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Original Research

MiR-29 promotes ovarian carcinoma cell proliferation through the PTEN pathway

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Summary

The objective of this study was to investigate the role of miR-29 in ovarian carcinoma progression. Cell proliferation was measured with a cell counter. mRNA expression of miR-29, phosphatase and tensin homolog (PTEN), and proliferating cell nuclear antigen (PCNA) was measured by RT-PCR. Protein expression was detected by Western blot. The results demonstrated that miR-29 expression was upregulated in ovarian carcinoma and that miR-29 promoted cell proliferation. Consistently, miR-29 silencing decreased ovarian carcinoma cell proliferation. Mechanistically, we found PTEN expression was decreased following miR-29 overexpression and PTEN expression was increased following miR-29 silencing. Importantly, overexpression of PTEN was sufficient to inhibit ovarian carcinoma cell proliferation, demonstrating a key role for PTEN downstream of miR-29. Therefore, our study highlights the miR-29-PTEN pathway as a critical mediator of ovarian cancer cell proliferation, providing a new therapeutic target for patients with ovarian carcinoma.

Key words: Ovarian carcinoma; MiR-29; PTEN; Cell proliferation.

Introduction

Ovarian cancer is often asymptomatic or causes only non-specific symptoms at the earliest stages. Therefore, patients with ovarian cancer are often diagnosed at advanced stages of the disease [1]. Currently, cytoreductive surgery and combination chemotherapies are standard strategies for the treatment of ovarian carcinoma. Unfortunately, relapse following chemotherapy is common and remaining treatment options are mostly exploratory [2, 3]. Therefore, it is necessary to better understand the signaling pathways that drive aberrant growth and survival of ovarian cancer to develop new therapeutic targets.

miRNAs have roles in the regulation of cell proliferation, invasion, and apoptosis of variable cancers by targeting several different mRNAs [4]. The consequence of directly targeting different miRNAs include both promoting and inhibiting tumor development and is largely context-dependent [5, 6]. The miR-29 family consists of three mature members: miR-29a, miR-29b, and miR-29c. A number of laboratory studies have demonstrated that miR-29 plays a critical role in reducing the expression of tumor suppressors in glioblastoma, bladder cancer, breast cancer, and pancreatic cancer [7-9]. However, a role of miR-29 in ovarian cancer has not been reported.

Phosphatase and tensin homolog (PTEN) is a tumor suppressor that is often expressed at low levels in a variety of cancers [10]. PTEN has been demonstrated to be regulated by miR-21 and miR-32 in prostate and gastric cancer, respectively [11, 12]. However, the effects, if any, of PTEN on ovarian cancer have been scarcely investigated, nor has a mechanistic link between PTEN and miR-29 in ovarian cancer been previously reported.

In this study, the roles of miR-29 and PTEN in ovarian cancer were studied, as were the potential effects of these two genes on modulating cell proliferation and survival of ovarian cancer cells. Overall, the results in this study provide insights into the aberrant signaling and potential treatment strategies in ovarian cancer.

Materials and Methods

Fresh tumor tissues were collected according to the institutional IRB-approved protocol from 120 patients who underwent surgical resection in the Second Affiliated Hospital of Zhengzhou University. All patients had not been treated before surgery and the tissues were diagnosed as ovarian carcinoma by pathologists. Tumor tissues were placed in lysing matrix D tubes with RIPA buffer, protease inhibitor, and halt phosphatase inhibitors and incubated and processed in the FastPrep-24. The lysates were then aliquoted and stored at -80 °C. This study was approved by the Ethics Committee of Second Affiliated Hospital of Zhengzhou University and all patients signed the written informed consent prior to surgery.

The ovarian carcinoma cell line SK-OV-3 and the immortalized human ovarian epithelial cells SV40 were purchased from a cell bank. SK-OV-3 and SV40 cells were cultured in RPMI1640 medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin at 37 °C, 95% air/5% CO2. miR-29 mimic or inhibitor was transfected into cells using lipofectamine 3000 reagent for 48 hours, according to the manufacturer's protocol. A vector containing the cDNA of PTEN was utilized to express

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PTEN.

Cell number was measured with a cell counter. Every two days. RNA was extracted with 100 μl TRIzol reagent and complementary DNA was generated by adding 0.5 μg of the total RNA to SuperScript master mix and performing reverse transcription. Quantitative PCR was performed using SYBR Green Supermix. The comparative *C*t value method to quantify the expression of RNA species in different samples. The mRNA levels were normalized to that of a housekeeping gene, β-actin.

