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Research Article

CircRNA_0058063 functions as a ceRNA in bladder cancer progression via targeting miR-486-3p/FOXP4 axis

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Emerging evidence has uncovered critical regulatory roles of circular RNAs (circRNAs) function as dynamic scaffolding molecules in tumorigenesis and progression. However, the aberrant expression and clinical significance of hsa_circ_0058063 (circRNA_0058063) in bladder cancer (BC) remain poorly understood. circRNA expression was analyzed via a microarray in cancerous tissue and non-carcinoma tissues. Luciferase reporter assays and RNA immunoprecipitation (RIP) were both conducted to uncover the function of circRNA_0058063 in BC. circRNA_0058063 was overexpressed in BC tissues compared with adjacent normal tissues. Knockdown of circRNA_0058063 dramatically decreased cell proliferation and invasion, and promoted apoptosis in 5637 and BIU-87 cell lines. Furthermore, mechanistic investigations showed that circRNA_0058063 and FOXP4 could directly bind to miR-486-3p, demonstrating that circRNA_0058063 regulated FOXP4 expression by competitively binding to miR-486-3p. Taken together, circRNA_0058063 functions by sponging miR-486-3p in BC progression, which could act as a new biomarker and further developed to be a therapeutic target in BC.

Background

Bladder cancer (BC) was ranked as the main factors that are responsible for the cancer-related deaths worldwide, with roughly 74,000 developed cases in the United States in 2015 [1,2]. The risk of BC increases with age, peaking between 50 and 70 years old, and men are three times higher than women [3]. The recurrence rate is as high as over 70% within 5 years, and bladder cancer is required for lifelong cystoscopy [4].

Circular RNAs (circRNAs) represent a sort of non-coding RNA, which are widespread presented in mammals [5]. circRNAs are formed by RNA splicing, which occurs on a so called 'head-to-tail' splicing joint where the receptor splicing site at exon 5′ ends is linked to the donor site at downstream exon 3′ ends. [6]. Most circRNAs (∼85%) resided with known protein-coding genes with structures have intact exons, suggesting that RNA polymerase II (RNA pol II) transcribes them and their biogenesis may be mediated by the splice [7–9]. In the past two decades, they have been considered non-functional due to splicing errors. To date, numerous circRNAs have been continuously explored in various cell lines and tissue species [10]. However, their biological processes and potential functions are poorly understood. Several circRNAs have been reported to regulate the progression of BC. For example, circUBXN7 were identified to suppress BC cancer viability and metastasis [11]. circSLC8A1 acts as a ceRNA of miR-130b/miR-494 to regulate PTEN expression and thus inhibits BC progression [12]. It has been reported that circFAM114A2 represses BC carcinogenesis through regulating ΔNP63 by sponging miR-762 (Liu et al.). Another study also reported that by binding to microRNA-589-5p circ_0091017 suppresses BC cell growth, migration, as well

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as invasion (Zhang et al.). However, the mechanistic function of major circRNAs in BC is merely understood. In hepatocellular carcinoma, circTRIM33–12 has thus far been verified to induce tumorigenesis via its action as a buffering molecule for miR-191 [13]. Specifically, in lung cancer circRNA circPRKCI stimulates tumor cell growth through a ceRNA mechanism [14]. In pancreatic cancer, circ-LDLRAD3 was found to be significantly associated with lymphatic/venous invasion and metastasis [15]. Taken together, all these explorations potentiate circRNA's regulatory roles as ceRNA in cancer progression.

In this research, we reanalyzed the previous microarray datasets and found that circRNA_0058063 was aberrantly expressed in both different BC cell lines and BC carcinoma tissues. Silencing of circRNA_0058063 signaling blocked cell proliferation but promoted cell apoptosis. Furthermore, circRNA_0058063 could bind to miR-486-3p and regulate the expression of FOXP4. Therefore, circRNA_0058063 would be a potent candidate for future diagnostic and therapeutic purpose of BC.

Methods

Patient specimens and cell culture

All of 94 cases of BC cancerous tissues and the non-cancerous normal tissues were retrospectively analyzed from the First Affiliated Hospital of Wenzhou Medical University during April 2013 to Oct 2014. BC cell lines (BIU-87, 5637 and RT-112) were purchased from the American Type Culture Collection (ATCC, Manassas, VA, U.S.A.). 293T cell line was donated by Dr Teng of Shanghai Eighth People's Hospital (Shanghai, China). Cells were cultured in DMEM/F12 supplemented with 10% FBS (Gibco, Grand Island, NY, U.S.A.), penicillin (100 μg/ml, Gibco) and streptomycin (100 μg/ml, Gibco) at 37°C in 95% humidified air and 5% CO_2. All studies were conducted by following the protocol provided by the manufacturer. Studies are performed in accordance with the Declaration of Helsinki and adhere to local ethical regulations. We have acquired the written informed consent from each participant before the initiation of this experimental study. The protocol of the current investigation was authorized by the Institutional Review Board of the First Affiliated Hospital of Wenzhou Medical University.

Plasmids

Full-length FOXP4 was inserted in pcDNA3.1 plasmid to construct the overexpression plasmid. Transfection was performed using Lipofectamine 2000 (Invitrogen) according to the manufacturer's introductions.

