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Circular RNA Myosin Light Chain Kinase (MYLK) Promotes Prostate Cancer Progression through Modulating Mir-29a Expression

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Statistical Analysis C
Data Interpretation D
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Background: In developed countries, prostate cancer (PCa) is a frequently diagnosed cancer with the second highest fatality rate. Circular RNAs (circRNAs) are a class of endogenous non-coding RNAs (ncRNAs) stably expressed in cells and involved in a series of carcinomas. However, few research studies have reported on the role of circRNAs in PCa.

Material/Methods: We used qRT-PCR to detect the expression of circMYLK (circRNA ID: hsa_circ_0141940) and miR-29a in PCa tissues and cell lines. MTT, colony formation, and TUNEL assays were performed to analysis the cell viability of PCa cells. Transwell and wound scratch assays were performed to investigate the cell invasion and migration of PCa cells.

Results: In the present study, we confirmed that circMYLK expression level was significantly higher in PCa samples and PCa cells than in normal tissues and normal prostatic cells. The upregulated circRNA-MYLK promoted PCa cells proliferation, invasion, and migration; however, si-circRNA-MYLK significantly accelerated the PCa cell apoptosis. We also observed that the aforementioned function of circRNA-MYLK on PCa cells was affected through targeting miR-29a.

Conclusions: We confirmed circRNA-MYLK was an oncogene in PCa and revealed a novel mechanism underlying circRNA-MYLK in PC progression.

MeSH Keywords: **MicroRNAs • Prostatic Neoplasms • RNA, Untranslated**

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Background

Prostate cancer (PCa) is considered one of the most life-threatening malignancies, with the highest percentage of localized tumor diagnosed in males [1,2]. In recent years, the molecular mechanisms, especially the genetic changes in the process of PCa, have been studied extensively. A series of non-coding RNAs, such as miRNAs and lncRNAs, were considered to take part in the PCa carcinogenesis. For example, miR-135a and miR-27a were identified as tumor suppressors that mediate the development and progression of PCa, while mir-301a and mir-301b promoted radioresistance of PCa [3–5]. LncRNA HCG11, lncRNA LINC01138, and SUZ12P1 were considered to play important roles in regulating PCa carcinogenesis through promoting proliferation and inhibiting apoptosis of PC cells [6,7]. In addition, many biomarkers including miRNAs have been investigated as diagnostic value in prostate cancer [8–10]. However, few reports have described the role of circular RNAs (circRNAs) in PCa.

The circRNAs are a class of RNAs that stably exist in cells, and characteristically lack 5'-3' ends and poly A tail [11]. In the past few years, the characteristics of circRNAs have garnered much scientific attention. An increasing number of research studies have confirmed that circRNAs take part in the development and progression of a variety of diseases such as diabetes, heart failure, osteoarthritis, neurological disorders, and especially carcinomas [12–16].

It has been widely identified that circRNAs can play a crucial role in regulating gene expressions by acting as microRNA (miRNA) sponges, RNA-binding protein (RBP) sequestering agents, and transcription regulators [17–20]. And the circRNA-miRNA-mRNA axis is considered to play an important role in cancer-related pathways. In addition, recent studies have suggested that enhanced expression of miR-29a inhibited the process of PCa [21,22].

In our study, we identified that circRNA-MYLK (circRNA ID: hsa_circ_0141940) expression was significantly upregulated in PCa tissues and PCa cells. The upregulated circRNA-MYLK enhanced PC cells proliferation, colony formation, invasive, migration, and the ability of wound healing; whereas si-circRNA-MYLK remarkably inhibited the aforementioned viabilities of PCa cells. On the contrary, upregulation of circRNA-MYLK remarkably decreased the apoptotic of PCa cells; whereas knockdown of circRNA-MYLK increased the apoptotic of PCa cells.

Moreover, we observed that the aforementioned function of circRNA-MYLK on PCa cells was affected through targeting miR-29a. Our data revealed a new mechanism underlying circRNA-MYLK in the progression of PCa and provided strong evidence for circRNA-MYLK as a tool to use to diagnose and treat PCa.

Material and Methods

PCa tissues and cell culture

Seventeen paired PCa and matched non-tumor normal tissues were collected from XiangYa Hospital, Central South University. This study was approved by the ethics committee of XiangYa Hospital, Central South University and written informed consent was obtained from all patients. The four PCa cell lines (DU145, LNCaP, PC-3, and PC-3MIE8 cells) and normal prostatic cell line (WPMY-1 cell) were maintained in RPMI 1640 medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal calf serum (Sigma Chemical Co., St. Louis, MO, USA) and 1% penicillin-streptomycin G (Invitrogen Life Technologies, Carlsbad, CA, USA). All cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2.

RNA transfection

For RNA transfection, 2×10^5 PC-3 or PC-3M IE8 cells were cultured in six-well plates with antibiotic-free complete medium. Then the PC-3 cells were transfected with circMYLK or mock vector, and PC-3M IE8 cells were transfected with si-circMYLK or mock vector. Transfection was performed by using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Then circMYLK and si-circMYLK sequences were synthesized by GenePharma (Shanghai, China). For miRNA transfection, the miR-29a mimics and miRNA controls (miR-NC) were purchased from Shanghai GeneChem Co., Ltd. (Shanghai, China). PC-3 cells were seeded in six-well plates (1×10^5 cells/well) and were transfected with miR-29a mimics and miR-NC using Lipofectamine 3000 reagent (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's protocol. After 48 hours, the cells were harvested for further experiments.

