ACTA ABP BIOCHIMICA POLONICA

Vol. 68, No 4/2021
673–677
https://doi.org/10.18388/abp.2020_5546

Regular paper

MiR-198 inhibits proliferation, invasion and migration of ovarian cancer cells by regulating the PI3K/Akt signaling pathway

Huichao Xiao, Yafeng Zheng, Jiming Chen and Huaji Shen✉

Department of Gynaecology, Changzhou No.2 People's Hospital, Changzhou, Jiangsu 213000, China

Objective: The specific objective of this investigation is to explore the impact of miR-198 on proliferation, migration as well as invasion of ovarian cancer (OC) cells. Methods: OC tissue and adjacent normal tissue samples from OC patients were collected, and normal human ovarian epithelial cell IOSE80 and OC cell lines SKOV3, Caov3, A2780 and OVCAR3 were selected in this study for investigation. MiR-198 expression level was assessed using RT-qPCR. MTT, colony formation assay, Transwell and wound healing assay, and flow cytometry were adopted to analyze the role of miR-198 in OVCAR cell proliferation, invasion, migration, as well as apoptosis. Meanwhile, the levels of P13K/Akt signaling pathway-related proteins were determined by western blotting. Results: A significant decrease in miR-198 level was revealed in the OC tissues and cells, contributing to the promotion of OVCAR3 cells in terms of proliferation, migration, invasion, and inhibition of apoptosis. MiR-198 overexpression had an opposite effect on these biological processes of OVCAR3 cells. Further study found that down-regulation of miR-198 caused a significant increase in the activity of PI3K/Akt signaling pathway in the OVCAR3 cells. In contrast, overexpressed miR-198 led to inhibition of this pathway's activity. Conclusion: MiR-198 may possess an ability to inhibit activation of the P13K/Akt pathway, thus suppressing the OC cell proliferation, migration, as well as invasion.

Key words: ovarian cancer; miR-198; PI3K/Akt signaling pathway; proliferation; invasion; migration

Received: 16 November, 2020; revised: 20 January, 2021; accepted: 25 January, 2021; available on-line: 28 June, 2021

✉e-mail: 13401376106@163.com

Abbreviations: EMT, epithelial-mesenchymal transition; miRNAs, MicroRNAs; OC, ovarian cancer; oncomirs, oncogenic miRNAs; ZEB1, zinc finger E-box binding homeobox 1

INTRODUCTION

Ovarian cancer (OC) is a common cancer and the fifth leading cause of death from malignant tumors in females (Siegel *et al.*, 2018). OC subtypes are mainly divided into epithelial OC and non-epithelial OC. Epithelial OC is the most common and its histological types include serous (62%), mucinous (5%), clear cell (8%), endometrioid (20%), and other types (5%) (Program, 2005, Ramalingam, 2016). Among them, most serous OC are diagnosed at an advanced stage and are highly malignant, namely high-grade serous OC (Köbel *et al.*, 2010). The serous subtype accounts for 80% of OC deaths, and in patients with advanced serous OC the 5-year survival rate (15%) is low (Sopik *et al.*, 2015). Although aggressive treatment is currently possible with surgery and adjuvant chemotherapy, the 5-year survival rate (35%-40%) remains unsatisfactory for patients at Stage III or IV. What's worse, most patients relapse after treatment and eventually become chemoresistant (Waldmann *et al.*, 2013). The results of clinical trials have shown no significant increase in the overall survival rate of OC patients after conventional treatment, which suggests that the conventional treatment may have reached a plateau (Odunsi, 2017). Therefore, it is of great significance to find new therapeutic strategies for the diagnosis, treatment and prognosis of OC patients.

MicroRNAs (miRNAs) participate in mediating gene expression in a range of developmental and physiological processes, and their dysregulation is associated with all types of cancer. Interaction of miRNAs and their target mRNAs induces degradation and/or translational repression of mRNA (Svoronos *et al.*, 2016). Oncogenic miRNAs (oncomirs) usually refer to miRNAs that target coding transcripts of tumor suppressor proteins, showing up-regulation in different cancers (Frixa *et al.*, 2015). In contrast, miRNAs with tumor suppressor functions can down-regulate oncogenes and are frequently lost in cancer (Almeida *et al.*, 2011). Clinically, miRNAs pose important effects on various cancers, such as OC (Svoronos *et al.*, 2016). MiRNAs have a regulatory impact upon OC invasion and metastasis. For example, miR-7 can cause a decrease in epithelial growth factor receptor expression to inhibit activity of the AKT-ERK1/2 pathway and reverse the epithelial-mesenchymal transition (EMT), thus eventually inhibiting tumor metastasis (Loganathan *et al.*, 2012). MiR-150 suppresses a tendency of OC cells to invade and metastasize by negatively regulating the zinc finger E-box binding homeobox 1 (ZEB1) (Jin *et al.*, 2014). And in patients with epithelial OC, low miR-146a and miR-198 expression is considered to be a marker predicting poor prognosis (Wilczyński *et al.*, 2017, Xu *et al.*, 2020). In addition, in other human malignancies, such as the non-small cell lung cancer (Wang *et al.*, 2019), prostate cancer (Ray *et al.*, 2019) and breast cancer (Niu *et al.*, 2019), miR-198 was found to participate in their development and progression. However, the biological function of miR-198 in OC and its potential mechanism are still unclear.