Microarray analysis

We used microarray datasets for identification of candidate circRNAs at platform GPL19978 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE92675). Four pairs of BC carcinoma tissues and paired para-carcinoma tissues were selected for detection of the expression and potential circRNAs functions.

qRT-PCR

Total RNA was extracted using TRIzol reagent (Invitrogen, U.S.A.). Then, cDNA was reverse-transcribed by the Prime Script RT reagent Kit (New England Biolabs, MA, U.S.A.) and subjected to RT-PCR measurement using PowerUp SYBR Green Master Mix (Takara Bio, Dalian, China). The relative expressions of each gene were acquired by using the 2-ΔΔCt method. The primers were listed below:

5′-TATGATCCTGTTTGGTGGTCGGCA-3′ (forward), 5′-TGGACCAAGATGGGTAGCTTGTGA-3′ (reverse) for circRNA_0058063
5′-CAATGTACGTTCGCTATCGGC-3′ (forward), 5′-CTCTCACGCACTTAATGCGAT-3′ (reverse) for miR-486-3p
5′-CGGCACAGCAGCTGAACTTA-3′ (forward), 5′-GCAACACCTGAAAAAGTGTGA-3′ (reverse) for FOXP4
5′-CTCGCTACACCTCAATACATCG-3′ (forward), 5′-GCGCCATAAGTCTAGTATTGAGA-3′ (reverse) for GAPDH

Luciferase assay

siRNA for circRNA_0058063 or scramble siRNA (si-NC) was synthesized by Invitrogen. The miR-486-3p mimic and the negative control (miR-NC) were synthesized by GenePharma (Shanghai, China). Lipofectamine 2000 (Invitrogen, Carlsbad, CA) system was used to conduct cell transfection assay by following the manufacturer's protocol.

CCK-8 assay

As previously described [16], 2×10^3 cells were split and cultured in 96-well plates at 24 h before its use for CCK-8 assay. After 48 h of transfection, 10 μl of CCK-8 solution was then pipetted into the medium. Absorbance was detected at 450 nM on a Spectra Max 250 spectrophotometer (Molecular Devices, U.S.A.). After cells were fixed for 15 min in 4% of paraformaldehyde, the colonies were stained by 0.05% Crystal Violet (20 min) followed by visualized under a microscope.

Flow cytometry

The rate of apoptosis of the regulatory cells was examined. Both flow cytometry and annexin V/propidium iodide staining were applied to detect cell death rate by using the Annexin V-fluorescein isothiocynate Apoptosis Detection Kit (KeyGen, Nanjing, China).

Transwell assay

At the beginning 20% fetal calf serum was put into the lower chamber, and the conditioned cells were suspended in a medium without FBS and inoculated onto an upper chamber. Forty-eight hours after adding the cells, get rid of the Matrigel in the upper chamber, at the same time the attached cells of invading ability were fixed, and Crystal Violet was applied to stain the cells, and then it was subjected for imaging with the aid of a microscope.

RNA immunoprecipitation (RIP) assay

With the aid of Lipofectamine 2000 transfection system, 1×10^7 cells were incubated in the RIP buffer transfected with MS2bs-circRNA_0058063mt or MS2bs-circRNA_0058063, and control MS2bs-Rluc. After 48 h of transfection, RIP was conducted by using the Magna RIP RNA-binding protein immunoprecipitation kit (Millipore). For the RIP assay of Ago2, anti-Ago2 antibody (Millipore) was applied to RIP assay after the transfection for 48 h.

Western blot analysis

First, proteins were subjected to electrophoresis with 10% SDS-PAGE and then were transferred onto the PVDF membrane (Millipore Corp., MA, U.S.A.) for 1 h. Then, the membranes were blocked with 5% non-fat milk prepared by washing buffer for 1 h. Next, the membranes were prepared to be incubated with corresponding antibodies against FOXP4 (1:500, Abcam, U.S.A.) to detect the relative protein expression of targets. Anti-β-actin antibody (1:1000, Affinity, U.S.A.) was applied as loading control. After incubating with primary antibody at 4°C overnight, the membranes were washed with tris-buffered saline for 4 times and each time for 5 min. At last, specific corresponding secondary antibodies (1:2000, CST, U.S.A.) were further incubated with the membrane at room temperature. Protein bands were visualized and analyzed using Quantity One 4.6.2 software (Bio-Rad, Hercules, CA, U.S.A.).

Statistical analysis

SPSS 21.0 software for Windows (SPSS Inc.) was utilized to conduct statistical analysis. Data sets were expressed as mean ± S.E.M. The differences between two groups or more were compared by two-tail *t* test and $\chi 2$ test, respectively. Kaplan–Meier survival curves were analyzed using log-rank tests. High/low circRNA_0058063 expression groups were divided according to the medium expression values. Statistical differences were taken when $P < 0.05$. Triplicate experiments were performed in any of the assay unless otherwise stated.

Results

circRNA_0058063 is up-regulated in BC progression

A total of 312 circRNAs were differentially expressed (Fold change > 2, $P < 0.05$) with the analysis of circRNA microarray from GEO database. About 195 circRNAs were up-regulated, and 117 circRNAs were significantly down-regulated in four bladder cancer samples. The top dysregulated circRNAs were presented in the heat map (Figure 1A). Our results indicated that circRNA_0058063's expression level was augmented in both BC cell lines (Figure 1B) and the tissues (Figure 1C). Kaplan–Meier analysis showed 94 cases of BC patients with the higher expression level of circRNA_0058063 had a relatively low 5-year OS, indicating that circRNA_0058063 expression was negatively correlated with carcinogenesis in individual BC patients (Figure 1D). And it was further confirmed that higher circRNA_0058063 expression contributes to advanced stage and metastasis of the disease (Table 1). Taken together, it potentiates circRNA_0058063's critical role in the regulation of BC.