RNA extraction and quantitative RT-PCR (qRT-PCR)

RNA was extracted from all tissues and cells using TRIzol reagent (Life Technologies, CA, USA) and reversely transcribed to cDNA by real-time (RT)-PCR assay. The manual of NovoScript® Reverse Transcriptase was used for reverse transcription and the SYBR-Green PCR Master Mix kit (Takara) was used for quantitative RT-PCR. The reactions were performed on ABI 7500 system (Applied Biosystems, Carlsbad, CA, USA). The data were counted using $2^{-\Delta\Delta Ct}$ calculation [23]. The primer sequences used were miR-29a:
5'-TGCGCTAGCACCATCTGAAAT-3' (forward) and
5'-CAGTGCAGGGTCCGAGGT-3' (reverse);
U6: 5'-CTCGCTTCGGCAGCACA-3' (forward) and
5'-AACGCTTCACGAATTTGCGT-3' (reverse);
circMYLK: 5'-CAGTGCATGCTGTTTGTTCA-3' (forward) and
5'-tcggagccttgacttccag-3' (reverse);

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GAPDH: 5'-TATGATGATATCAAGAGGGTAGT-3' (forward) and 5'-TGTATCCAAACTCATTGTCATAC-3' (reverse).

Cell proliferation assay

Cell proliferation was assessed by Cell Counting Kit-8 assay. Transfected cells were seeded into 96-well plates (5×10^3 cells/well) with complete medium for different point-in-time, respectively. After 10 µL CCK-8 was added to the wells and incubated at 37°C for two hours, the absorbance was observed at 450 nm with Microplate Reader ELx808.

Apoptosis assay

After transfection for 48 hours, the cells were removed and washed with PBS. Then the cells were fixed with formaldehyde and permeabilized with ethanol. The level of apoptosis was determined with the TUNEL staining Kit in accordance with the manufacturer's instructions. The positive cells were counted in four fields.

Colony formation assay

For the colony formation assay, the same densities of different vector transfected PC-3 and PC-3M IE8 cells were seeded in six-well plates. After seven days, the cells were stained with crystal violet. The formation ability was evaluated by the average mount.

Migration and invasion assays

In total, 5×10^4 PC-3 cells or PC-3M IE8 cells were seeded in the upper compartment of Transwell chambers with serum-free media. In the lower compartment of Transwell chambers, the complete medium was supplemented with 10% FBS. After incubation for 24 hours, the migratory and invasive cells on the bottom surface of the filters were fixed by 4% paraformaldehyde, and stained by 0.1% crystal violet solution. Four random fields were counted for each group. The experiments were performed in triplicate.

Wound healing assay

The different vectors transfected PC-3 cells or PC-3M IE8 cells were seeded in six-well plates. Then the disinfected Eppendorf tip was used to create small linear wounds. After washing the cell debris with FBS-free medium three times, the wound areas were photographed under a microscope. The results were analyzed by image analysis software.

Statistical analysis

The data were analyzed by the Student's *t*-test and analysis of variance (ANOVA) using SPSS 15.0 software (SPSS, Chicago, IL, USA). Pearson's correlation coefficient was used to calculate the correlation between miR-29a and circRNA-MYLK. Each experiment was repeated at least three times. All results were summarized and presented as means ±SD. A value of $p<0.05$. was considered statistically significant.

Results

CircRNA-MYLK expression was upregulated in PCa

The qRT-PCR assay was used to detect circRNA-MYLK expression levels in tumor samples and PCa cell lines. We randomly selected 17 paired PCa tissues and adjacent normal tissues, and detected the mRNA expression of circRNA-MYLK. We found that the relative expression of circRNA-MYLK was significantly upregulated in tumor samples compared with the match normal prostate tissues (Figure 1A, 1B). mRNA expression levels of circRNA-MYLK in noncancerous prostatic cell line WPMY-1 and four PCa cell lines including DU145, LNCaP, PC-3, and PC-3MIE8 were detected by RT-PCR. The result showed the expression of circRNA-MYLK was significantly higher in four PCa cell lines compared with the WPMY-1 cells (Figure 1C). In addition, we found that the increase of circRNA-MYLK levels was minimal in PC3 cells and maximal in PC-3MIE8 cells. Consequently, we chose these two cell lines for further study. Relative circRNA-MYLK expression was significantly upregulated in the PC-3 cells transfected with circRNA-MYLK overexpression vector. However, MYLK expression was remarkably downregulated in the PC-3MIE8 cells transfected with si-circRNA-MYLK vector (Figure 1D).

The effects of circRNA-MYLK on PCa cell proliferation and apoptosis

We further studied the effects of circRNA-MYLK on PCa cell proliferation and apoptosis. The transfection efficiency was determined by qRT-PCR. The result showed that circRNA-MYLK overexpression significantly enhanced PCa cells proliferation and colony formation, whereas si-circRNA-MYLK remarkably inhibited cell viability (Figure 2A–2D). On the contrary, upregulation of circRNA-MYLK significantly decreased the apoptotic cells number, whereas knockdown of circRNA-MYLK remarkably increased the apoptotic cells number (Figure 2E).

The effects of circRNA-MYLK on PCa cell invasion and migration

Then we further researched the effects of circRNA-MYLK on PCa cell invasion and migration. We found that upregulation of circRNA-MYLK noticeably increased the invasive and migratory number of PC cells, however, downregulation of circRNA-MYLK significantly decreased the invasive and migratory

