Circular RNA circ-Foxo3 induced cell apoptosis in urothelial carcinoma via interaction with miR-191-5p

Background: Circular RNAs (circRNAs) play a critical role in cancer. Emerging evidence has shown circ-Foxo3, a circRNA, was dysregulated in a variety of tumor types. However, the exact role of circ-Foxo3 in bladder cancer has never been studied.

Methods: We measured the expression level of circ-Foxo3 in human and murine bladder cancer tissues and in various human bladder cancer cell lines. We induced bladder cancer in mice by a carcinogen N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN). circ-Foxo3 was overexpressed in mice by lentiviral gene transfer and in cultured cells via overexpression plasmid. The effect of circ-Foxo3 on apoptosis was examined via apoptotic marker staining, Western blot, and flow cytometry. We further characterized the interaction between circ-Foxo3 and miR-191 and its functional impact on bladder cancer cells.

Results: circ-Foxo3 was downregulated in bladder cancer in vivo and in vitro, and was upregulated in response to apoptotic stress. Overexpression of circ-Foxo3 promoted bladder cancer cell apoptosis in BBN mice and in human bladder cancer cell lines. miR-191-5p suppressed circ-Foxo3 expression and the pro-apoptotic effect of circ-Foxo3 in bladder cancer cells via directly targeting the 3’-untranslated region (3’-UTR) of circ-Foxo3.

Conclusion: circ-Foxo3 was downregulated in bladder cancer in vivo and in vitro, and promoted bladder cancer apoptosis via direct interaction with miR-191. circ-Foxo3 could be a potential therapeutic target for bladder cancer.

Keywords: bladder cancer, circular RNA, apoptosis, circ-Foxo3, miR-191

Background
Bladder cancer is the 9th most commonly diagnosed cancer worldwide and the 4th most common cancer in men.1,2 Globally, about 430,000 new bladder cancer cases are diagnosed annually.1 In the United States, over 80,000 new cases of bladder cancer are diagnosed each year, and over 17,000 patients die from bladder cancer annually.2 Urothelial carcinoma, also known as transitional cell carcinoma, is the most common type of bladder cancer. Urothelial carcinoma originates from the urothelial cells that line the inner layer of urinary tract. The development of urothelial carcinoma is predisposed by certain risk factors, including smoking and exposure to certain industrial carcinogens such as aromatic amines. To date, there is no definitive cure to urothelial carcinoma, and its mortality rate has maintained stable. Therefore, identifying early diagnostic markers and novel therapeutic targets for bladder cancer, particularly urothelial carcinoma, remains a major public health need.

Circular RNA (circRNA) is a type of single-stranded RNA that forms a closed loop by joining the 5’ and 3’ ends of a linear RNA. CircRNAs are ubiquitously expressed from archaea to eukaryotes and are evolutionary conserved, strongly...
suggesting their functional importance.\textsuperscript{3} CircRNA can be protein-coding or non-coding.\textsuperscript{4,5} Although the detailed function of most circRNAs remains unclear, one major role of those non-coding circRNAs is shown to be gene regulation,\textsuperscript{6} potentially by interaction with microRNAs or RNA-binding proteins.\textsuperscript{7,8}

circ-Foxo3 is a circRNA derived from the FOXO3 gene that also encodes the linear FOXO3 mRNA.\textsuperscript{9} The expression of circ-Foxo3 appeared independent of the expression of FOXO3 mRNA, and may have regulatory function on targets beyond the linear FOXO3 mRNA.\textsuperscript{10} Recently, emerging evidence has indicated that circ-Foxo3 was detectable in multiple cancers, and that circ-Foxo3 was associated with cell cycle retardation or apoptosis.\textsuperscript{10–14} However, the role of circ-Foxo3 in bladder cancer has yet to be fully understood.

Here, we explored the role of circ-Foxo3 in bladder cancer. We found circ-Foxo3 was dysregulated in bladder cancer tissue in vivo and in vitro. circ-Foxo3 was a direct target of the microRNA miR-191-5p. Together, a miR-191-circ-Foxo3 axis appeared critical to bladder cancer apoptosis.