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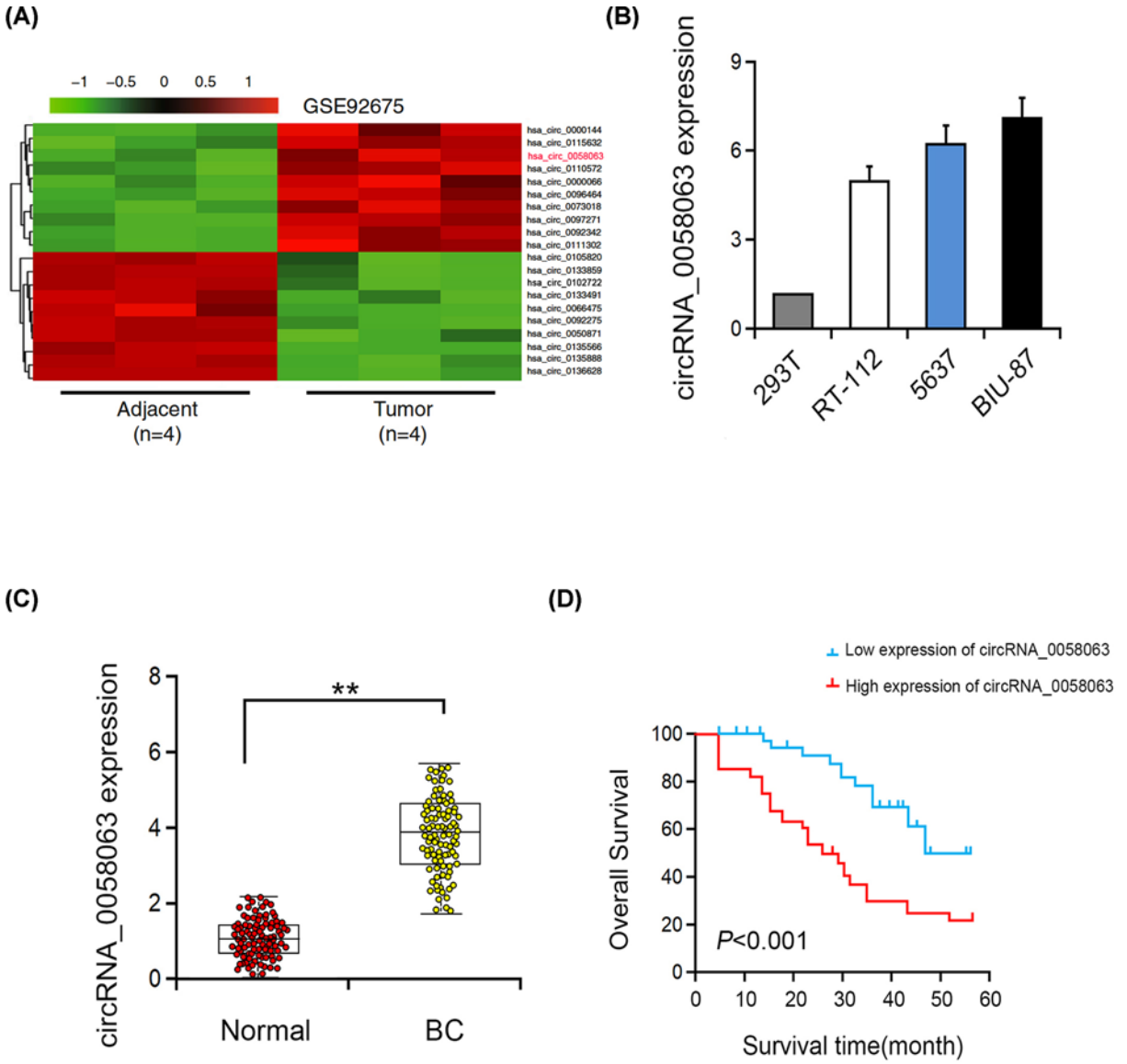

Figure 1. Relative circRNA_0058063 expression in BC tissues and cell lines

(**A**) The cluster heatmap demonstrated the differentiated expression of circRNAs (>2-fold change) between cancerous tissues and control normal tissues in BC patients. (**B**) Expression of circRNA_0058063 in the BC cell lines. (**C**) Expression level of circRNA_0058063 in BC tissues. (**D**) Kaplan–Meier survival analysis in BC tissues.

circRNA_0058063 knockdown suppressed cell proliferation and invasion

Since the expression of circRNA_0058063 was induced in BC, the expression of circRNA_0058063 was silenced in BC to determine the regulatory events. As shown in Figure 2A, silencing of circRNA_0058063 was successfully achieved. Moreover, silencing of circRNA_0058063 inhibited the proliferation rate of 5637 and BIU-87 cell lines (Figure 2B). Transwell assay showed that inhibition of cell invasion by circRNA_0058063 (Figure 2C). Apoptosis assays indicated that inhibition of circRNA_0058063 contributed to elevate cell apoptosis (Figure 2D). Therefore, it would be interesting to conclude that silencing of circRNA_0058063 inhibits both 5637 and BIU-87 cell proliferation and cell invasion, and promotes apoptosis of 5637 and BIU-87 cell lines during BC progress *in vitro*.

circRNA_0058063 acts as a sponge for miR-486-3p

The intracellular residence of circRNA_0058063 was further explored, and it was revealed that circRNA_0058063 was mainly distributed in the cytoplasm (Figure 3A), suggesting that circRNA_0058063 may act as a miRNA sponge to in-

Table 1 Association between circRNA_0058063 expression and different clinicopathological characteristics of bladder cancer patients

Variable	*n*	Relative circRNA_0058063 level	*P* value
Gender			
Female	44	5.24 ± 1.27	
Male	50	5.51 ± 1.48	0.721
Age (years)			
<60	58	5.12 ± 1.36	
≥60	36	5.64 ± 1.41	0.135
Number of tumors			
Single	71	4.89 ± 1.32	
Multiple	23	5.84 ± 1.71	0.118
Stage			
Ta-T1	80	4.13 ± 1.26	
≥T2	14	8.28 ± 1.47	0.003
Grade			
G1/2	37	3.51 ± 1.23	
G3	57	7.97 ± 1.45	0.004

duce miRNAs. Based on the results, the Circular RNA Interactome (https://circinteractome.nia.nih.gov/index.html) was applied to study the underlying mechanism of circRNA/miRNA interactions and we found the complementary site of miR-486-3p in the circRNA_0058063 sequence. (Figure 3B). miR-486-3p was chosen for further studies as it has been shown to a biomarker for prediction and prognosis of BC (Jiang et al.). Furthermore, miR-486-3p expression was mitigated in 5637 and BIU-87 cell lines and BC tissues (Figure 3C,D). Next, the direct binding relationship between miR-486-3p and circRNA_0058063 was confirmed with a luciferase reporter assay. When luciferase reporter gene and the miR-486-3p mimic were co-transfected into the 5637 and BIU-87 cell lines, the luciferase intensities were greatly reduced (Figure 3E). To elucidate whether the binding of circRNA_0058063 and miR-486-3p was direct or indirect, a RIP assay was conducted. It was indicated that miR-486-3p was predominantly resided at the site of MS2bs-circRNA_0058063, potentiating a direct binding of circRNA_0058063 and miR-486-3p (Figure 3F). In summary, our data confirm that circRNA_0058063 can directly inhibit the miR-486-3p expression by directly combining to the binding site.