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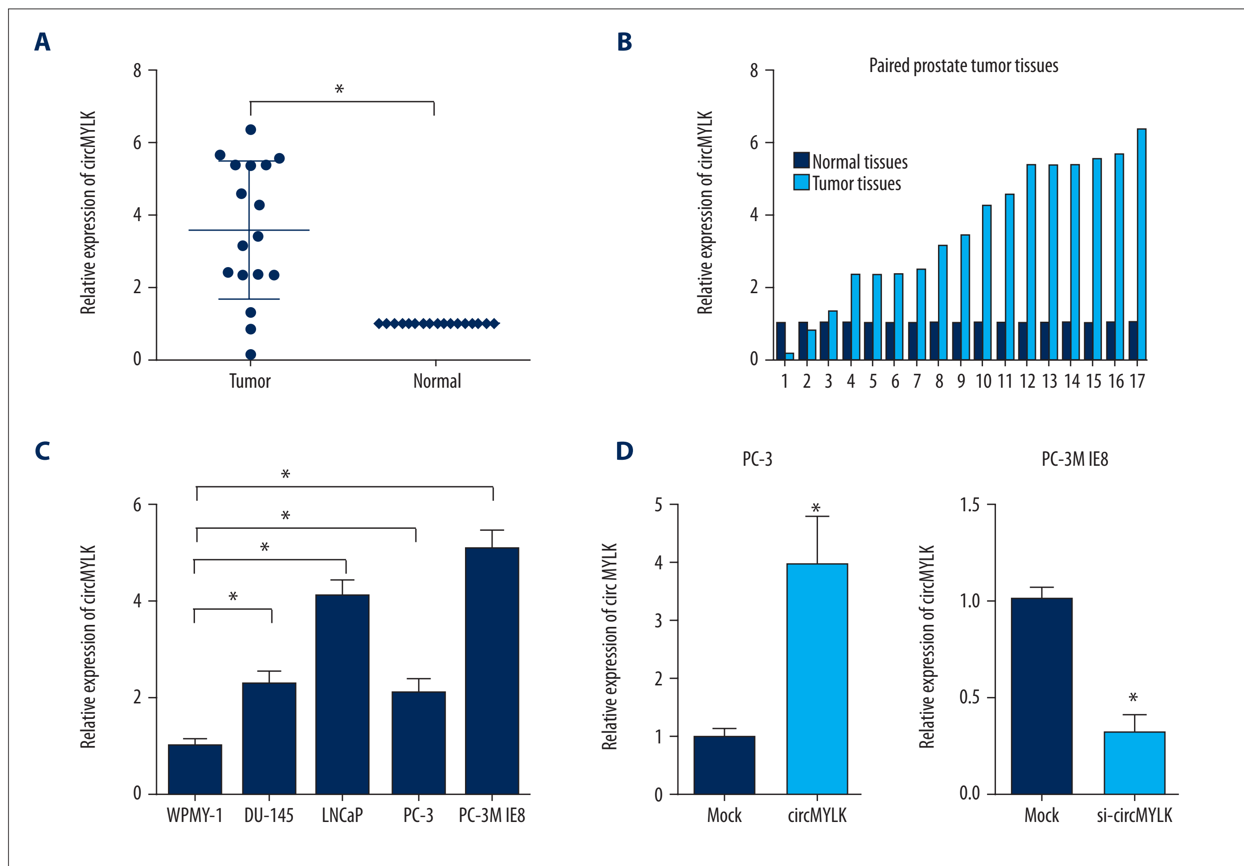

Figure 1. CircRNA-MYLK expression was upregulated in prostate cancer. (**A, B**) qRT-PCR was performed to evaluate the expression levels of circRNA-MYLK from 17 prostate cancers tumor tissues compared with their corresponding adjacent prostate normal tissues. (**C**) Relative expression level of circRNA-MYLK was measured in normal prostate epithelial cell line WPMY-1 and four prostate cancer cell lines, DU145, LNCaP, PC-3, and PC-3MIE8 by qRT-PCR. (**D, E**) Relative circRNA-MYLK expression was measured in circRNA-MYLK or mock vector transfected PC-3 cells and si-circRNA-MYLK or mock vector transfected PC-3MIE8 cells by qRT-PCR. Actin was used as control. The error bars indicate the mean ±SD. Experiments were performed in triplicate; * p<0.05 compared with the control group.

number of PCa cells (Figures 3A–5D). Moreover, overexpression of circRNA-MYLK remarkably enhanced wound healing ability of PC cells, whereas si-circRNA-MYLK noticeably inhibited the PC cell motility (Figure 5E, 5F).

CircRNA-MYLK promoted PCa cells proliferation and invasion through targeting miR-29a

According to miRBase prediction, circRNA-MYLK possessed a complementary sequence to miR-29a seed region (Figure. 4A up-panel). Our data revealed that miR-29a expression was significantly decreased in PCa cells transfected with circRNA-MYLK overexpression vector, whereas si-circRNA-MYLK played an opposite role in PCa cells (Figure 4A, 4B). We used miR-29a mimics to analyze if circRNA-MYLK affected the proliferation, invasion and migration of PC cells through inhibiting miR-29a. PC-3 cells were transfected with circRNA-MYLK overexpression vector and miR-29a mimics or miR-NC, and we found that miR-29a mimics reversed the promotive effect of circRNA-MYLK mediated PC-3 cell proliferation, invasion, and migration (Figure 4C–4E). Consequently, we predict circRNA-MYLK promoted PCa cell proliferation, invasion and migration by downregulating the expression of miR-29a.

MiR-29a expression is downregulated in PCa

The qRT-PCR assay revealed that the relative expression levels of miR-29a in noncancerous prostatic cell line (WPMY-1 cells) was significantly higher compared to the four PCa cell lines (DU145, LNCaP, PC-3, and PC-3MIE8 cells) (Figure 5A). Moreover, miR-29a expression was significantly lower in tumor samples compared with the match normal prostate tissues (Figure 5B, 5C). What's more, we identified that miR-29a expression was negatively correlated with the expression level of circRNA-MYLK (Figure 5D). The relative fold change in the expression of circRNA-MYLK and miR-29a among the 17