Protein was quantified with the BCA protein assay kit. Equal amounts of protein (50 μg) from each sample were subjected to electrophoresis on 4-12% (v/v) SDS-polyacrylamide gels. Protein was then transferred from the gels to PVDF membranes. Membranes were blocked with 5% non-fat milk at room temperature for one hour, incubated with the indicated primary antibodies, and subsequently incubated with the indicated secondary antibody for one hour. Finally, an enhanced chemiluminescence kit (ECL) was used to detect antibody binding.

Data was represented as the mean ± SD of triplicate samples and analyzed by one-way analysis of variance followed by Dunnett's *t*-test with SPSS 19.0 software. $p < 0.05$ was considered to indicate a statistically significant difference.

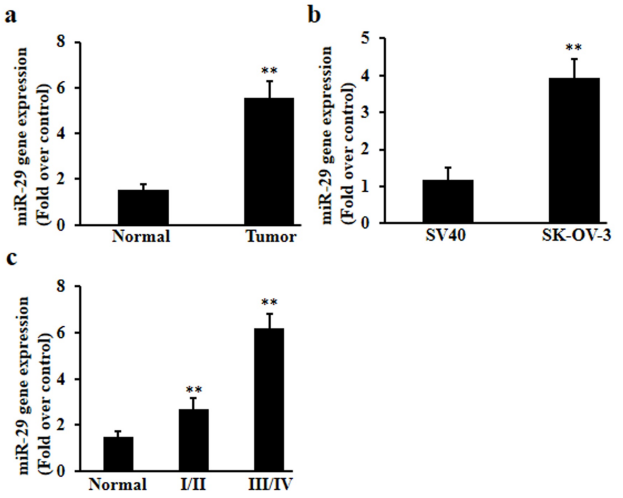

Figure 1. — Expression of miR-29 is increased in ovarian carcinoma. (A). Expression of miR-29 is increased in ovarian carcinoma tissues (n = 120). (B). Expression of miR-29 is increased in ovarian cancer cells. (C). bb Quantification of miR-29 expression at different stages of ovarian carcinoma. $^{**}p < 0.01$.

Results

miR-29 level was detected in ovarian carcinoma tumor tissues and cells using qRT-PCR. Compared to the normal tissues, miR-29 gene expression in ovarian carcinoma tissues were significantly increased. MiR-29 expression in ovarian carcinoma cells also increased. MiR-29 expression was detected in various stages of ovarian carcinoma and results showed a higher expression of miR-29 at a more advanced tumor stage (Figure 1), indicating that miR-29 might participate in ovarian carcinoma progression.

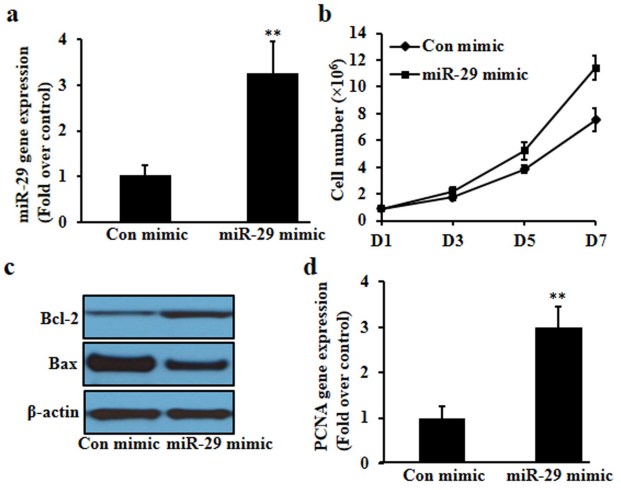

Figure 2. — MiR-29 overexpression promotes ovarian carcinoma cell proliferation. (A). Quantification of miR-29 mRNA levels in SK- OV-3 cells treated with the miR-29 mimic. (B, C). Overexpression of miR-29 promoted cell proliferation and inhibition of apoptotic proteins; (D). Overexpression of miR-29 increased PCNA expression. $^{**}p < 0.01$ miR29 overexpressed cells compared to control cells.