Methods

Human bladder cancer samples

The study involving human samples was approved by the Institutional Review Board of The First Affiliated Hospital of Harbin Medical University. Written informed consent was obtained from all patients upon recruiting. Thirty pairs of fresh bladder cancer tissues and adjacent normal bladder tissue were excised during partial or radical cystectomy from patients with confirmed diagnosis of urothelial carcinoma at the Department of Urology of The First Affiliated Hospital of Harbin Medical University between 2016 and 2017. None of the patients had medication or radiation therapy prior to surgery. The specimens were snap-frozen in liquid nitrogen immediately after excision. Pathological and histological diagnoses were performed by two pathologists independently, and the diagnosis of urothelial carcinoma was confirmed in all samples. The grades and stages of specimens were classified using 2004 World Health Organization Consensus Classification and Staging System.

Cell culture and treatment

Three cell lines of human transitional cell carcinoma, T24, UM-UC-3 and J82, and a normal human uroepithelial cell line SV-HUC-1, were obtained from the American Type Culture Collection (ATCC, USA). All cells were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (Gibco, USA). Cells were kept in an incubator at 37°C with humidified atmosphere containing 5% CO\textsubscript{2}. Medium was replaced every 2–3 days.

Cells receiving transfection were treated in RPMI-1640 basal medium (serum-free) containing 0.4 mM hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}), or 1 μg/mL doxorubicin (DOX), or 2 μg/mL cisplatin (CP) for 24 h.

Bladder tumor model in mice

All animal protocols were approved by the Ethical Committee of The First Affiliated Hospital of Harbin Medical University and adhered to the Guide for the Care and Use of Laboratory Animals (NRC 2011, 8th Edition). Male, 8-week-old C57BL/6 mice were obtained from The First Affiliated Hospital of Harbin Medical University. Mice were housed in a temperature-controlled environment with 12-h light/dark cycles and unrestricted access to food and drinking water. To induce carcinogenesis in mouse bladder, 0.05% N-butyl-N-(4-hydroxybutyl) nitrosamine (BBN; Tokyo Chemical Industry Co Ltd, Japan) was added to the drinking water for 17 weeks until tissue harvesting. BBN-free water (vehicle) was added in the drinking water in equal volume for 17 weeks as control treatment. The induction of bladder cancer was histologically confirmed at tissue harvesting.

Histology

Mice were euthanized by CO\textsubscript{2} inhalation. The bladders were exposed. A small fraction of bladder tumor tissue and the adjacent normal bladder tissue was removed for RNA extraction. The remaining bladder was inflated and soaked in 4% formaldehyde in phosphate-buffered saline (PBS) overnight. Fixed bladders were cut in half, positioned in cassettes, soaked in 70% alcohol overnight, and embedded in paraffin. The paraffin-embedded blocks were cut into 6-μm-thick serial sections, stained with hematoxylin and eosin (H&E), and gold enhanced for visualization by light microscopy.

Circrna vectors

The circ-Foxo3 over-expression plasmid was constructed by sub-cloning the human circ-Foxo3 cDNA (synthesized by TSINGKE, China) onto a pCD-ciR circRNA expression vector (Geneseed Biotech, China), which has a front circular frame and a back circular frame to ensure functional circRNA transcription. The sequence-verified plasmid was transfected into cells using Lipofectamine 2000.
The following primary antibodies were used: anti-cleaved-caspase3 (1:1000), anti-Bcl2 (1:1000), anti-Bax (1:1000), and anti-GAPDH (1:1000) (all from Abcam, UK).
Results

circ-Foxo3 was downregulated in bladder cancer in vivo and in vitro

We first examined the expression of circ-Foxo3 in bladder cancer tissues and cell lines. RT-qPCR showed the RNA expression of circ-Foxo3 was significantly downregulated in tumor tissues isolated from bladder cancer patients as compared with the adjacent normal bladder tissues (Figure 1A). circ-Foxo3 expression was also reduced in murine bladder tumors induced by 17-week oral treatment of BBN, a carcinogen that effectively induces bladder cancer (Figure 1B).