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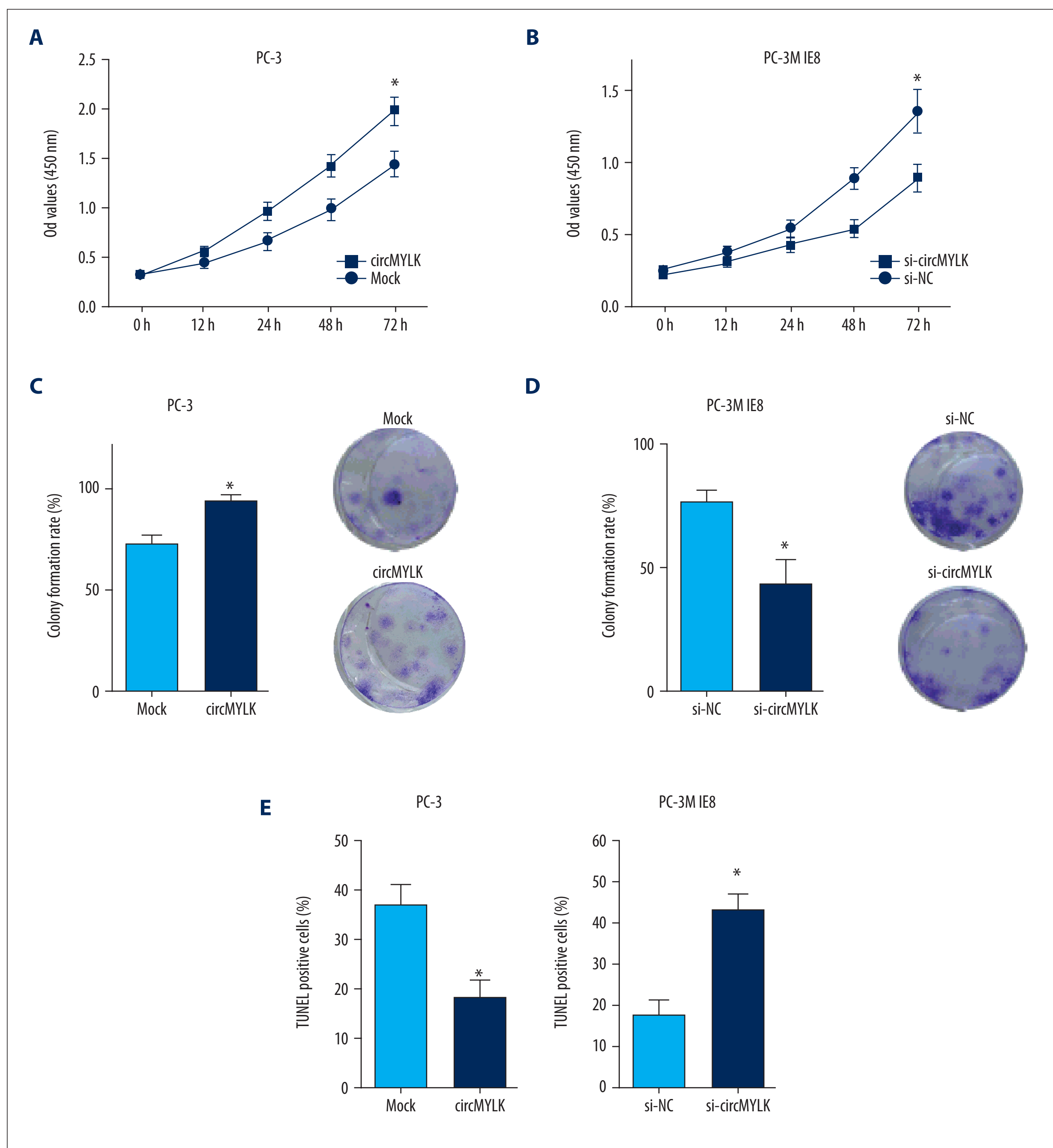

Figure 2. The effects of circRNA-MYLK on prostate cancer cell proliferation and apoptosis. (**A**) The growth curves were measured by CCK-8 assays. The growth curves of PC-3 cells transfected with circRNA-MYLK or mock vector. (**B**) The growth curves of PC-3MIE8 cells transfected with si-circRNA-MYLK or mock vector. (**C, D**) Colony formation assays to test the proliferation of circRNA-MYLK or mock vector transfected PC-3 cells and si-circRNA-MYLK or mock vector transfected PC-3MIE8 cells. (**E**) The percent of FITC-labeled TUNEL positive circRNA-MYLK or mock vector transfected PC-3 cells and si-circRNA-MYLK or mock vector transfected PC-3MIE8 cells. The error bars indicate the mean ±SD. Experiments were performed in triplicate; * $p<0.05$ compared with the control group.