circRNA_0058063 regulates FOXP4 by miR-486-3p

To investigate whether circRNA_0058063 modifies miR-486-3p to regulate target gene expression, the potential targets of miR-486-3p were predicted by using TargetScan and FOXP4 was selected to be a potential candidate (Figure 4A). FOXP4 has been demonstrated to play an important role in the progression of multiple cancers, such liver cancer and oral squamous cell carcinoma, and breast cancer (Qi et al.; Xu et al.; Ma et al.); however, its function in bladder cancer remains unknown. We detected FOXP4 expression level and found that the expression of FOXP4 was significantly increased in the BC cell lines (Figure 4B) and tissues (Figure 4C). Overexpression of miR-486-3p inhibited the expression of FOXP4 and reversed the up-regulation of FOXP4 induced by transfection with FOXP4-overexpressing plasmid (Figure 4D). Next, the luciferase reporter assay suggested that the luciferase activity of FOXP4 was significantly decreased by the miR-486-3p mimic (Figure 4E). Next, the RIP assay for Ago2 showed circRNA_0058063, FOXP4 and miR-486-3p were mainly enriched to Ago2 (Figure 4F). This result indicated that circRNA_0058063 and FOXP4 were subjected to Ago2-related RISC, and then it promoted their interaction with miR-486-3p. Furthermore, inhibition of circRNA_0058063 lowered the recruitment of Ago2 to circRNA_0058063, but elevated the recruitment of Ago2 enrichment to FOXP4 (Figure 4G), which revealed that circRNA_0058063 can act as a ceRNA and antagonize with FOXP4 to bind with miRNA. In addition, the expression of FOXP4 was detected when circRNA_0058063 was silenced and it was found that FOXP4 expression was significantly reduced, but it was significantly reversed by miR-486-3p LNA (Figure 4H). Taken together, circRNA_0058063 tightly regulated the expression of FOXP4 as a ceRNA by inducing miR-486-3p (Figure 5).

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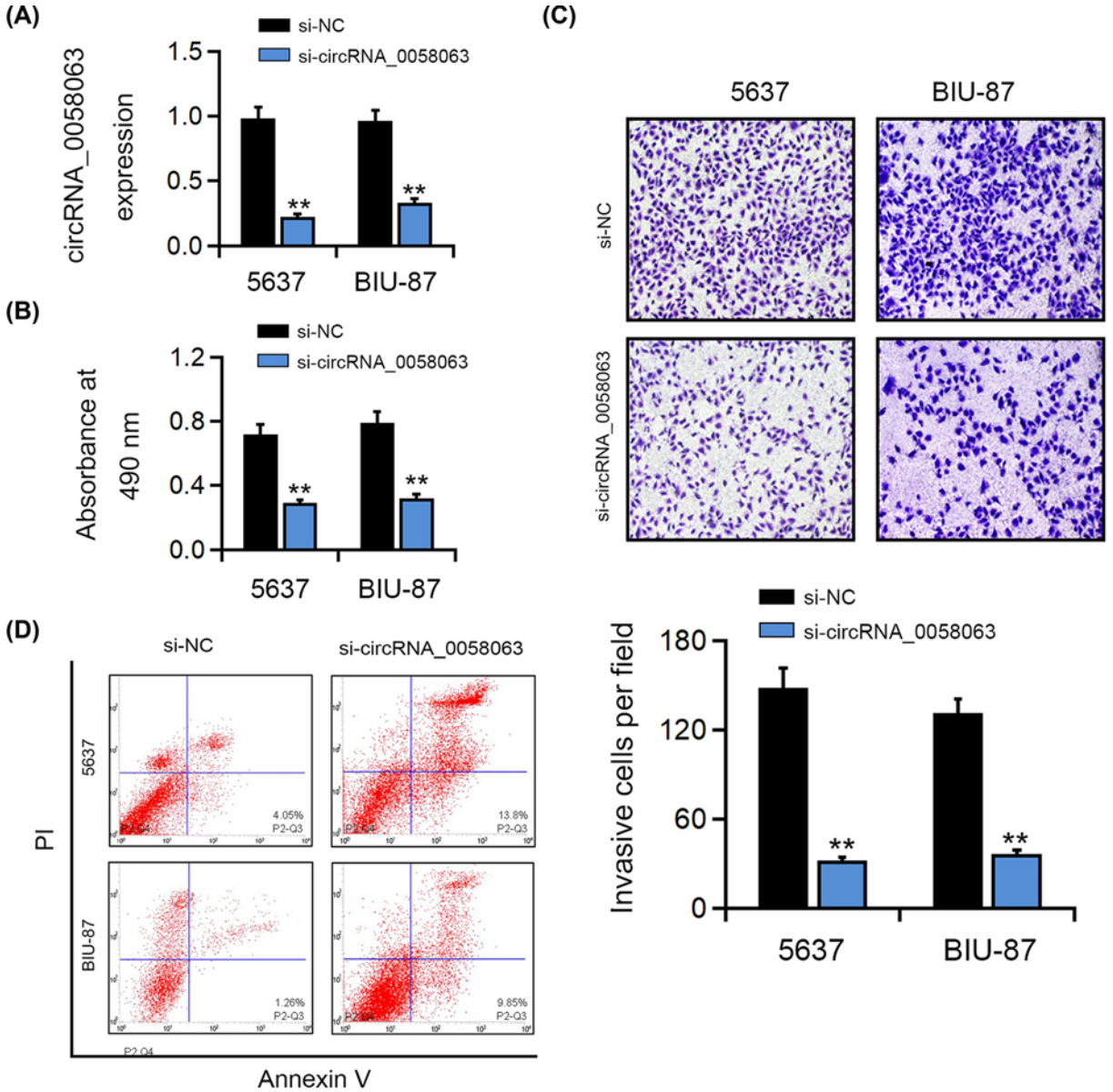

Figure 2. Effect of circRNA_0058063 knockdown on BC cell proliferation, migration and apoptosis

(**A**) si-NC or si-circRNA_0058063 were transfected into BC cells. (**B**) Cell proliferation was detected by CCK-8 assay. (**C**) Cell migration was detected by Transwell assay. (**D**) Flow cytometry assay was applied to detect cell apoptosis 48 h after transfection; $^{**}P<0.01$.