We also measured circ-Foxo3 expression in bladder cancer in vitro. The expression of circ-Foxo3 RNA was significantly down-regulated in human bladder cancer cell lines T24, UM-UC-3, and J82, as compared with the immortalized normal bladder cell line SV-HUC-1 (Figure 1C).

circ-Foxo3 was upregulated in response to apoptotic stress

Next, we explored the role of circ-Foxo3 in apoptosis. We first tested the level of apoptosis in J82 cells treated with doxorubicin, cisplatin or H2O2. Western blot showed the apoptotic protein markers was markedly increased following these treatments, as indicated by increased cleaved-caspase3 expression and increased ratio of the pro-apoptotic protein Bax over the pro-survival protein Bcl2 (Figure 2A).

We further characterized the apoptotic stress in additional cell lines. The expression of circ-Foxo3 was significantly upregulated in bladder cancer cell lines T24, UM-UC-3 and J82 as well as the normal bladder epithelial cell line SV-HUC-1 upon treatment with doxorubicin (Figure 2B), cisplatin (Figure 2C), and H2O2 (Figure 2D).

To test the effect of circ-Foxo3 on apoptosis in vivo, we injected control (circControl-GFP) or circ-Foxo3 (circFoxo3-GFP) lentiviral vectors for somatic gene transfer in mice. We then treated mice with oral BBN for 17 weeks to induce bladder cancer. Somatic gene transfer of circ-Foxo3 significantly increased cell apoptosis in the bladder tissue of BBN mice as measured by TUNEL staining (Figure 3A). Likewise, Western blot showed that the expression the cleaved-caspase3 and the ratio of Bax to Bcl2 was increased in mice receiving circ-Foxo3 lentiviral injection (Figure 3B).

We then examined the effect of circ-Foxo3 overexpression on apoptosis in vitro. We transiently overexpressed circ-Foxo3 RNA in human bladder cancer cell lines with a circ-Foxo3 plasmid (Figure 4A). Overexpression of circ-Foxo3 promoted bladder cancer cell apoptosis, as measured by Western blot of apoptotic markers (Figure 4B) and flow cytometry (Figure 4C).

Furthermore, we examined the viability of bladder cancer cell lines. Overexpression of circ-Foxo3 significantly reduced bladder cancer cell viability as measured by the CCK-8 assay (Figure 4D).

miR-191-5p suppressed circ-Foxo3 expression in bladder cancer cells

Our preliminary analysis revealed a strong negative correlation between the expression of circ-Foxo3 and miR-191-5p RNA in 30 tumor biopsies from bladder cancer patients (Figure 5A). miR-191-5p is a novel microRNA that was implicated in a variety of solid tumors.18–24 The strong

Figure 1 circ-Foxo3 was downregulated in bladder cancer in vivo and in vitro. (A) Expression of circ-Foxo3 RNA in tumor tissues and adjacent normal bladder tissues taken from bladder cancer patients (n=30). (B) Expression of circ-Foxo3 RNA in tumor tissues and adjacent normal bladder tissues from mice treated with vehicle or BBN for 17 weeks (n=10). (C) Expression of circ-Foxo3 RNA in different bladder cell lines (n=5). Mean ± SD, *p<0.05.
The correlation between circ-Foxo3 and miR-191-5p RNA expression prompted us to further examine their potential functional interaction in bladder cancer cells.

In an attempt to search for the direct interaction between circ-Foxo3 and miR-191-5p RNA, we identified a predicted miR-191-5p target site within the 3′-UTR of circ-Foxo3 RNA using the TargetScan algorithm (http://www.targetscan.org) (Figure 5B). To experimentally validate the interaction, we designed luciferase reporters coupled with either wild-type (WT) or mutant (MUT) 3′-UTR sequence of circ-Foxo3, and

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**Figure 2** circ-Foxo3 was upregulated in response to apoptotic stress. (A) Western blot of cleaved-caspase3 (c-caspase3), Bax, Bcl2 and GAPDH expression in J82 cells treated with vehicle (Ctrl), doxorubicin (Dox; 1 μg/mL), cisplatin (CP; 2 μg/mL), and H2O2 (H2O2; 0.4 mM). The band intensity relative to GAPDH, and the ratio of Bax/Bcl2 band intensity were shown on the right. (B–D) Expression of circ-Foxo3 RNA level in bladder cell lines treated with vehicle (B–D), doxorubicin (B), cisplatin (C), and H2O2 (D), respectively (n=5). Mean ± SD, *p<0.05.

