
8 Liu J, Wang H, Xiao S, Zhang S, Qi Y, Wang M. Circ_0000231 promotes paclitaxel resistance in ovarian cancer by regulating miR-140/RAP1B. Am J Cancer Res. 2023;13(3):872–85.
9 Pei S, Ma C, Chen J, Hu X, Du M, Xu T, et al. CircFOXM1 acts as a ceRNA to upregulate SMAD2 and promote the progression of nasopharyngeal carcinoma. Mol Genet Genomic Med. 2022;10(5):e1914.
10 Li X, Zeng X, Xu Y, Wang B, Zhao Y, Lai X, et al. Mechanisms and rejuvenation strategies for aged hematopoietic stem cells. J Hematol Oncol. 2020r;13:31.
11 Hsu MT, Coca-Prados M. Electron microscopic evidence for the circular form of RNA in the cytoplasm of eukaryotic cells. Nature. 1979;280(5720):339–40.
12 Meng S, Zhou H, Feng Z, Xu Z, Tang Y, Li P, et al. CircRNA: functions and properties of a novel potential biomarker for cancer. Mol Cancer. 2017;16:94.
13 Ghafouri-Fard S, Dinger ME, Maleki P, Taheri M, Hajiesmaeili M. Emerging role of circular RNAs in the pathobiology of lung cancer. Biomedicine & Pharmacotherapy. 2021;141:111805.
14 Li Z, Ruan Y, Zhang H, Shen Y, Li T, Xiao B. Tumor-suppressive circular RNAs: Mechanisms underlying their suppression of tumor occurrence and use as therapeutic targets. Cancer Science. 2019;110(12):3630–8.
15 Yuan G, Ding W, Sun B, Zhu L, Gao Y, Chen L. Upregulated circRNA_102231 promotes gastric cancer progression and its clinical significance. Bioengineered. 2021;12(1):4936–45.
16 Li H, Lei Y, Chen N, Guo G, Xiang X, Huang Y. circRNA-CPA4 Regulates Cell Proliferation and Apoptosis of Non-small Cell Lung Cancer via the miR-1183/PDPK1 Axis. Biochem Genet. DOI: 10.1007/s10528-023-10641-0
17 Yang H, He P, Luo W, Liu S, Yang Y. circRNA TATA-box binding protein associated factor 15 acts as an oncogene to facilitate bladder cancer progression through targeting miR-502-5p/high mobility group box 3. Molecular Carcinogenesis. 2024;63(4):629–46.

18 Peng G, Meng H, Pan H, Wang W. CircRNA 001418 Promoted Cell Growth and Metastasis of Bladder Carcinoma via EphA2 by miR-1297. Current Molecular Pharmacology. 2021;14(1):68–78.
19 Liu Z, Yang Y, Yang Z, Xia S, Lin D, Xiao B, et al. Novel circRNA_0071196/miRNA−19b−3p/CIT axis is associated with proliferation and migration of bladder cancer. International Journal of Oncology. 2020;57(3):767–79.
20 Gong P, Xu R, Zhuang Q, He X. A novel circular RNA (hsa_circRNA_102336), a plausible biomarker, promotes the tumorigenesis by sponging miR-515-5p in human bladder cancer. Biomedicine & Pharmacotherapy. 2020;126:110059.
21 Yu XY, Ma CQ, Sheng YH. circRNA CRIM1 regulates the migration and invasion of bladder cancer by targeting miR182/Foxo3a axis. Clin Transl Oncol. 2022;24(6):1195–203.
22 Xu Z-Q, Yang M-G, Liu H-J, Su C-Q. Circular RNA hsa_circ_0003221 (circPTK2) promotes the proliferation and migration of bladder cancer cells. Journal of Cellular Biochemistry. 2018;119(4):3317–25.
23 Wan P, Bai X, Yang C, He T, Luo L, Wang Y, et al. miR-129-5p inhibits proliferation, migration, and invasion in rectal adenocarcinoma cells through targeting E2F7. J Cell Physiol. 2020;235(7–8):5689–701.
