MiR-596 activated by EP300 controls the tumorigenesis in epithelial ovarian cancer by declining BRD4 and KPNA4

Deying Wang, Yulan Cui, Aili Xu, Lin Zhao and Peiling Li*

Abstract

**Background:** Epithelial ovarian cancer (EOC), a subclass of ovarian cancer (OC), is usually diagnosed at advanced stages due to the lack of effective screening means. Mounting reports have disclosed the vitally important roles of microRNAs (miRNAs) in carcinogenesis. Here, we aimed to find out possible miRNAs participating in EOC development.

**Methods:** qRT-PCR and western blot respectively examined the mRNA and protein levels of studied genes. CCK-8, colony formation, flow cytometry, TUNEL and spheroid formation assays were appropriately employed for examining cell proliferation, cell cycle, apoptosis and stemness. The interaction between molecules was affirmed by luciferase reporter, RNA pull down and ChIP assays.

**Results:** In consistent with the observation of a past study, miR-596 expression was relatively low in EOC cells. Up-regulating miR-596 suppressed EOC cell proliferation and stemness. EP300 transcriptionally activated miR-596 to serve as a tumor-repressor in EOC. Then BRD4 and KPNA4, whose knockdown led to restraining effects on cell growth and stemness, were both revealed to be targeted by miR-596 in EOC. Lastly, rescue assays affirmed the tumor-restraining role of miR-596-BRD4/KPNA4 axis in EOC.

**Conclusion:** EP300-activated miR-596 hampered cell growth and stemness via targeting BRD4 and KPNA4 in EOC, proofing miR-596 as a promising therapeutic target in treating EOC patients.

**Keywords:** Epithelial ovarian cancer (EOC), EP300, miR-596, BRD4, KPNA4

Background

Ovarian cancer (OC) is a deadly carcinoma in gynecological system [1, 2], among which epithelial ovarian cancer (EOC) is a typical subclass with the most mortality [3]. Owing to the lack of early symptoms and valid biomarkers, EOC patients often develop into advanced stages when diagnosed, which finally results in disappointing prognosis [4]. Therefore, the identification of available biomarkers for treating EOC patients is the urgent task.

Unlike long noncoding RNAs (lncRNAs), microRNAs (miRs) are noncoding RNAs (ncRNAs) with approximately 20 nt in length [5, 6]. They are widely known to recognize specific sequences in the 3′UTR of target message RNAs (mRNAs) to induce the silence of these mRNAs, so as to elicit restraining or facilitating roles in carcinomas [7]. Moreover, miRNAs with abnormal expression have been proved to affect cellular activities in diverse human cancers [8, 9]. For examples, circRNA AGFG1 acts as a sponge of miR-195-5p to promote triple-negative breast cancer progression through regulating CCNE1 expression [10]; miR-760 suppresses human colorectal cancer cell growth by targeting BATF3/AP-1/cyclinD1 signaling [11]; MiR-4319 suppresses the
malignancy of triple-negative breast cancer by regulating self-renewal of cancer stem cells [12]. Recently, several researches have demonstrated the tumor suppressive role of miR-596 in malignancies such as gastric cancer [13] and melanoma [14]. Besides, a former report revealed that miR-596 is expressed at a low level in EOC [15]. Yet, no in-depth investigations have been made on the function of miR-596 in EOC.

Our paper aimed to further expose the role and related mechanism of miR-596 in EOC. We firstly affirmed the low expression of miR-596 in EOC cells. Functional assays displayed that overexpression of miR-596 repressed EOC cell proliferation and stemness. To seek the reason for miR-596 down-regulation, we discovered the transcription activation of EP300 on miR-596, which was weakened along with reduced EP300 level in EOC cells. Subsequently, the negative regulation of miR-596 on BRD4 and KPNA4 was revealed. Rescue experiments validated that miR-596 hindered carcinogenesis in EOC through targeting BRD4 and KPNA4.

**Methods**

**Cell lines**

All cell lines including human EOC cell lines (SKOV3, OVCAR3 and A2780) and human ovarian surface epithelial cell line (HOSEpiC) were procured from Chinese Academy of Sciences (Shanghai, China) and allowed to grow in RPMI-1640 medium. 10% fetal bovine serum (FBS; Gibco, Grand Island, NY) was incorporated into the culture medium for cell logarithmic growth at 37°C in the air with 5% CO₂.