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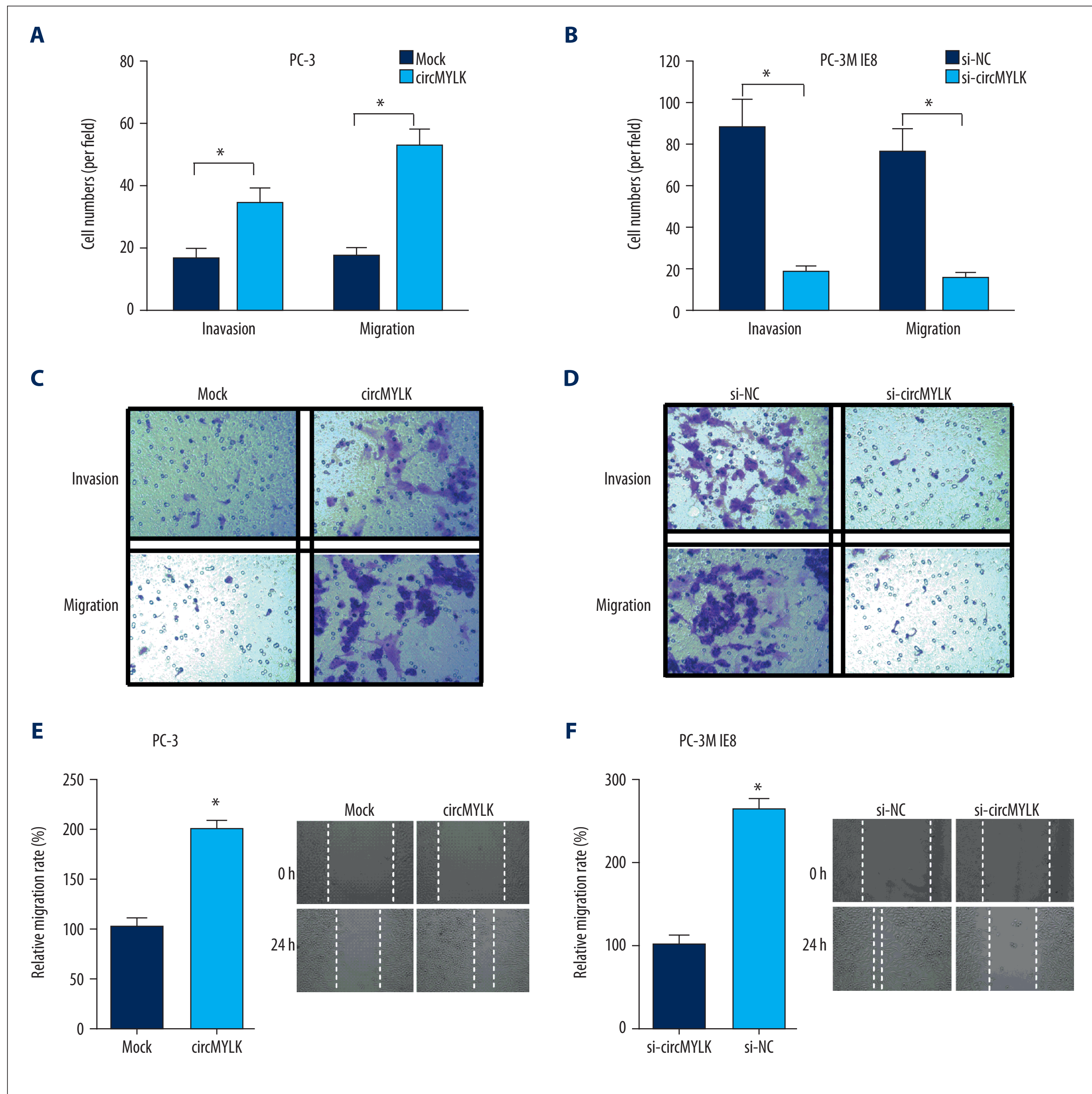

Figure 3. The effects of circRNA-MYLK on prostate cancer cell invasion and migration. (**A, C**) Cell invasion and migration assays were performed using Transwell chamber. The invasion and migration of PC-3 cells after transfection with circRNA-MYLK or mock vector. (**B, D**) The invasion and migration of PC-3MIE8 cells after transfection with circRNA-MYLK or mock vector. (**E**) The relative migration rate of cells was examined by wound healing assay. The relative migration rate of PC-3 cells after transfection with circRNA-MYLK or mock vector. (**F**) The relative migration rate of PC-3MIE8 cells after transfection with circRNA-MYLK or mock vector. * $p<0.05$ compared with the control group.

paired PCa tissue and adjacent normal tissues were examined and compared by qRT-PCR. The result suggested that the relative fold change of circRNA-MYLK in PC tissues/normal tissues was almost greater than one, however, the relative fold change of miR-29a in PC tissues/normal tissues was almost smaller than one (Figure 5E). In this study, we confirmed that in prostate normal low-expression of circRNA-MYLK suppressed PC cell growth, invasion, and migration by upregulated miR-29a. On the contrary, in prostate tumor high-expression of circRNA-MYLK promoted PC cell growth, invasion, and migration by downregulated miR-29a (Figure 5E). In conclusion, we predict circRNA-MYLK promoted PC cell proliferation, colony formation, invasion, and migration by downregulating the expression of miR-29a.

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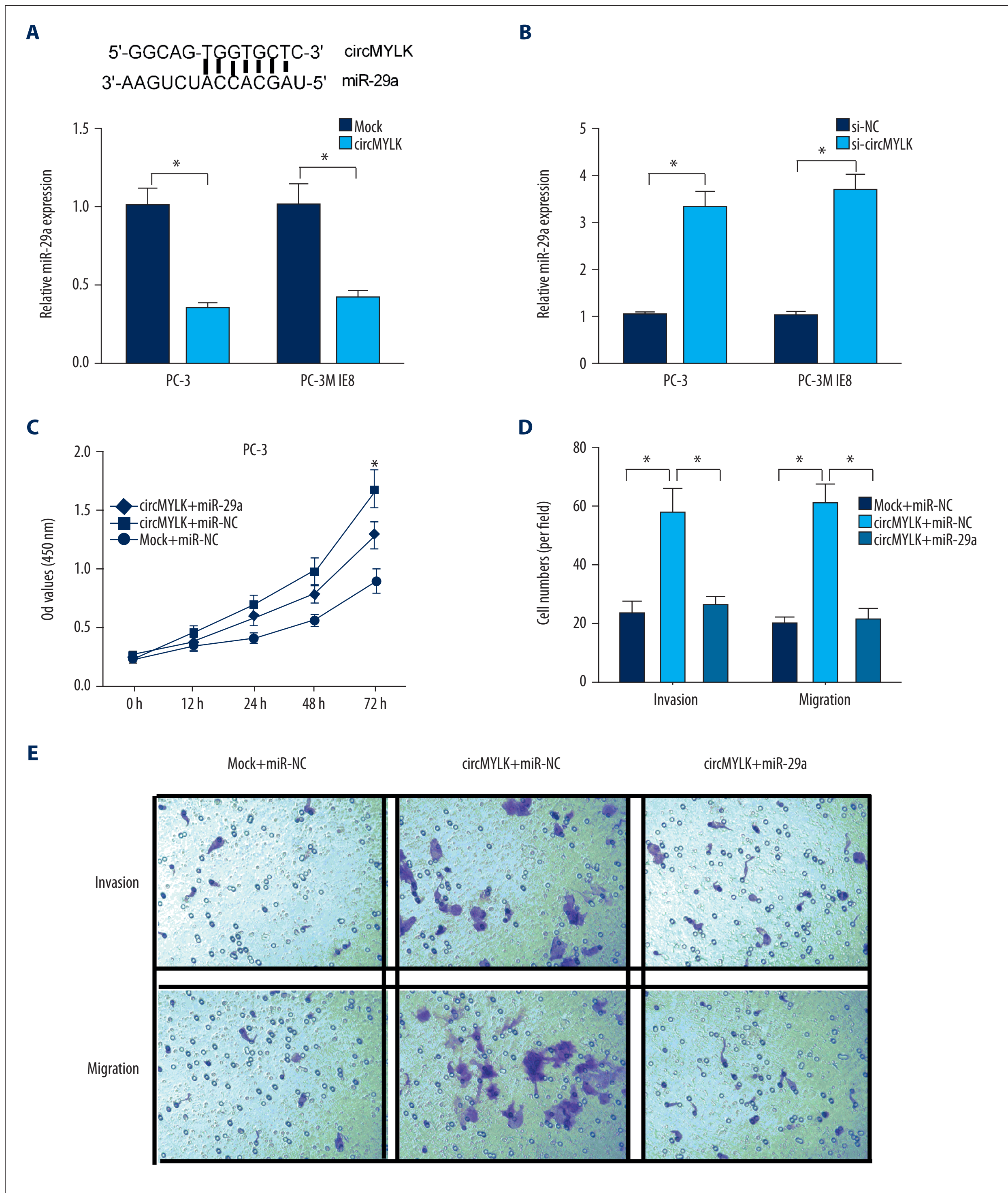