Discussion

An abundant of researches have explored circRNA's crucial functions in the occurrence and progression of diverse cancers [17,18]. In lung cancer, the regulatory roles of circRNA have been uncovered in various cell biological processes [19]. During the progression of glioma, circPTN blocked cancer growth and metastasis [20]. Several studies were performed to investigate the function of circRNAs in bladder cancer (Liu et al.; Lu et al.; Liu et al.; Zhang et al.). However, the exploration of circRNAs in BC is still rare. Therefore, we utilized the circRNA expression microarray assay in BC and indicated that circRNA_0058063 was up-regulated in BC. Hsa_circ_0058063 is located at the domain of chr2: 216177220-216213972 according to the human reference genome (GRCh38.p13) and is thought to originate from the gene ATIC, which residues at chromosome 2q35. Therefore, hsa_circ_0058063 was named as 'circRNA_0058063'. Further investigations demonstrated that silencing of circRNA_0058063 blocked cell proliferation

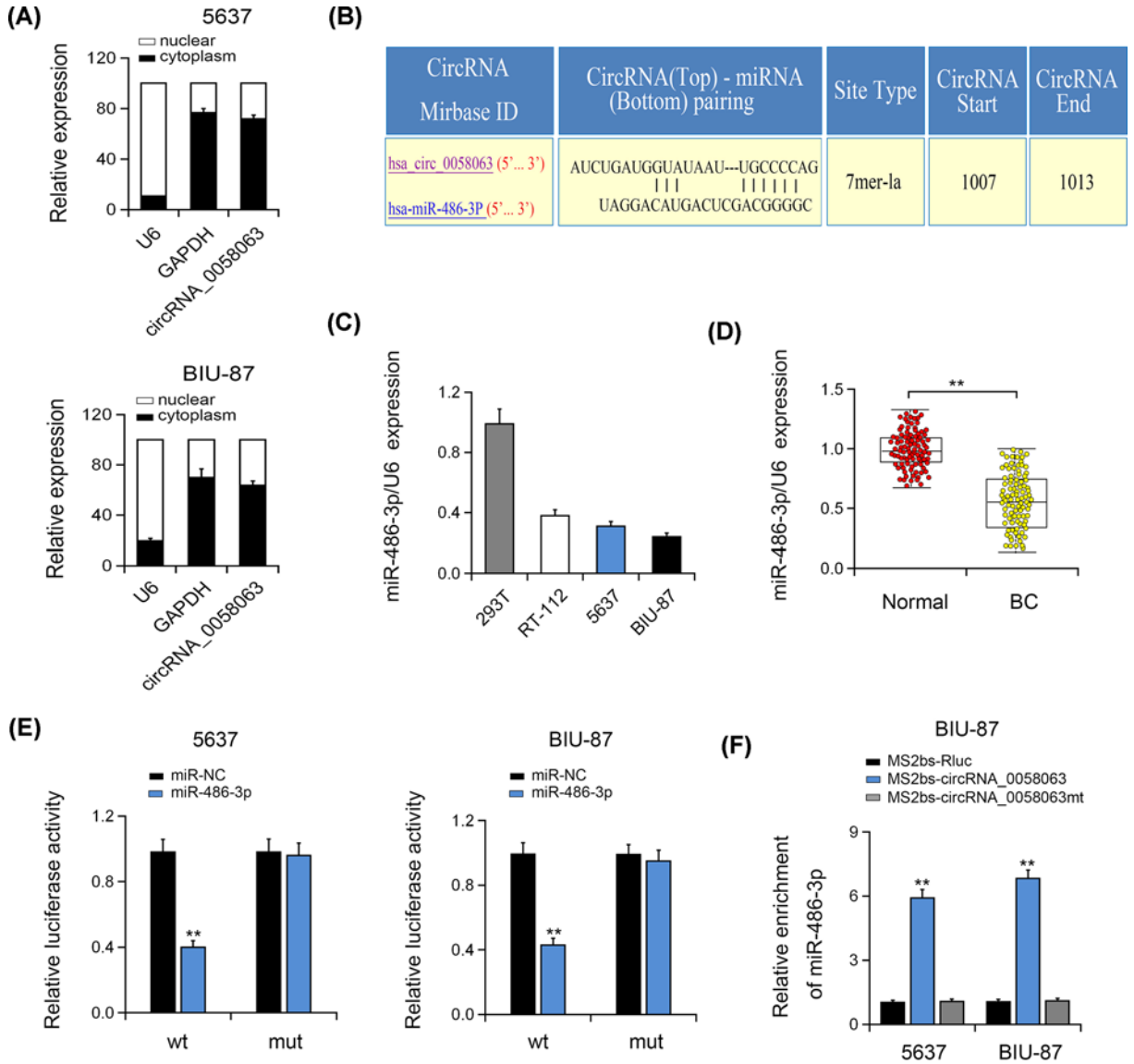

Figure 3. CircRNA_0058063 acts as a sponge for miR-486-3p

(**A**) Expression levels of circRNA_0058063 in the nuclear and cytoplasmic fractions detected by qRT-PCR. (**B**) Binding sites of cirRNA_0058063 for miR-486-3p. (**C** and **D**) Expressions of miR-486-3p in BC cell lines and specimen. (**E**) Luciferase reporter assay in BC cells after transfection with miR-486-3p mimics and luciferase reporter gene containing circRNA_0058063 (wt/mut). (**F**) RIP assay in BC cells transfected with MS2bs-circRNA_0058063 wt/mut; $^{**}P < 0.01$.

and cell invasion, and promoted cell death, suggesting that circRNA_0058063 can modulate BC progression. Therefore, circRNA_0058063 potentiates its potential as a target for pathological detection and treatment.