**qRT-PCR**

Different RNA expression levels were interrogated by qRT-PCR, with GAPDH/U6 as the normalized control using 2^{-ΔΔCt} method. The total RNA sample was collected from A2780 and OVCAR3 cells using Trizol reagent (Invitrogen, Carlsbad, CA) for achieving the first-strand cDNA synthesis using PrimeScript Reverse Transcriptase kit (Takara, Shiga, China). qPCR was implemented with the 5 μL of SYBR Green Master Mix (Invitrogen) on ABI Step One Plus instrument (Applied Biosystems, Foster City, CA). The sequences of primers used were shown in Additional file 1: Table S1.

**Transfection**

Confluent EOC cells (A2780 and OVCAR3) were prepared for 48 h of transfection in 6-well culture plates with the help of Lipofectamine2000 (Invitrogen). To elevate or reduce miR-596 expression, miR-596 mimics or inhibitor, as well as the corresponding negative control (miR-NC), were constructed by GenePharma (Shanghai, China). To overexpress EP300, BRD4 or KPNA4, indicated cDNA sequence was inserted into the pcDNA3.1 vector (Invitrogen) to acquire pcDNA3.1/EP300 (EP300), pcDNA3.1/BRD4 (BRD4) or pcDNA3.1/KPNA4 (KPNA4), and the empty pcDNA3.1 vector was seen as the negative control. In the meantime, short hairpin RNAs (shRNAs) specifically against EP300 (shEP300), BRD4 (shBRD4) or KPNA4 (shKPNA4), as well as corresponding nonspecific shRNA control (sh-NC), were obtained from the Ribobio (Guangzhou, China). The sequences of shRNAs were shown in Additional file 2: Table S2.

**CCK-8 assay**

For CCK-8 assay, the 96-well culture plates were plated with the transfected EOC cells (1 × 10³ per well), followed by adding 10 μL of CCK8 solution (Dojindo, Tokyo, Japan) at the specific times. After 4 h, the microplate reader was used to probe the absorption at 450 nm.

**Colonies formation assay**

EOC cells were cultured in 6-well plates for about 2 weeks. After that, cells were then rinsed in phosphate buffer saline (PBS) and fixed in 4% paraformaldehyde for 15 min. After crystal violet staining for 30 min, the number of formed colonies was calculated manually.

**Flow cytometry analyses**

The apoptosis rate and cell cycle distribution of EOC cells were analyzed with flow cytometry with BD AccuriC6 (BD Biosciences, San Jose, CA) and FlowJo 7.6 software (BD Biosciences). Before that, cell apoptosis was determined using Annexin V/PE/7AAD Apoptosis Kit, while cell cycle distribution was tested by PI staining and CycleTEST™ PLUS DNA reagent kit (BD Biosciences).

**TUNEL analysis**

After transfection, EOC cells were subjected to In-Situ Cell Death Detection Kit (Roche, Basel, Switzerland) based on the user guide. Cell nuclei were double-stained with TUNEL and DAPI (Beyotime, Shanghai, China). Images of TUNEL-positive cells were captured by fluorescence microscope (NIKON, Tokyo, Japan).

**3-Dimensional (3-D) spheroid formation assay**

The spheroid formation assay was performed in accordance with a former protocol [16]. Simply put, 3 × 10⁴ cells in Matrigel (BD Biosciences, Bedford, MA, USA) with reduced growth factor were added into culture medium with enriched growth factor for two-week incubation, with the medium changed every 2 days. The images of formed spheroids were obtained under a microscope.
Animal study
Total of 12 nude male mice (aged 6 weeks) were purchased from Slac Laboratories (Shanghai, China) for the in vivo experiments approved by the Ethics Committee of the Second Affiliated Hospital of Harbin Medical University. In short, four kinds of A2780 cells, which were respectively transfected with miR-NC, miR-596 mimics, miR-596 mimics + BRD4 or miR-596 mimics + KPNA4, were inoculated on the backs of the mice. Tumor volume was recorded every 4 days, and mice were sacrificed 4 weeks later. Afterwards, the xenografts were separated from mice, pictured and weighed for further analysis.