Figure 4. CircRNA-MYLK promoted PC cells proliferation and invasion through targeting miR-29a. (**A**) Schematic of putative binding site between circRNA-MYLK and miR-29a predicted by miRBase (**up-panel**). The relative miR-29a expression was analyzed using qRT-PCR in PC-3 cells and PC-3MIE8 cells transfected with circRNA-MYLK or mock vector. (**B**) The relative miR-29a expressions in PC-3 cells and PC-3MIE8 cells transfected with si-circRNA-MYLK or mock vector. (**C**) The growth curves of PC-3 cells were measured after transfection with circRNA-MYLK with/without miR-29a overexpression plasmid using CCK-8 assays. (**D, F**) Cell invasion and migration assays were performed in PC-3 cells transfected with circRNA-MYLK with/without miR-29a overexpression plasmid using Transwell chamber, respectively. * $p<0.05$ compared with the miR-24-3p group.

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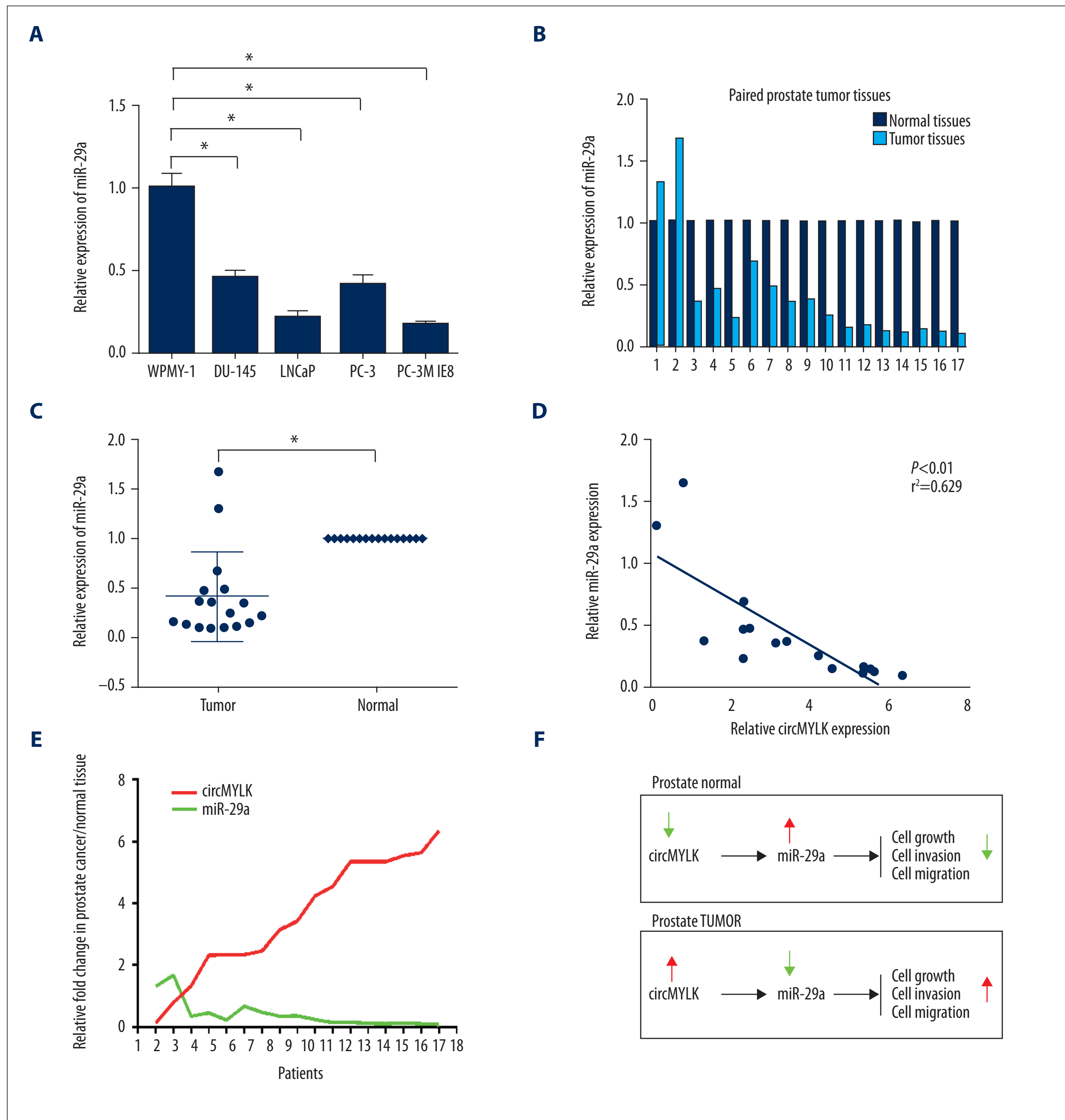