The reported researches have indicated that circRNA regulates gene expression through cavernous miRNAs. In colorectal cancer, CCDC66 promotes cancer growth and metastasis through targeting miRNA-33b and miR-93 [21]. Bi et al. proved that circRNA_102171 regulates cell proliferation and apoptosis, and causes malignant transformation by blocking CTNNBIP1 in papillary thyroid carcinoma [22]. miR-486-3p has been reported to have a critical role in several cancers, such as lung, liver, breast cancers (ElKhouly et al.). It has also been reported that lncRNA-PVT1 promotes the proliferation of cervical cancer cells by increasing ECM1 expression via sponging miR-486-3p (Wang et al.). In oral cancer, overexpression of miR-486-3p inhibits oral cancer cell growth and induces apoptosis by down-regulating of DDR1 (Chou et al.). Here, we found that circRNA_0058063 directly bound with miR-486-3p and worked as a miRNA sponge.

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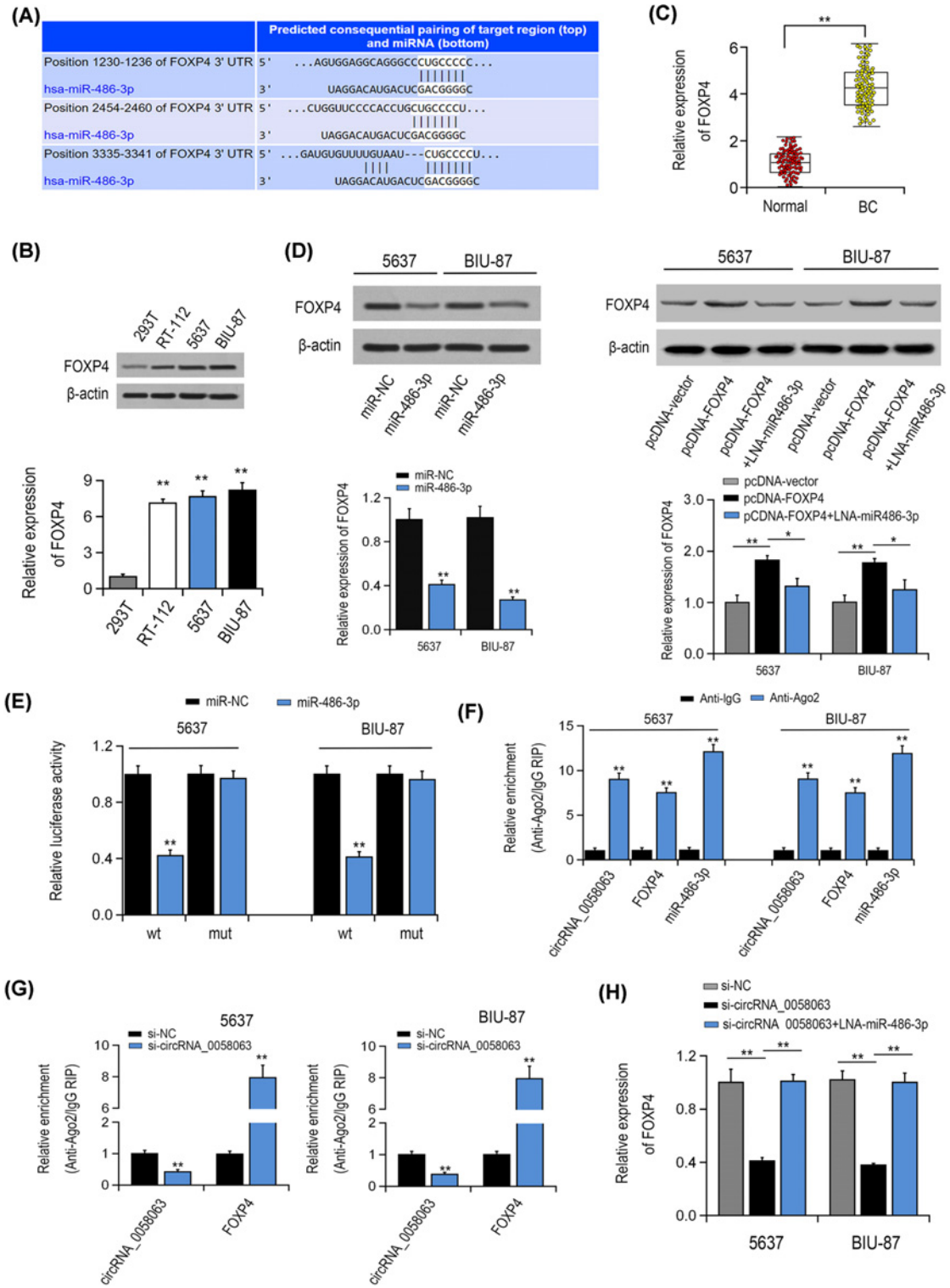

Figure 4. circRNA_0058063 regulates FOXP4 by miR-486-3p

(**A**) Possible binding sites for miR-486-3p in FOXP4. (**B**) Expression of FOXP4 in BC cell lines was determined by Western blot (up) and qRT-PCR (down). (**C**) FOXP4 expression in BC tissues. (**D**) Western blot (up) and qRT-PCR (down) were used to assess the expression of FOXP4 after transfection with miR-486-3p mimic and/or pcDNA-FOXP4. (**E**) Luciferase reporter assay for cells co-transfected with the miR-486-3p mimic and the luciferase reporter gene containing the mutant construct (mut) or FOXP4 3′-UTR (wt). (**F**) RIP assay displayed the enrichment of circRNA_0058063, FOXP4 and miR-486-3p on Ago2. (**G**) RIP assay on Ago2. (**H**) Expression of FOXP4 was determined in cells transfected with si-circRNA_0058063 and/or miR-486-3p by qRT-PCR; $^{**}P < 0.01$.