Dual-luciferase reporter assays
For miR-596 promoter analysis, the EOC cells (A2780, OVCAR3) were collected for co-transfection in 24-well plates with reporter vector containing miR-596 promoter, pRL-TK-Renilla and transfection plasmids (shEP300, sh-NC, EP300, pcDNA3.1). The 3′-UTR of BRD4 or KPNA4 sequences with wild-type (WT) or mutant (Mut) miR-596 interacting sites were utilized for inserting to pmirGLO-luciferase vectors (Promega, Madison, WI) to form the reporter vectors BRD4 (WT/Mut) or KPNA4 (WT/Mut), followed by co-transfection into EOC cells with miR-596 mimics or miR-NC. The Dual Luciferase Reporter Assay System (Promega) was implemented 48 h post-transfection to test the luciferase activity.

ChIP assay
EOC cells treated with 4% formaldehyde were cultured for 10 min to acquire DNA–protein cross-links, which were then sheared into chromatin fragments of 200–500 bp. Immunoprecipitation was performed using the H3K27ac-specific antibody (Millipore, Bedford, MA), with anti-IgG as the negative control. DNA fragments in the final precipitates recovered by magnetic beads were dissected by qRT-PCR.

RNA pull-down experiments
RNA pull-down assay was implemented by applying the in vitro biotin-labeled RNAs (including sense and antisense miR-596 sequences and a nonsense RNA sequence), termed as Bio-miR-596 sense, Bio-miR-596 antisense (AS) and Bio-NC. After that, the biotinylated RNAs were mixed with cellular extracts and streptavidin-crossed beads, and qRT-PCR analysis of the captured RNAs was followed.

Western blot
RIPA lysis buffer incorporating protease inhibitor was applied for total protein extraction from A2780 and OVCAR3 cells. Protein samples were then electrophoresed for separation on 10% SDS-PAGE, followed by transferring to polyvinylidene fluoride (PVDF) membranes. After sealing with 5% defatted milk, membranes were blotted with primary antibodies (1:2000; Abcam, Cambridge, MA) against Bax, Bcl2, cleaved-PARP, EP300, BRD4, KPNA4 and GAPDH, and then probed with corresponding secondary antibodies (1:5000; Abcam). The antigen–antibody complex was measured by chemiluminescent detection system (Bio-Rad, Hercules, CA).

Statistical analyses
Results were given as mean ± standard deviation from 3 various replications. Prism for windows, version 6.0 (GraphPad, San Diego, CA) was implemented for data integration and analysis by use of student’s t test and analysis of variance (ANOVA). The statistical data were thought as significant when P value was less than 0.05.

Results
Overexpression of miR-596 hampered the proliferation ability of EOC cells
Considering the reduced expression of miR-596 in epithelial ovarian cancer (EOC) [15], we decided to further explore the impacts of miR-596 on the biological behaviors of EOC cells. Firstly, qRT-PCR proved that miR-596 expression was indeed decreased in EOC cells in comparison with the normal HOSEpiC cells (Fig. 1a). Then the expression of miR-596 was elevated in A2780 and OVCAR3 cells by miR-596 mimics for subsequent loss-of-function assays (Fig. 1b). The results of CCK-8 assay exhibited that cell viability was significantly repressed by miR-596 overexpression (Fig. 1c). Meanwhile, cell proliferation was distinctly inhibited when miR-596 was up-regulated (Fig. 1d). As for cell cycle, flow cytometry analysis was performed and the outcomes demonstrated that the proportion of cells at G0/G1 phase was increased...
in miR-596 mimics groups (Fig. 1e), suggesting the arrest of cell cycle induced by enhanced miR-596. Nonetheless, cell apoptosis was evidently induced by ectopic expression of miR-596, since more apoptotic cells and TUNEL positive cells were observed after the transfection of miR-596 mimics (Fig. 1f, g). Additionally, western blot analyzed that Bax and cleaved-PARP protein levels were improved while Bcl2 protein level was lessoned after miR-596 was increased (Fig. 1h). Afterwards, we further tested the impact of miR-596 on EOC cell stemness, which is a specific character of malignant cells [17]. As anticipated, the number of spheroids formed in these two EOC cells was apparently declined under ectopic miR-596 expression (Additional file 3: Figure S1A). Altogether, miR-596 hampered cell growth and stemness in EOC.