**Figure 3** circ-Foxo3 promoted bladder tumor cell apoptosis in BBN mice. Male, 8-week-old C57BL/6 mice received control (circControl-GFP) and circ-Foxo3 (circFoxo3-GFP) somatic gene transfer by lentiviral injection, followed by exposure to oral vehicle or BBN treatment for 17 weeks. (A) The bladder tumor cell apoptosis was evaluated by the TUNEL assay (left) and quantified (right; n=10). (B) Western blot of cleaved-caspase3 (c-caspase3), Bax, Bcl2 and GAPDH expression in murine bladder tissues. The band intensity relative to GAPDH, and the ratio of Bax/Bcl2 band intensity were quantified (n=5). Mean ± SD, *p<0.05, **p<0.01.
co-transfected the reporters with a miR-191-5p mimic or inhibitor in bladder cancer cells. The expression of miR-191-5p was significantly enhanced by miR-191-5p mimic and reduced by its inhibitor (Figure 5C). In J82 bladder cancer cells, co-transfection of miR-191-5p significantly suppressed wild-type, but not mutant, circ-Foxo3 luciferase reporter activity (Figure 5D). These results suggested that the 3′-UTR of circ-Foxo3 was a direct binding target of miR-191-5p.

Similarly, ectopic overexpression of miR-191 significantly suppressed the expression of circ-Foxo3 in T24, UM-UC-3 and J82 cells, while silencing of miR-191 significantly enhanced expression of circ-Foxo3 (Figure 5E). These data
suggest that transcription of circ-Foxo3 RNA is suppressed by miR-191 in bladder cancer cells.

miR-191-5p suppressed apoptosis via inhibiting circ-Foxo3 in bladder cancer cells

To elucidate the functional relevance of the interaction between miR-191 and circ-Foxo3, we transfected bladder cancer cells with the miR-191-5p mimic, and treated cells with cisplatin to induce apoptosis. Western blot showed cisplatin-induced apoptosis was significantly attenuated by overexpression of miR-191 (Figure 6A). Notably, overexpression of circ-Foxo3 alone also increased apoptosis (Figure 6B). Co-transfection with miR-191 mimic, however, effectively suppressed apoptosis induced by circ-Foxo3 as indicated by Western blot (Figure 6B) and flow cytometry (Figure 6C). Consistent with the cell apoptosis measurements, CCK-8 assay showed that ectopic overexpression of circ-Foxo3 significantly reduced cell viability, which was partially rescued by concurrent transfection of miR-191 mimic in T24, UM-UC-3 and J82 cells, suggesting a pro-survival effect of miR-191 on bladder cancer cells that was partially dependent on targeting of circ-Foxo3 (Figure 6D).

Discussion

The primary finding of our study is that circ-Foxo3 regulated bladder cancer growth in vivo and in vitro. The expression of circ-Foxo3 was lower in bladder cancer tissues and bladder cancer cell lines, and was upregulated in response to apoptotic stress. Overexpression of circ-Foxo3 promoted bladder cancer apoptosis in vivo and in vitro, which was partially attributable to the direct targeting of circ-Foxo3 by miR-191.

Our study is the first to identify a critical role of a circRNA in bladder cancer growth. Several recent studies reported that circ-Foxo3 regulated progression and proliferation of breast cancer, lung cancer, and gastric cancer. Our current data showed circ-Foxo3 was also dysregulated in bladder cancer and that the dysregulation of circ-Foxo3 could potentially contribute to various solid tumors as a common pathogenic factor.