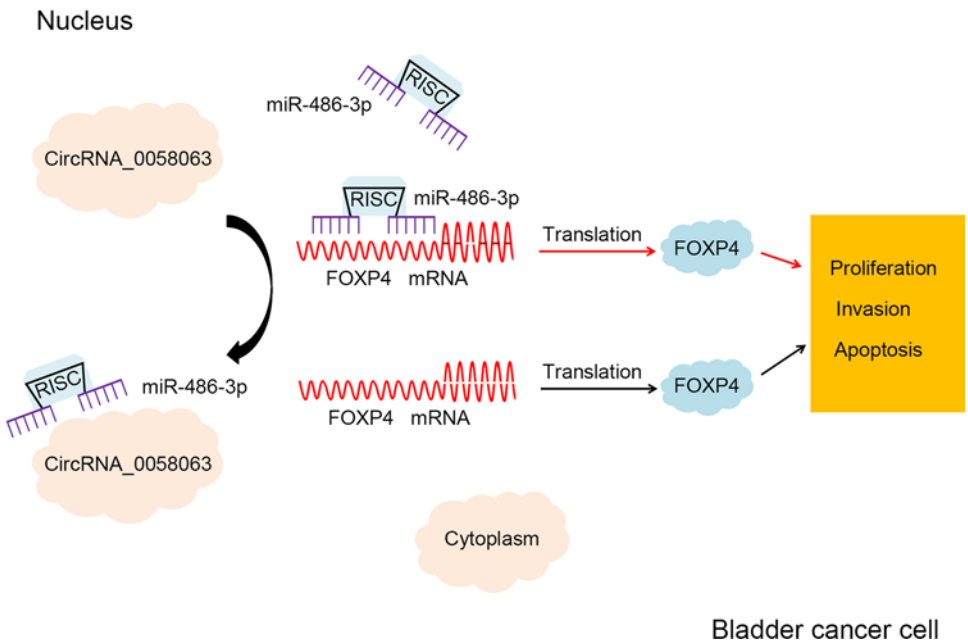

Figure 5. The mechanism schematic model by circRNA_0058063/ miR-486-3p/FOXP4 in BC

It was reported that microtubule-associated proteins (MAPs) mainly reside in the cytoplasmic fraction and bind with tubulin and facilitate their aggregation [23]. Others have suggested MAP's involvement in the control of mitochondrial balance or apoptosis [24]. Previous studies have reported that FOXP4 overexpresses and regulates tumor cell growth in non-small cell lung cancer [25]. FOXP4 has been certified in the progression of prostate cancer [26]. It has been also reported that FOXP4 enhances the migration and invasion of breast cancer cells via promoting EMT (2019). In the present study, the abundance of FOXP4 was found to be elevated in BC. Moreover, our data demonstrated that FOXP4 was a novel target gene of miR-486-3p. Further exploration suggested circRNA_0058063 acts as a bait for miR-486-3p to modulate FOXP4 expression. Inhibition of circRNA_0058063 decreased FOXP4's expression, which was overturned by silencing of miR-486-3p.

Conclusion

To sum up, the expression of circRNA_0058063 was elevated in BC. Silencing of circRNA_0058063 blockeds cell proliferation and cell invasion, however promoted cell death. Mechanistically, circRNA_0058063 regulates the expression of FOXP4 by the sponge miR-486-3p. circRNA_0058063 potentiates as a valuable candidate for the early detection and prognosis of BC.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Author Contribution

Conception and intellectual input: Y.T.Y.; Designed and performance of experimentation: L.H.T.; Data collection and manuscript drafting: H.H., L.Y.P. Statistical analyses and data interpretation: L.Y.Y. All authors read and approved the final manuscript.

Ethics Approval

The protocol of the current investigation was authorized by the Institutional Review Board of the First Affiliated Hospital of Wenzhou Medical University. Written informed consent was obtained from all the participants in the study.

Data Availability

The authors declare that the data supporting the findings of this study are available within the article.

Abbreviations

BC, bladder cancer; circRNA, circular RNA; RIP, RNA immunoprecipitation; RNA pol II, RNA polymerase II.