**EP300 activated miR-596 expression at transcriptional level**

Considering the down-regulation of miR-596 in EOC, we assumed that there might be some transcription factors affecting the transcription activity of miR-596. From UCSC genome browser, we observed that EP300, which could act as a histone acetyltransferase to modulate transcription activation, was a possible transcription factor of miR-596. Hence, we sought to investigate the association between EP300 and miR-596 in EOC. Prior to that, we validated the knockdown and overexpression efficiencies of EP300 expression in A2780 and OVCAR3 cells (Fig. 2a). The results of luciferase reporter assay indicated that the luciferase activity of miR-596 promoter was impaired by silenced EP300 but strengthened by overexpressed EP300 (Fig. 2b). Since EP300 has been indicated to function through modulating H3K27 acetylation (H3K27ac) on gene promoter [18, 19], we then assessed H3K27ac status of miR-596 promoter via ChIP assay. It manifested that less H3K27ac in miR-596 promoter was observed in A2780 and OVCAR3 cells than that in control cells (Fig. 2c). Moreover, EP300 expression at both mRNA and protein levels was overtly underexpressed in EOC cells relative to normal HOSEpiC cells (Fig. 2d). Importantly, miR-596 expression was accordingly lessened or enlarged after EP300 expression was silenced or enhanced (Fig. 2e). To view the impact of EP300/miR-596 axis on EOC cellular activities, we conducted subsequent rescue assays in EP300-overexpressed cells via suppressing miR-596 by miR-596 inhibitor (Fig. 2f). Consequently, we unveiled that the hindered viability of EOC cells with enhanced EP300 was regained when miR-596 was further inhibited (Fig. 2g). Besides, cell cycle progression that was previously arrested by EP300 upregulation was recovered in response to miR-596 inhibition (Fig. 2h). Also, the accelerating effect of EP300 overexpression on cell apoptosis was offset by miR-596 inhibitor (Fig. 2i, j). More importantly, EP300 elevation resulted in hampered spheroid formation ability of both EOC cells, while such inhibitory impact was then offset by miR-596 suppression (Additional file 3: Figure S1B). These data suggested that EP300 promoted miR-596 expression to restrain cell growth in EOC.

**MiR-596 targeted BRD4 and KPNA4 in EOC**

Thereafter, we screened the targets of miR-596 via mirDIP database, and then analyzed the expression of top 500 targets in three EOC cells relative to normal HOSEpiC cells. As presented in the heatmap, five among them showed high expression in these EOC cells BRD4, BPTF, KPNA4, ETNK1 and BCAT1 (Fig. 3a). However, only two of the five candidates, BRD4 and KPNA4, exhibited elevated expression in both A2780 and OVCAR3 cells under miR-596 inhibition (Fig. 3b), hinting that BRD4 and KPNA4 were the most probable targets of miR-596 in EOC. Further, western blotting elucidated the high expression of BRD4 protein in EOC cells (Fig. 3c). Moreover, inhibition or elevation of miR-596 augmented or declined the mRNA and protein expression of BRD4 in the two EOC cells, respectively (Fig. 3d, e). Next, the binding sites between BRD4 and miR-596, as well as the mutant sequence of BRD4 were represented in Fig. 3f. After that, luciferase reporter and RNA pull-down assays were utilized to confirm the interplay between miR-596 and BRD4. Results indicated that in both A2780 and OVCAR3 cells, miR-596 up-regulation only impaired the luciferase activity of reporters with wild-type BRD4 sequence (Fig. 3g).
Also, BRD4 mRNA could merely be pulled down by Bio-miR-596 sense probe (Fig. 3h). As for KPNA4, we observed that KPNA4 protein was markedly elevated in EOC cells (Fig. 3i). Further, KPNA4 expression at mRNA and protein levels displayed similar changes as BRD4 in response to miR-596 inhibition or miR-596 upregulation (Fig. 3j, k). Likewise, the luciferase activity of KPNA4 (WT) was specifically decreased by miR-596 mimics and KPNA4 mRNA was merely pulled down by biotinylated sense miR-596 (Fig. 3l–n). These findings told us that BRD4 and KPNA4 were two targets of miR-596 in EOC.