Figure 5. MiR-29a expression is downregulated in prostate cancer. (**A**) The relative expression level of miR-29a was measured in normal prostate epithelial cell line WPMY-1 and four prostate cancer cell lines, DU145, LNCaP, PC-3, and PC-3MIE8 by qRT-PCR. (**B, C**) qRT-PCR was performed to evaluate the expression levels of miR-29a from 17 prostate cancers tumor tissues compared with their corresponding adjacent prostate normal tissues. (**D**) The negative correlation between miR-29a and circRNA-MYLK in clinical samples was determined by Pearson's correlation coefficient (R^2=0.629, *p*<0.01). (**E**) Data represents the relative fold change of circRNA-MYLK and miR-29a mRNA expression in prostate cancer tissues/normal tissues. (**F**) Schematic representation of the possible pathogenetic mechanism of prostate cancer. * *p*<0.05 compared with the control group.

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Discussion

In the past few years, there has been an increasing number of research studies that have focused on the role of circRNAs in carcinomas. In the present study, we found that circRNA-MYLK was significantly upregulated in human PCa tissues and PCa cells. Functionally, our result revealed that the upregulation level of circRNA-MYLK could enhance PCa cells proliferation, colony formation, invasion, migration, and the ability of wound healing; whereas it inhibited the apoptosis *in vitro* studies. However, knockdown of the circRNA-MYLK by siRNA exerted a contrary role. Moreover, we confirmed that the promotive function of circRNA-MYLK on PCa cells was affected through downregulating the miR-29a expression. Thus, our results suggested that circRNA-MYLK played an important role in the development and progression of PCa.

Recently, mounting evidence has demonstrated that circRNAs play important roles in cancer cell proliferation, apoptosis, invasion, and migration, including gastric cancer, colon cancer, human hepatocellular carcinoma, and PCa. Chen et al. suggested that circPVT1 acted as a carcinogenic gene in gastric cancer through promoting gastric cancer cells proliferation [24]. Hsiao et al. confirmed that circCCDC66 promoted colon cancer cell proliferation, invasion, and migration and acted as an oncogene [25]. Yao et al. considered that circZKSCAN1 repressed cell proliferation, promoted apoptosis, and acted as a tumor suppressor gene in human hepatocellular carcinoma [26]. Kong et al. found that circ-SMARCA5 promoted cell cycle, inhibited cell apoptosis, and acted as an oncogene in PCa [27].

Reports have shown that the circRNA-miRNA-mRNA axis plays a crucial role in cancer-related pathways. For instance, Chen et al. considered that CircRNA_100290 acted as a carcinogenic gene in human oral squamous cell carcinoma (OSCC) by regulating the activity of miR-29b [28]. Zhong et al. confirmed that circRNA TCF25 bound to miR-103a-3p/miR-107 and promoted expression of targets genes related to cell proliferation, migration, and invasion in bladder carcinoma [29]. Wan et al. found that cir-ITCH inhibited lung cancer cell proliferation and function as a tumor suppressor gene by regulating the activities of miR-7 and miR-214 [30]. Wang et al. identified that circHIAT1 could inhibit clear cell renal cell carcinoma cell migration and invasion through binding to miR-195-5p/29a-3p/29c-3p to upregulate CDC42 expression [31].

Currently, miR-29a has been shown to be involved in different kinds of cancers, such as chronic lymphocytic leukemia [32], acute myeloid leukemia [33], and some solid tumors [22,34]. And there are a lot of reports about the function of miR-29a on prostate cancer. Nishikawa et al. showed that miR-29s functioned as tumor suppressors in PCa and inhibited PCa cells migration and invasion through regulation of LAMC1 [35]. Ahmed et al. demonstrated that miR-29a acted as a tumor suppressor gene in metastatic prostate cancer by regulating the TRAF4 expression [21]. In the present study, our results showed that miR-29a was downregulated in PCa cells and tumor samples compared with the match normal prostate tissues. In addition, we found that miR-29a expression was negatively correlated with the expression level of circRNA-MYLK. In conclusion, we suggested that circRNA-MYLK promoted PCa cell proliferation, invasion, and migration; whereas it inhibited PCa cell apoptosis by downregulating the expression of miR-29a.

Conclusions

In conclusion, we confirmed that circRNA-MYLK was an oncogene in PCa cells and revealed a novel mechanism underlying circRNA-MYLK in PC progression.

Conflict of interests

None.

References:

1. Ferlay J, Soerjomataram I, Dikshit R et al: Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer, 2015; 136: E359–86
2. Siegel RL, Miller KD, Jemal A: Cancer Statistics, 2017. Cancer J Clin, 2017; 67: 7–30
3. Wan X, Huang W, Yang S et al: Androgen-induced miR-27A acted as a tumor suppressor by targeting MAP2K4 and mediated prostate cancer progression. Int J Biochem Cell Biol, 2016; 79: 249–60
4. Wan X, Pu H, Huang W et al: Androgen-induced miR-135a acts as a tumor suppressor through downregulating RBAK and MMP11, and mediates resistance to androgen deprivation therapy. Oncotarget, 2016; 7: 51284–300
5. Wang W, Liu M, Guan Y, Wu Q: Hypoxia-responsive mir-301a and mir-301b promote radioresistance of prostate cancer cells via downregulating ndrg2. Med Sci Monit, 2016; 22: 2126–32
6. Wan X, Huang W, Yang S et al: Identification of androgen-responsive lncRNAs as diagnostic and prognostic markers for prostate cancer. Oncotarget, 2016; 7: 60503–18
7. Zhang Y, Zhang P, Wan X et al: Downregulation of long non-coding RNA HCG11 predicts a poor prognosis in prostate cancer. Biomed Pharmacother, 2016; 83: 936–41
8. Akbayir S, Muslu N, Erden S, Bozlu M: Diagnostic value of micrornas in prostate cancer patients with prostate specific antigen (psa) levels between 2, and 10 ng/ml. Turk J Urol, 2016; 42: 247–55
9. Lin YL, Li YL, Ma JG: Aberrant promoter methylation of protocadherin8 (pcdh8) in serum is a potential prognostic marker for low gleason score prostate cancer. Med Sci Monit, 2018; 24: 4895–900
10. Liang J, Zhang J, Ruan J et al: Cpne1 is a useful prognostic marker and is associated with tnf receptor-associated factor 2 (traf2) expression in prostate cancer. Med Sci Monit, 2018; 24: 5504–14
11. Chen LL, Yang L: Regulation of circRNA biogenesis. RNA Biol, 2015; 12: 381–88
12. Li J, Yang J, Zhou P et al: Circular RNAs in cancer: Novel insights into origins, properties, functions and implications. Am J Cancer Res, 2015; 5: 472–80