24 Liao C, Long Z, Zhang X, Cheng J, Qi F, Wu S, et al. LncARSR sponges miR-129-5p to promote proliferation and metastasis of bladder cancer cells through increasing SOX4 expression. Int J Biol Sci. 2020;16(1):1–11.
25 Cao J, Wang Q, Wu G, Li S, Wang Q. miR-129-5p inhibits gemcitabine resistance and promotes cell apoptosis of bladder cancer cells by targeting Wnt5a. Int Urol Nephrol. 2018;50(10):1811–9.
26 Kong Y-L, Wang H-D, Gao M, Rong S-Z, Li X-X. LncRNA XIST promotes bladder cancer progression by modulating miR-129-5p/TNFSF10 axis. Discov Oncol. 2024;15(1):65.
27 Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001;25(4):402–8.
28 Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020;70(1):7–30.
29 Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer Statistics, 2021. CA Cancer J Clin. 2021;71(1):7–33.
30 Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. CA Cancer J Clin. 2022;72(1):7–33.
31 Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. CA Cancer J Clin. 2023;73(1):17–48.
32 Siegel RL, Giaquinto AN, Jemal A. Cancer statistics, 2024. CA Cancer J Clin. 2024;74(1):12–49.
33 Lenis AT, Lec PM, Chamie K, MSHS M. Bladder Cancer: A Review. JAMA. 2020;324(19):1980–91.
34 Fan H-N, Zhao X-Y, Liang R, Chen X-Y, Zhang J, Chen N-W, et al. CircPTK2 inhibits the tumorigenesis and metastasis of gastric cancer by sponging miR-134-5p and activating CELF2/PTEN signaling. Pathology - Research and Practice. 2021;227:153615.
35 Gao L, Xia T, Qin M, Xue X, Jiang L, Zhu X. CircPTK2 Suppresses the Progression of Gastric Cancer by Targeting the MiR-196a-3p/AATK Axis. Front Oncol. DOI: 10.3389/fonc.2021.706415
36 Yu D, Zhang C. Circular RNA PTK2 Accelerates Cell Proliferation and Inhibits Cell Apoptosis in Gastric Carcinoma via miR-139-3p. Dig Dis Sci. 2021;66(5):1499–509.
37 Gong S-X, Yang F-S, Qiu D-D. CircPTK2 accelerates tumorigenesis of colorectal cancer by upregulating AKT2 expression via miR-506-3p. The Kaohsiung Journal of Medical Sciences. 2022;38(11):1060–9.
38 Yang H, Li X, Meng Q, Sun H, Wu S, Hu W, et al. CircPTK2 (hsa_circ_0005273) as a novel therapeutic target for metastatic colorectal cancer. Molecular Cancer. 2020;19(1):13.
39 Yang Z, Jin J, Chang T. CircPTK2 (hsa_circ_0003221) Contributes to Laryngeal Squamous Cell Carcinoma by the miR-1278/YAP1 Axis. Journal of Oncology. 2021;2021:e2408384.
40 Wang L, Tong X, Zhou Z, Wang S, Lei Z, Zhang T, et al. Circular RNA hsa_circ_0008305 (circPTK2) inhibits TGF-β-induced epithelial-mesenchymal transition and metastasis by controlling TIF1γ in non-small cell lung cancer. Molecular Cancer. 2018;17(1):140.
41 Gong T-T, Sun F-Z, -Y Chen J, Liu J-F, Yan Y, Li D, et al. The circular RNA circPTK2 inhibits EMT in hepatocellular carcinoma by acting as a ceRNA and sponging miR-92a to upregulate E-cadherin. Eur Rev Med Pharmacol Sci. 2020;24(18):9333–42.
42 Meng X, Xiao W, Sun J, Li W, Yuan H, Yu T, et al. CircPTK2/PABPC1/SETDB1 axis promotes EMT-mediated tumor metastasis and gemcitabine resistance in bladder cancer. Cancer Letters. 2023;554:216023.