Inhibiting BRD4 and KPNA4 hindered EOC cell growth

In order to probe the functional role of BRD4 and KPNA4 in EOC, loss-of-function experiments were conducted. BRD4 mRNA and protein expression was firstly downregulated in A2780 and OVCAR3 cells by transfecting with shBRD4 (Fig. 4a). Then, we observed that after BRD4 was silenced, cell viability was weakened and the number of colonies formed by these cells was reduced (Fig. 4b, c), which meant that cell proliferation was controlled under BRD4 deficiency. Besides, the loss of BRD4 led to cell cycle arrested at G0/G1 phase (Fig. 4d). On the contrary, cell apoptosis was activated after BRD4 knockdown, which was affirmed by increased apoptotic rate and TUNEL positive cell proportion (Fig. 4e, f). Moreover, such phenomenon was further proved by the increased Bax and cleaved-PARP protein levels and reduced Bcl2 protein levels in face of BRD4 depletion (Fig. 4g). In the meantime, it manifested that the absence of BRD4 mitigated the stemness of EOC cells (Additional file 3: Figure S1C). Similarly, we also uncovered that KPNA4 silence inhibited cell proliferation, induced cell cycle arrest, accelerated cell apoptosis and impaired cell stemness in EOC (Fig. 4h–n and Additional file 3: Figure S1D). All in all, BRD4 and KPNA4 served restraining roles in EOC cell growth.

Ectopic expression of BRD4 or KPNA4 reversed the repressive influence of elevated miR-596 on EOC tumorigenesis

Before the rescue experiments, BRD4 and KPNA4 were separately overexpressed in A2780 cells via transfecting with particular pcDNA3.1 overexpressing vectors (Fig. 5a, b). Thereafter, we disclosed that cell viability impaired by miR-596 mimics was rescued by upregulating either BRD4 or KPNA4 (Fig. 5c). Meanwhile, the reduced number of colonies due to miR-596 upregulation was recovered by enhancement of BRD4 or KPNA4 (Fig. 5d). Also, the arrest of cell cycle resulted from miR-596 upregulation was relieved in response to further overexpression of BRD4 or KPNA4 (Fig. 5e). In addition, according to the outcomes of flow cytometry analysis and TUNEL assay, the active impact of miR-596 overexpression on cell apoptosis was neutralized when BRD4 or KPNA4 was augmented (Fig. 5f–g). Meanwhile, it was evidenced that the effects of miR-596 up-regulation on the level of several apoptosis-related proteins (Bax, Bcl2 and cleaved-PARP) were counteracted after increasing the expression of BRD4 or KPNA4 (Fig. 5h). Further, we proved that the suppressed stemness in miR-596-upregulated EOC cells was recovered under the overexpression of BRD4 or KPNA4 (Additional file 3: Figure S1E). To further proof the significance of miR-596/BRD4/KPNA4 axis in EOC tumorigenesis, we then carried out in vivo experiments. As a result, we viewed that the size, growth rate and weight of tumors came from miR-596-elevated cells were all lessened compared to control group, whereas upregulation of BRD4 or KPNA4 could reverse above reductions (Additional file 3: Figure S1F, G). In summary, BRD4 and KPNA4 was implicated in miR-596-controlled tumorigenesis in EOC.
Discussion
Mounting miRs have been indicated to be dysregulated in EOC to elicit either promotive or repressive effects [20–22]. For example, miR-506 restrains multiple targets associated with epithelial-to-mesenchymal transition (EMT) in EOC [23]. MiR-211 hinders cell proliferation by blocking cell-cycle progression in EOC via Cyclin D1 and CDK6 [24]. In this paper, we focused on the role of miR-596 in EOC, given that miR-596 was reported to be under-expressed in EOC [15]. Consistently, we also proved the down-regulation of miR-596 in EOC cells. More importantly, our work detected
Fig. 4  BRD4 or KPNA4 knockdown served restraining effects on the growth of EOC cells. 

a The transfection of shBRD4 suppressed BRD4 expression at mRNA and protein levels in A2780 and OVCAR3 cells, as detected via qRT-PCR and western blot.

b–g The results of functional assays demonstrated that depleted BRD4 impaired EOC cell proliferation, blocked cell cycle progression, and facilitated cell apoptosis. As examined by qRT-PCR and western blot, the expression of KPNA4 was weakened by shKPNA4 compared to that in the control group (shNC).

h–n The suppressive impact of KPNA4 knockdown on the growth of A2780 and OVCAR3 cells was assessed by CCK-8, colony formation and TUNEL assays and flow cytometry and western blot analyses. **P < 0.01
Fig. 5 MiR-596 regulated cell proliferation, cell cycle and apoptosis in EOC via targeting BRD4 and KPNA4. 