To date, the detailed mechanism by which circ-Foxo3 regulates tumor progression has not be well understood. Several prior reports suggest the mechanism appears to be multifold. circ-Foxo3 has been shown to increase cellular senescence, to arrest cancer cell cycle progression by binding to the cell cycle proteins CDK2 and p21, and to inhibit angiogenesis. Our data showed in bladder cancer tissue and...
Figure 6 miR-191-5p suppressed the pro-apoptotic effect of circ-Foxo3 in bladder cancer cells. (A) T24, UM-UC-3, and J82 bladder cancer cell lines were transfected with miR-191 mimic (10 nM) and treated with cisplatin (CP, 2 μg/mL). The expression of cleaved-caspase3 (c-caspase3) was examined by Western blot and quantified relative to GAPDH (n=5). (B–C) T24, UM-UC-3, and J82 bladder cancer cell lines were co-transfected with miR-191 mimic and a circ-Foxo3 over-expression plasmid (n=5). The expression of cleaved-caspase3 (c-caspase3) was examined by Western blot (B) and apoptosis was analyzed by flow cytometry (C). (D) Cell viability measured by the CCK-8 assay in T24, UM-UC-3, and J82 bladder cancer cell lines receiving miR-191 mimic or circ-Foxo3 transfection (n=5). Mean ± SD, *p<0.05, **p<0.01.
bladder cancer cell lines, one major effect of circ-Foxo3 was the induction of apoptosis, indicated by increased cleaved-caspase3 and Bax/Bcl2 ratio. Our results was consistent with the pro-apoptotic effect of circ-Foxo3 in breast carcinoma biopsies and in multiple cancer cell lines. Interestingly, in breast cancer cells, circ-Foxo3 also increased Foxo3 protein level and promoted p53 ubiquitination and subsequent degradation, suggesting an alternative pathway by which circ-Foxo3 induces cancer cell apoptosis.

The role of non-coding circRNAs as potential microRNA “sponges” have been recognized. Compared with most linear RNAs, circRNAs have no 3’ ends and are therefore more resistant to exonuclease degradation. In addition, circRNAs are characterized by high sponging capacity (sequence containing multiple microRNA binding sites) and relatively high expression level. All these characteristics indicate circRNAs may be more effective microRNA-sponges than the linear non-coding RNAs. The sponging effect of circRNAs appears to be microRNA- and tissue-specific: certain circRNAs only “sponge up” microRNAs of a particular family in particular tissues. However, the exact microRNA-sponging spectrum of circ-Foxo3 has been controversial. For instance, in non-small cell lung cancer, circ-Foxo3 sequesters miR-155; in breast cancer, circ-Foxo3 sequesters eight miRNAs. We found for the first time that in bladder cancer cells, there was a strong negative correlation between the expression of circ-Foxo3 and miR-191-5p. Our experiments confirmed the 3’-UTR of circ-Foxo3 was a direct binding site for miR-191, and the pro-apoptotic effect of circ-Foxo3 could be effectively blocked by miR-191 mimic. These results highlighted a critical role of the direct interaction between circ-Foxo3 and miR-191 in regulating bladder cancer apoptosis. Given that the aberrant expression of microRNAs in cancers are highly tissue-specific, this particular interaction between circ-Foxo3 and miR-191 may be indicative of a highly specific therapeutic target for bladder cancer.

Our study has several limitations. First, there may be alternative pathways by which circ-Foxo3 promotes cancer apoptosis, which were not examined in our study. In addition, our focus of bladder-cancer-related microRNA is limited to miR-191 and non-exhaustive. There could potentially be many other functionally important microRNAs that interact with circ-Foxo3 in bladder cancer cells.

**Conclusion**
circ-Foxo3 regulated bladder cancer growth in vivo and in vitro. Overexpression of circ-Foxo3 promoted bladder cancer apoptosis, which was partially attributable to the direct targeting of circ-Foxo3 by miR-191.

**Ethics approval and consent to participate**
The present study was approved by the Ethics Committee of The First Affiliated Hospital of Harbin Medical University. The research has been carried out in accordance with the World Medical Association Declaration of Helsinki. All patients and healthy volunteers provided written informed consent prior to their inclusion within the study.

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

**Abbreviations**
circRNA, circular RNA; 3’-UTR, 3’-untranslated region; ANOVA, analysis of variance; BBN, N-butyl-N-(4-hydroxybutyl)nitrosamine; PBS, phosphate-buffered saline; RT-qPCR, Reverse transcription-quantitative polymerase chain reaction; SD, standard deviation; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.

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**Disclosure**
The authors declare that they have no competing interests in this work.