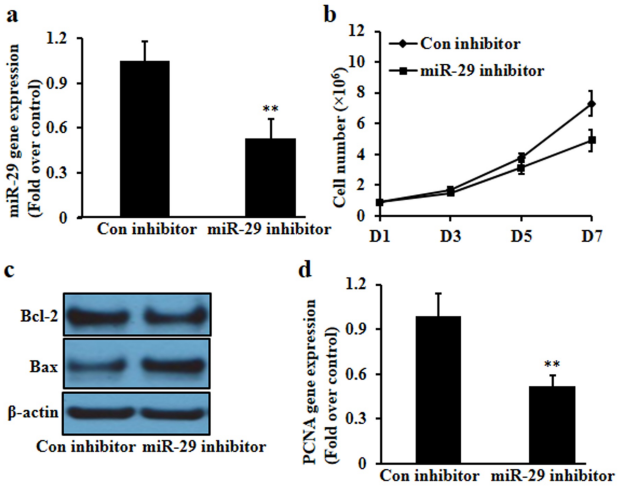

Figure 3. — Silencing of miR-29 inhibits ovarian carcinoma cell proliferation. A. Quantification of miR-29 mRNA level in SK-OV- 3 cells treated with miR-29 inhibitor. B, C. Silencing of miR-29 inhibits cell proliferation and increases the expression of apoptotic proteins. D. Silencing of miR-29 decreases PCNA expression. $^{**}p < 0.01$ comparing miR-29 inhibitor to control.

We next evaluated whether miR-29 was involved in ovarian cancer cell proliferation and survival. To do so,

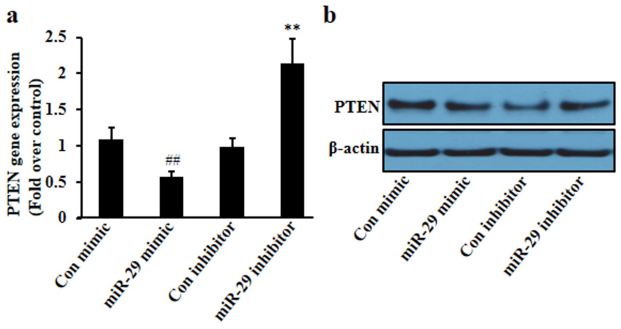

Figure 4. — PTEN is a target of miR-29 in ovarian cancer cells. mRNA and protein expression of PTEN in SK-OV-3 cells after overexpression or silencing of miR-29. $##p < 0.01$ comparing miR-29 inhibitor to mimic; $**p < 0.01$ comparing miR-29 inhibitor to control.

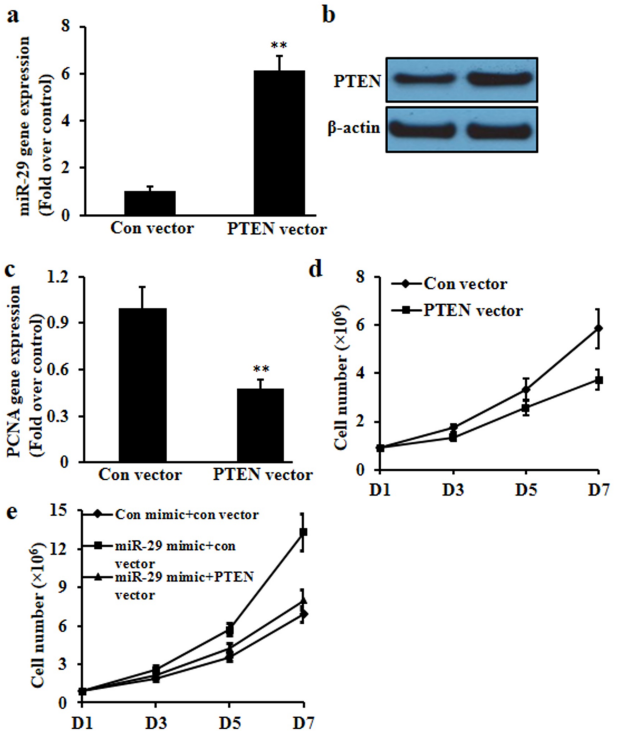

Figure 5. — PTEN attenuates the proliferative effects of miR-29 on ovarian cancer cells. (A, B). mRNA and protein expression of PTEN in SK-OV-3 cells after overexpression of PTEN; (C). Overexpression of PTEN inhibited PCNA expression in SK-OV-3 cells; (D, E). Overexpression of PTEN inhibited cell proliferation. $**p < 0.01$ *vs.* Con vector.

we expressed exogenous miR-29 and evaluated viable cells as well as the expression of key apoptotic proteins. Proliferating cell nuclear antigen (PCNA) expression was also significantly increased in cells with overexpressed miR- 29 (Figure 2). These results indicated that miR-29 promotes cell proliferation in ovarian cancer cells.