References

1 Sin, M.L.Y., Mach, K.E., Sinha, R., Wu, F., Trivedi, D.R., Altobelli, E. et al. (2017) Deep Sequencing of Urinary RNAs for Bladder Cancer Molecular Diagnostics. *Clin. Cancer Res.* **23**, 3700–3710, https://doi.org/10.1158/1078-0432.CCR-16-2610
2 Flaig, T.W., Kamat, A.M., Hansel, D., Ingersoll, M.A., Barton Grossman, H., Mendelsohn, C. et al. (2017) Proceedings of the 3rd Annual Albert Institute for Bladder Cancer Research Symposium. *Bladder Cancer* **3**, 211–223, https://doi.org/10.3233/BLC-170111
3 Smith, S.G. and Zaharoff, D.A. (2016) Future directions in bladder cancer immunotherapy: towards adaptive immunity. *Immunotherapy* **8**, 351–365, https://doi.org/10.2217/imt.15.122
4 Lorenzatti Hiles, G., Cates, A.L., El-Sawy, L., Day, K.C., Broses, L.J., Han, A.L. et al. (2019) A surgical orthotopic approach for studying the invasive progression of human bladder cancer. *Nat. Protoc.* **14**, 738–755, https://doi.org/10.1038/s41596-018-0112-8
5 Kristensen, L.S., Hansen, T.B., Veno, M.T. and Kjems, J. (2018) Circular RNAs in cancer: opportunities and challenges in the field. *Oncogene* **37**, 555–565, https://doi.org/10.1038/onc.2017.361
6 Zhang, Y., Liang, W., Zhang, P., Chen, J., Qian, H., Zhang, X. et al. (2017) Circular RNAs: emerging cancer biomarkers and targets. *J. Experiment. Clin. Cancer Res.: CR* **36**, 152
7 Zhong, Y., Du, Y., Yang, X., Mo, Y., Fan, C., Xiong, F. et al. (2018) Circular RNAs function as ceRNAs to regulate and control human cancer progression. *Mol. Cancer* **17**, 79, https://doi.org/10.1186/s12943-018-0827-8
8 Dong, Y., He, D., Peng, Z., Peng, W., Shi, W., Wang, J. et al. (2017) Circular RNAs in cancer: an emerging key player. *J. Hematol. Oncol.* **10**, 2
9 Cui, X., Wang, J., Guo, Z., Li, M., Li, M., Liu, S. et al. (2018) Emerging function and potential diagnostic value of circular RNAs in cancer. *Mol. Cancer* **17**, 123, https://doi.org/10.1186/s12943-018-0877-y
10 Xu, C., Haque, F., Jasinski, D.L., Binzel, D.W., Shu, D. and Guo, P. (2018) Favorable biodistribution, specific targeting and conditional endosomal escape of RNA nanoparticles in cancer therapy. *Cancer Lett.* **414**, 57–70, https://doi.org/10.1016/j.canlet.2017.09.043
11 Liu, H., Chen, D., Bi, J., Han, J., Yang, M., Dong, W. et al. (2018) Circular RNA circUBXN7 represses cell growth and invasion by sponging miR-1247-3p to enhance B4GALT3 expression in bladder cancer. *Aging* **10**, 2606–2623, https://doi.org/10.18632/aging.101573
12 Lu, Q., Liu, T., Feng, H., Yang, R., Zhao, X., Chen, W. et al. (2019) Circular RNA circSLC8A1 acts as a sponge of miR-130b/miR-494 in suppressing bladder cancer progression via regulating PTEN. *Mol. Cancer* **18**, 111, https://doi.org/10.1186/s12943-019-1040-0
13 Zhang, P.F., Wei, C.Y., Huang, X.Y., Peng, R., Yang, X., Lu, J.C. et al. (2019) Circular RNA circTRIM33-12 acts as the sponge of MicroRNA-191 to suppress hepatocellular carcinoma progression. *Mol. Cancer* **18**, 105, https://doi.org/10.1186/s12943-019-1031-1
14 Zhang, X., Yang, H., Zhao, L., Li, G. and Duan, Y. (2019) Circular RNA PRKCI promotes glioma cell progression by inhibiting microRNA-545. *Cell Death. Dis.* **10**, 616
15 Yang, F., Liu, D.Y., Guo, J.T., Ge, N., Zhu, P., Liu, X. et al. (2017) Circular RNA circ-LDLRAD3 as a biomarker in diagnosis of pancreatic cancer. *World J. Gastroenterol.* **23**, 8345–8354, https://doi.org/10.3748/wjg.v23.i47.8345
16 Liu, H., Bi, J., Dong, W., Yang, M., Shi, J., Jiang, N. et al. (2018) Invasion-related circular RNA circFNDC3B inhibits bladder cancer progression through the miR-1178-3p/G3BP2/SRC/FAK axis. *Mol. Cancer* **17**, 161, https://doi.org/10.1186/s12943-018-0908-8
17 Qin, C., Liu, C.B., Yang, D.G., Gao, F., Zhang, X., Zhang, C. et al. (2018) Circular RNA Expression Alteration and Bioinformatics Analysis in Rats After Traumatic Spinal Cord Injury. *Front. Mol. Neurosci.* **11**, 497, https://doi.org/10.3389/fnmol.2018.00497
18 Liu, J., Liu, T., Wang, X. and He, A. (2017) Circles reshaping the RNA world: from waste to treasure. *Mol. Cancer* **16**, 58, https://doi.org/10.1186/s12943-017-0630-y
19 Smid, M., Wilting, S.M., Uhr, K., Rodriguez-Gonzalez, F.G., de Weerd, V., Prager-Van der Smissen, W.J.C. et al. (2019) The circular RNome of primary breast cancer. *Genome Res.* **29**, 356–366, https://doi.org/10.1101/gr.238121.118
20 Chen, J., Chen, T., Zhu, Y., Li, Y., Zhang, Y., Wang, Y. et al. (2019) circPTN sponges miR-145-5p/miR-330-5p to promote proliferation and stemness in glioma. *J. Experiment. Clin. Cancer Res.: CR* **38**, 398
21 Hsiao, K.Y., Lin, Y.C., Gupta, S.K., Chang, N., Yen, L., Sun, H.S. et al. (2017) Noncoding Effects of Circular RNA CCDC66 Promote Colon Cancer Growth and Metastasis. *Cancer Res.* **77**, 2339–2350, https://doi.org/10.1158/0008-5472.CAN-16-1883
22 Bi, W., Huang, J., Nie, C., Liu, B., He, G., Han, J. et al. (2018) CircRNA circRNA_102171 promotes papillary thyroid cancer progression through modulating CTNNBIP1-dependent activation of beta-catenin pathway. *J. Exp. Clin. Cancer Res.: CR* **37**, 275
23 Devanna, P., Middelbeek, J. and Vernes, S.C. (2014) FOXP2 drives neuronal differentiation by interacting with retinoic acid signaling pathways. *Front. Cell. Neurosci.* **8**, 305, https://doi.org/10.3389/fncel.2014.00305
24 Hu, J., Chu, Z., Han, J., Zhang, Q., Zhang, D., Dang, Y. et al. (2014) Phosphorylation-dependent mitochondrial translocation of MAP4 is an early step in hypoxia-induced apoptosis in cardiomyocytes. *Cell Death. Dis.* **5**, e1424
25 Wu, X., Xiao, Y., Zhou, Y., Zhou, Z. and Yan, W. (2019) LncRNA FOXP4-AS1 is activated by PAX5 and promotes the growth of prostate cancer by sequestering miR-3184-5p to upregulate FOXP4. *Cell Death. Dis.* **10**, 472
26 Hicks, C., Miele, L., Koganti, T. and Vijayakumar, S. (2013) Comprehensive assessment and network analysis of the emerging genetic susceptibility landscape of prostate cancer. *Cancer Informatics* **12**, 175–191, https://doi.org/10.4137/CIN.S12128