**a**, **b** Elevation on the mRNA and protein levels of BRD4 or KPNA4 severally induced by transfection of pcDNA3.1/BRD4 (BRD4) or pcDNA3.1/KPNA4 (KPNA4) in A2780 cells was determined by qRT-PCR and western blot. 

**c** The results of CCK-8 and colony formation assays proved that miR-596 upregulation-restrained cell proliferation was reversed by overexpressing BRD4 or KPNA4. 

**d** The normalization of BRD4 or KPNA4 overexpression on cell cycle and apoptosis of miR-596-enhanced A2780 cells were confirmed by flow cytometry, TUNEL staining (Scale bar = 50 μm) and western blot. **P < 0.01**
that upregulating miR-596 hindered cell proliferation via hampering cell cycle progression and accelerating cell apoptosis, and also impaired cell stemness in EOC. From these findings, we firstly identified miR-596 as a novel tumor-inhibitor in EOC, which was similar to several previous reports regarding miR-596 function in malignancies including melanoma [14], oral cancer [25] and gastric cancer [13].

In order to find the possible upstream molecules regulating miR-596 in EOC, we browsed UCSC genome browser and figured out several transcription factors of miR-596. Herein, we validated the binding between EP300 and miR-596 promoter, and confirmed the positive regulation of EP300 on miR-596 in EOC cells. These results proved E1A-Binding Protein P300 (EP300) as the transcription activator of miR-596 in EOC. Interestingly, EP300, which acts as a histone acetyltransferase to activate gene transcription [26, 27], has been previously reported to exert an anti-cancer function in tumors like breast cancer [28, 29]. However, some literatures also suggested EP300 as a tumor-promoter in several malignancies [30, 31]. In this work, we unveiled that EP300 was downregulated in EOC cells, which was consistent with the discovery of a former study [32]. Moreover, it was elucidated that EP300 upregulation mitigated EOC cell growth and stemness, indicating it served an anti-cancer part in EOC. Further, the inhibitory impact of EP300 on EOC cellular behaviors could be counteracted by miR-596 suppression, highlighting the tumor-repressive role of EP300/miR-596 signaling in EOC.

Next, we attempted to discover the downstream mechanism whereby miR-596 worked in EOC. Fortunately, through bioinformatics analysis and experimental detections, we unveiled bromodomain containing 4 (BRD4) and karyopherin subunit alpha 4 (KPNA4) as the most potential two targets of miR-596 in EOC. Currently, several reports have elucidated the high expression and promotive function of BRD4 and KPNA4 in some diseases. For instance, NSD3-BRD4-CHD8 pathway functions in pelvic high-grade serous carcinomas originated from tubo-ovarian and endometrial [33]. Of note, BRD4 is predicted as a novel therapeutic target for regulating Notch3 signaling in OC [34]. Also, MiR-3619-5p inhibits cell proliferation and cisplatin resistance in cutaneous squamous cell cancer by targeting KPNA4 [35]. Increased miR-181 s reverses EMT via decreasing KPNA4 in glioblastoma [36]. In the present research, we similarly found the oncogenic roles of BRD4 and KPNA4 in EOC, evidenced by that silencing BRD4 or KPNA4 led to abrogated EOC cell growth and stemness. In the end, the rescue assays conducted both in vitro and in vivo verified that miR-596 restrained tumorigenesis in EOC via modulating BRD4/KPNA4 axis.

Conclusion
These findings implied that EP300-activated miR-596 negatively regulated cell growth and stemness in EOC through declining BRD4 and KPNA4. Although the conclusion provided promising therapeutic targets for treating EOC patients, future efforts are still necessary for deep comprehension of EOC pathology.
References

1. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J. Cancer statistics in China, 2015. CA Cancer J Clin. 2016;66(2):115–32.

2. Sieh W, Kobel M, Longacre TA, Bowtell DD, deCaprio A, Goodman MT, Hogdall E, Deen S, Wentzensen N, Myslobod KB, et al. Hormone-receptor expression and ovarian cancer survival: an Ovarian Tumor Tissue Analysis consortium study. Lancet Oncol. 2013;14(9):853–62.

3. Lin X, Tang X, Zheng T, Qiu J, Hua K. Long non-coding RNA NONHSAT076574 promotes invasion and metastasis in epithelial ovarian cancer. J Cancer. 2019;10(8):1930–40.