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13. Liu Q, Zhang X, Hu X et al: Circular RNA related to the chondrocyte ECM regulates MMP13 expression by functioning as a MiR-136 'Sponge' in human cartilage degradation. Sci Rep, 2016; 6: 22572
14. Wang K, Long B, Liu F et al: A circular RNA protects the heart from pathological hypertrophy and heart failure by targeting miR-223. Eur Heart J, 2016; 37: 2602–11
15. Xu H, Guo S, Li W, Yu P: The circular RNA Cdr1as, via miR-7 and its targets, regulates insulin transcription and secretion in islet cells. Sci Rep, 2015; 5: 12453
16. Zhao Y, Alexandrov PN, Jaber V, Lukiw WJ: Deficiency in the ubiquitin conjugating enzyme UBE2A in Alzheimer's disease (AD) is linked to deficits in a natural circular miRNA-7 sponge (circRNA; ciRS-7). Genes (Basel), 2016; 7(12): pii: E116
17. Du WW, Yang W, Liu E et al: Foxo3 circular RNA retards cell cycle progression via forming ternary complexes with p21 and CDK2. Nucleic Acids Res, 2016; 44: 2846–58
18. Hansen TB, Jensen TI, Clausen BH et al: Natural RNA circles function as efficient microRNA sponges. Nature, 2013; 495: 384–88
19. Holdt LM, Stahringer A, Sass K et al: Circular non-coding RNA ANRIL modulates ribosomal RNA maturation and atherosclerosis in humans. Nat Commun, 2016; 7: 12429
20. Li Z, Huang C, Bao C et al: Exon-intron circular RNAs regulate transcription in the nucleus. Nat Struct Mol Biol, 2015; 22: 256–64
21. Ahmed F, Shiraishi T, Vessella RL, Kulkarni P: Tumor necrosis factor receptor associated factor-4: An adapter protein overexpressed in metastatic prostate cancer is regulated by microRNA-29a. Oncol Rep, 2013; 30: 2963–68
22. Sengupta S, den Boon JA, Chen IH et al: MicroRNA 29c is down-regulated in nasopharyngeal carcinomas, up-regulating mRNAs encoding extracellular matrix proteins. Proc Natl Acad Sci USA, 2008; 105: 5874–78
23. 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: 402–8
24. Chen J, Li Y, Zheng Q et al: Circular RNA profile identifies circPVT1 as a proliferative factor and prognostic marker in gastric cancer. Cancer Lett, 2017; 388: 208–19
25. Hsiao KY, Lin YC, Gupta SK et al: Noncoding effects of circular RNA CCDC66 promote colon cancer growth and metastasis. Cancer Res, 2017; 77: 2339–50
26. Li Y, Zheng Q, Bao C et al: Circular RNA is enriched and stable in exosomes: A promising biomarker for cancer diagnosis. Cell Res, 2015; 25: 981–84
27. Kong Z, Wan X, Zhang Y et al: Androgen-responsive circular RNA circSMARCA5 is up-regulated and promotes cell proliferation in prostate cancer. Biochem Biophys Res Commun, 2017; 493: 1217–23
28. Chen L, Zhang S, Wu J et al: circRNA_100290 plays a role in oral cancer by functioning as a sponge of the miR-29 family. Oncogene, 2017; 36: 4551–61
29. Zhong Z, Lv M, Chen J: Screening differential circular RNA expression profiles reveals the regulatory role of circTCF25-miR-103a-3p/miR-107-CDK6 pathway in bladder carcinoma. Sci Rep, 2016; 6: 30919
30. Wan L, Zhang L, Fan K et al: Circular RNA-ITCH suppresses lung cancer proliferation via inhibiting the Wnt/beta-Catenin pathway. Biomed Res Int, 2016; 2016: 1579490
31. Wang K, Sun Y, Tao W et al: Androgen receptor (AR) promotes clear cell renal cell carcinoma (ccRCC) migration and invasion via altering the circHIAT1/miR-195-5p/29a-3p/29c-3p/CDC42 signals. Cancer Lett, 2017; 394: 1–12
32. Santanam U, Zanesi N, Efanov A et al: Chronic lymphocytic leukemia modeled in mouse by targeted miR-29 expression. Proc Natl Acad Sci USA, 2010; 107: 12210–15
33. Garzon R, Liu S, Fabbri M et al: MicroRNA-29b induces global DNA hypomethylation and tumor suppressor gene reexpression in acute myeloid leukemia by targeting directly DNMT3A and 3B and indirectly DNMT1. Blood, 2009; 113: 6411–18
34. Iorio MV, Ferracin M, Liu CG et al: MicroRNA gene expression deregulation in human breast cancer. Cancer Res, 2005; 65: 7065–70
35. Nishikawa R, Goto Y, Kojima S et al: Tumor-suppressive microRNA-29s inhibit cancer cell migration and invasion via targeting LAMC1 in prostate cancer. Int J Oncol, 2014; 45: 401–10

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