**References**
1. Antoni S, Ferlay J, Soerjomataram I, Znaor A, Jemal A, Bray F. Bladder cancer incidence and mortality: a global overview and recent trends. *Eur Urol*. 2017;71(1):96–108. doi:10.1016/j.eururo.2016.06.010
2. Pal SK, Miller MJ, Agarwal N, et al. Clinical cancer advances 2019: annual report on progress against cancer from the American society of clinical oncology. *J Clin Oncol*. 2019;37(10):834–849. doi:10.1200/JCO.18.02037
3. Danan M, Schwartz S, Edelheit S, Sorek R. Transcriptome-wide discovery of circular RNAs in archaea. *Nucleic Acids Res*. 2012;40(7):3131–3142. doi:10.1093/nar/gkr1009
4. Pamudurti NR, Bartok O, Jens M, et al. Translation of CircRNAs. *Mol Cell*. 2017;66(1):9–21 e27. doi:10.1016/j.molcel.2017.02.021
5. Granados-Riveron JT, Aquino-Jarquin G. The complexity of the translation ability of circRNAs. *Biochim Biophys Acta*. 2016;1859(10):1245–1251. doi:10.1016/j.bbagen.2016.07.009
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6. Memczak S, Jens M, Elefnioti A, et al. Circular RNAs are a large class of animal RNAs with regulatory potency. Nature. 2013;495(7441):333–338. doi:10.1038/nature12198

7. Tang Z, Li X, Zhao J, et al. TRCirc: a resource for transcriptional regulation information of circRNAs. Brief Bioinform. 2018. doi:10.1093/bib/bby083

8. Wilusz JE, Sharp PA. Molecular biology: A circuitous route to noncoding RNA. Science. 2013;340(6131):440–441. doi:10.1126/science.1238522

9. Anderson MJ, Viars CS, Czekay S, Cavenee WK, Arden KC. Cloning and characterization of three human forkhead genes that comprise an FKHR-like gene subfamily. Genomics. 1998;47(2):187–199. doi:10.1006/geno.1997.5122

10. Du WW, Yang W, Liu E, Yang Z, Dhaliwal P, Yang BB. Foxo3 circular RNA retards cell cycle progression via forming ternary complexes with p21 and CDK2. Nucleic Acids Res. 2016;44(6):2846–2858. doi:10.1093/nar/gkw027

11. Lu WY. Roles of the circular RNA circFoxo3 in breast cancer progression. Cell Cycle. 2017;16(7):589–590. doi:10.1080/15384010.2017.1287895

12. Zhang Y, Zhao H, Zhang L. Identification of the tumorsuppressive function of circular RNA FOXO3 in nonsmall cell lung cancer through splicing miR155. Mol Med Rep. 2018;17(6):7692–7700. doi:10.3892/mmr.2018.8830

13. Du WW, Fang L, Yang W, et al. Induction of tumor apoptosis through a circular RNA enhancing Foxo3 activity. Cell Death Differ. 2017;24(2):357–370. doi:10.1038/cdd.2016.133

14. Du WW, Yang W, Chen Y, et al. Foxo3 circular RNA promotes cardiac senescence by modulating multiple factors associated with stress and senescence responses. Eur Heart J. 2017;38(18):1402–1412. doi:10.1093/eurheartj/ehw001

15. Bustin SA, Benes V, Garson JA, et al. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin Chem. 2009;55(4):611–622. doi:10.1373/clinchem.2008.112797

16. Wang L, Hou Y, Yin X, et al. Rottlerin inhibits cell growth and invasion via down-regulation of Cdc20 in glioma cells. Oncotarget. 2016;7(43):69770–69782. doi:10.18632/oncotarget.11974

17. Gavrichy Y, Sherman Y, Ben-Sasson SA. Identification of programmed cell death in situ via specific labeling of nuclear DNA fragmentation. J Cell Biol. 1992;119(3):493–501. doi:10.1083/jcb.119.3.493

18. Nagpal N, Ahmad HM, Chameettachal S, Sundar D, Ghosh S, Kulshreshtha R. HIF-inducible miR-191 promotes migration in breast cancer through complex regulation of TGFbeta-signaling in hypoxic microenvironment. Sci Rep. 2015;5:9650. doi:10.1038/srep09650

19. Liu Z, Huang S. Inhibition of miR-191 contributes to radiation-resistance of two lung cancer cell lines by altering autophagy activity. Cancer Cell Int. 2015;15(1):16. doi:10.1186/s12935-015-0165-5