4. Goff BA, Madell L, Muntz HG, Mellancoc CH. Ovarian carcinoma diagnosis. Cancer. 2000;89(10):2068–75.

5. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004;116(2):281–97.

6. Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by CDRs, indicates that thousands of human genes are microRNA targets. Cell. 2005;120(1):15–20.

7. Li BS, Zuo QF, Zhao YL, Xiao B, Zhanyong Y, Mao XH, Wu C, Yang SM, Zeng H, Zou QM, et al. MicroRNA-25 promotes gastric cancer migration, invasion and proliferation by directly targeting transducer of ERBB2, 1 and correlates with poor survival. Oncogene. 2015;34(20):2356–65.

8. Wu DW, Hsu NY, Wang YC, Lee MC, Cheng YY, Chen CY, Lee HC. c-Myc suppresses microRNA-29b to promote tumor aggressiveness and poor outcomes in non-small cell lung cancer by targeting FHT. Oncogene. 2015;34(16):2072–82.

9. Yang R, Xing L, Zheng X, Sun Y, Wang X, Chen J. The circular RNA circAGFG1 acts as a sponge of miR-195-5p to promote triple-negative breast cancer progression through regulating CNE1 expression. Mol Cancer. 2019;18(1):4.

10. Liu SM, Lin CH, Lu J, Zeng Y, Guo Q, Yuan W, et al. MiR-760 suppresses human colorectal cancer growth by targeting BATF3/AP-1/cyclinD1 signaling. J Exp Clin Cancer Res. 2018;37(1):83.

11. Cao L, Liu Y, Wang D, Huang L, Li F, Liu J, Zhang C, Shen Z, Gao Q, Yuan W, et al. MiR-760 suppresses human colorectal cancer growth by targeting BATF3/AP-1/cyclinD1 signaling. J Exp Clin Cancer Res. 2018;37(1):83.

12. Chi J, Li Y, Fan X, Ma J, Li J, Lu G, Zhang Y, Huang Y, Li W, Huang X, et al. MiR-4319 suppress the malignancy of triple-negative breast cancer by regulating self-renewal and tumorigenesis of stem cells. Cell Physiol Biochem. 2018;48(2):593–604.

13. Zhang Z, Dai DQ. MicroRNA-596 acts as a tumor suppressor in gastric cancer and is upregulated by promotor demethylation. World J Gastroenterol. 2019;25(10):3274–37.

14. Liu SM, Lin CH, Lu J, Lin YY, Tsai MS, Chen MH, Ma N. MiR-596 modulates melanoma growth by regulating cell survival and death. J Invest Dermatol. 2018;138(7):1801–3.

15. Sommerova L, Durikova H, Podhorec J, Hrstka R. MicroRNA analysis in osteosarcoma correlation analysis. Mol Clin Oncol. 2017;7(2):301–7.

16. Parashar D, Geethadevi A, Aure MR, Mishra J, George J, Chen C, Mishra MK, Tahin A, Zhao W, Nan B, et al. MiRNAS11-1b-3p activates an oncostatin signaling module for the progression of triple-negative breast cancer. Cell Rep. 2019;29(13):4389–406.

17. Choi SH, Gearhart MD, Cui Z, Bosnakovski D, Kim M, Schennum N, Kyba M, DUV4 recruits p300/CBP through its C-terminus and induces global H3K27 acetylation changes. Nucleic Acids Res. 2016;44(11):1561–73.

18. Choi SH, Gearhart MD, Cui Z, Bosnakovski D, Kim M, Schennum N, Kyba M, DUV4 recruits p300/CBP through its C-terminus and induces global H3K27 acetylation changes. Nucleic Acids Res. 2016;44(11):1561–73.

19. Choi SH, Gearhart MD, Cui Z, Bosnakovski D, Kim M, Schennum N, Kyba M. DUV4 recruits p300/CBP through its C-terminus and induces global H3K27 acetylation changes. Nucleic Acids Res. 2016;44(11):1561–73.

20. Choi SH, Gearhart MD, Cui Z, Bosnakovski D, Kim M, Schennum N, Kyba M, DUV4 recruits p300/CBP through its C-terminus and induces global H3K27 acetylation changes. Nucleic Acids Res. 2016;44(11):1561–73.

Publisher’s Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.