We further evaluated the effects of miR-29 on ovarian cancer cells by silencing miR-29 and MiR-29 gene silence inhibited cell proliferation and increased the expression of the apoptotic protein Bax and decreased the expression of anti-apoptotic protein Bcl-2. Consistent with the effects on PCNA following miR-29 expression, miR-29 silencing led to decreased PCNA expression (Figure 3).

Protein and mRNA expression of PTEN were detected in cells with overexpression or silencing of miR-29. Results demonstrated that PTEN expression decreased in cells with overexpression of miR-29 while PTEN expression increased when miR-29 was silenced (Figure 4).

Table 1. — *Patient characteristics.*

Factor	N (%) or Median (Range)
Age (range) at diagnosis (years)	55 (43-68)
Pathological type	
Serous cystadenocarcinoma	56 (47)
Mucinous cystadenocarcinoma	32 (27%)
Ovarian clear cell carcinoma	21 (17.5%)
Endometrioid ovarian carcinoma	11 (8)
Pathological stage	
I-II	28 (23%)
III-IV	92 (77%)
Pathological grading	
G1	26 (22%)
G2	43 (36%)
G3	51 (42%)

The role of PTEN was investigated in ovarian cancer cells through overexpression studies. Results demonstrated that overexpression of PTEN could inhibit ovarian carcinoma cell proliferation and PCNA expression. Subsequently, miR-29 mimic and a PTEN containing vector were co-administered into cells and results showed expressing PTEN with miR-29 was sufficient to reduce the proliferative effects of overexpression of miR-29 alone, indicating that PTEN attenuates the positive effect of miR-29 on cell proliferation (Figure 5).

Discussion

Epithelial ovarian cancer continues to portend poor outcomes with about 75% of patients diagnosed at an advanced stage of the disease. Although the initial response to chemotherapy os often good, overall survival remains low due to a high relapse rate [13-16]. Therefore, it is critical to better understand the central pathways that govern ovarian cancer cell proliferation and survival, with the goal to identify novel therapeutic targets.

miRNAs are non-coding, single-stranded RNAs that are approximately 19-25 nucleotides in length. miRNAs can regulate diverse processes in organisms through suppression of their target mRNAs. Many of these processes are critical in carcinogenesis, including cell differentiation,

proliferation, and apoptosis [17, 18]. Indeed, a number of miRNAs have been demonstrated to be vital to cancer cell proliferation and survival through control of their target mRNA expression. Among these miRNAs that have been shown to be intimately involved in cancer cell proliferation and survival, miR-29 has been found to be expressed abnormally in various tumors of diverse origin [19-21]. Interestingly, miR-29 has been linked to negative effects on cancer cell growth and survival. For instance, miR-29 inhibited hepatocellular carcinoma cell proliferation and migration by regulating IGF1R [22]. In addition, miR-29 also plays a tumor suppressive role in gliomas through targeting CDC42 [23]. However, the role of miR-29 in ovarian carcinoma has not been studied. In this study, our data indicate that miR-29 positively affects ovarian cell proliferation and survival. First, we found upregulation of miR-29 expression in ovarian carcinoma tissues compared to normal tissues. Second, we found that exogenous expression of miR-29 increases ovarian cancer cell proliferation, and third we found silencing of miR-29 inhibits ovarian cancer cell proliferation. Mechanistically, we found expression of the tumor suppressor PTEN was inhibited by miR-29, and, consistent with a key role of PTEN in miR-29-mediated effects, expression of PTEN was sufficient to inhibit ovarian cancer cell growth.

PTEN is a well characterized tumor suppressor in a variety of tumors. Noteworthy, other miRNAs have been demonstrated to suppress PTEN as well. For instance, PTEN is targeted by miR-28, and when this interaction is disrupted, it leads to gastric cancer cell proliferation and invasion [24]. Similarly, PTEN is targeted by miR-30a in renal cancer, contributing to renal cell cancer growth [25]. The present study demonstrated that in ovarian cancer, PTEN is targeted by miR-29, and the suppression of PTEN by miR-29 contributes to the proliferation and survival of ovarian cancer. As such, we have uncovered a novel interaction that contributes to ovarian cancer survival, and suggest the mIR-29-PTEN axis serves as an interesting new target for a recalcitrant cancer in need of new therapeutic approaches.

Ethics Approval and Consent to Participate

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of Second Affiliated Hospital of Zhengzhou University (approval number: 2015-096).

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Conflict of Interest

The authors declare no conflict of interest.

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