20. Song Z, Ren H, Gao S, Zhao X, Zhang H, Hao J. The clinical significance and regulation mechanism of hypoxia-inducible factor-1 and miR-191 expression in pancreatic cancer. Tumour Biol. 2014;35(11):11319–11328. doi:10.1007/s13277-014-2452-5

21. Di Leva G, Piovan C, Gasparini P, et al. Estrogen mediated-activation of miR-191/425 cluster modulates tumorigenicity of breast cancer cells depending on estrogen receptor status. PLoS Genet. 2013;9(3):e1003311. doi:10.1371/journal.pgen.1003311

22. Patnaik SK, Kannisto E, Yendamuri S, Blagosklonny MV. Overexpression of microRNA miR-30a or miR-191 in A549 lung cancer or BEAS-2B normal lung cell lines does not alter phenotype. PLoS One. 2010;5(2):e9219. doi:10.1371/journal.pone.0009219

23. Shen J, DiCioccio R, Odunsi K, Lele SB, Zhao H. Novel genetic variants in miR-191 gene and familial ovarian cancer. BMC Cancer. 2010;10:47. doi:10.1186/1471-2407-10-663

24. Chen P, Pan X, Zhao L, et al. MicroRNA-191-5p exerts a tumor suppressive role in renal cell carcinoma. Exp Ther Med. 2018;15(2):1686–1693. doi:10.3892/etm.2017.5581

25. Wang Y, Du WW, Li X, Yee AJ, Yang BB. Foxo3 activity promoted by non-coding effects of circular RNA and Foxo3 pseudogene in the inhibition of tumor growth and angiogenesis. Oncogene. 2016;35(30):3919–3931. doi:10.1038/onc.2015.460

26. Lu J, Zhang PY, Li P, et al. Circular RNA hsa_circ_0001368 suppresses the progression of gastric cancer by regulating miR-6506-5p/FOXO3 axis. Biochem Biophys Res Commun. 2019;512(1):29–33. doi:10.1016/j.bbrc.2019.02.111

27. Su H, Tao T, Yang Z, et al. Circular RNA CTFRC acts as the sponge of MicroRNA-107 to promote bladder carcinoma progression. Mol Cancer. 2019;18(1):27. doi:10.1186/s12943-019-1010-6

28. Han D, Li J, Wang H, et al. Circular RNA circMTO1 acts as the sponge of microRNA-9 to suppress hepatocellular carcinoma progression. Hepatology. 2017;66(4):1151–1164. doi:10.1002/hep.29270

29. Rao J, Cheng X, Zhu H, Wang L, Liu L. Circular RNA-0007874 (circMTO1) reverses chemoresistance to temozolomide by acting as a sponge of microRNA-630 in glioblastoma. Cell Biol Int. 2018. doi:10.1002/cbin.11080

30. Wang J, Zhao SY, Ouyang SS, Huang ZK, Luo Q, Liao L. [Circular RNA circHIPK3 acts as the sponge of microRNA-124 to promote human oral squamous cell carcinoma cells proliferation]. Zhonghua Kou Qiang Yi Xue Za Zhi. 2018;53(8):546–551. doi:10.3760/cma.j.issn.1000-0998.2018.08.009

31. Zhang X, Wang S, Wang H, et al. Circular RNA circNRIP1 acts as a microRNA-149-5p sponge to promote gastric cancer progression via the AKT1/mTOR pathway. Mol Cancer. 2019;18(1):20. doi:10.1186/s12943-019-1010-6

32. Ebert MS, Sharp PA. MicroRNA sponges: progress and possibilities. RNA. 2010;16(11):2043–2050. doi:10.1261/rna.2414110

33. Yoshino H, Seki N, Itsako T, Chiyomaru T, Nakagawa M, Enoki H. Aberrant expression of microRNAs in bladder cancer. Nat Rev Urol. 2013;10(7):396–404. doi:10.1038/nruro.2013.113

34. Amuran GG, Eyuboglu IP, Tinay I, Akkiprik M. New insights in bladder cancer diagnosis: urinary miRNAs and proteins. Med Sci. 2018;6(4):113.