The study presented here found low expression of miR-198 in OC. Furthermore, the impacts of miR-198 upon OC cell proliferation, invasion, migration, and apoptosis were determined by loss-of-function and gain-of-function experiments. Additionally, we examined the effects of miR-198 on the PI3K/Akt pathway activity. Presented results provide a theoretical reference for further understanding of the molecular mechanism of OC progression, and also propose a new therapeutic target for OC.

MATERIALS AND METHODS

Clinical sample collection

The OC tissues and corresponding non-tumor adjacent tissues were obtained from 60 OC patients who received no radiotherapy and chemotherapy before surgery between 2018 and 2019. The clinical tissues were stored in liquid nitrogen immediately after surgical collection for subsequent RT-qPCR to detect expression of related genes. All patients had given written informed consent for publication. An approval was obtained from the Ethics Committee of Changzhou No. 2 People's Hospital.

Cell culture and transfection

Human normal ovarian epithelial cells IOSE80, OC cell lines OVCAR3, SKOV3, Caov3, and A2780 were purchased from the Shanghai Cell Bank, Chinese Academy of Sciences (Shanghai, China). All cells were cultured in RPMI-1640 medium (Solarbio, Beijing, China), in a humidified incubator with 5% CO_2 at 37°C. The media were supplemented with 10% fetal bovine serum (FBS, Bioind, Kibbuiz, Israel) and 1% penicillin/streptomycin (Gibco, USA). All cells were divided into five groups: control group (control), miR-198 inhibitor NC group (in-NC), miR-198 inhibitor group (in-miR-198), miR-198 mimics NC group (NC), as well as miR-198 mimics group (miR-198). Before transfection, OVCAR3 cells were seeded in 6-well plates (1.0×10^5). When confluency reached 80–90%, OVCAR3 cells were transfected with mimics and inhibitor using the HiperFect transfection reagent (GIAGEN, Germany). Upon transfection completion, RT-qPCR was employed to analyze the transfection efficiency.

RT-qPCR

Trizol reagent (Invitrogen, Carlsbad, USA) was utilized for isolation of total cellular RNA. MiRNA First Strand cDNA Synthesis Kit (Vazyme, Nanjing, China) was then adopted to reverse transcribe RNA into cDNA. Subsequently, RT-qPCR measurements were performed using SYBR Green PCR Master Mix Kit (Takara, Japan) with the Applied Biosystems 7500 real-time PCR system (Foster, USA). Experimental parameters were as follows: 95°C for 2 minutes, then 95°C for 15 seconds and 60°C for 30 seconds, 40 cycles. Finally, $2^{-\Delta\Delta Ct}$ method was adopted to calculate relative expression of miR-198, with U6 as an endogenous reference. The primer sequences used in our study were as follows: U6, forward: 5'-GCTTCGGCAGCACATATACTAAAAT-3', reverse: 5'-CGCTTCAGAATTTGCGTGTCAT-3'; miR-198, forward: 5'-TCAAGTGCCTCAACCCATCT-3', reverse: 5'-ATCTCCTCTGTCTGGGT-3'.

MTT and colony formation assay

OVCAR3 cells were seeded into 96-well plates (2.0×10^3 cells/well). Cell proliferation was measured by using an MTT assay kit, and the absorbance values were measured at 450 nm with a spectrophotometric plate reader. For the colony formation assay, OVCAR3 cells were plated into 6-well plates (5.0×10^2 cells/well) and cultured in 10% FBS medium at 37°C for 14 days. Then, following the fixation step using 10% methanol, 0.5% crystal violet was employed to stain the cells. Colonies with >50 cells were counted under an inverted microscope. Each experiment was repeated at least three times.

Transwell assay

The invasive ability of the cells was analyzed by using matrigel-coated transwell culture inserts (pore size: 8 μm). After rinsing three times with PBS, the transfected cells were plated into the upper chamber, while 600 μL of 10% FBS medium was added to the lower chamber. Upon completion of 24- hour incubation, non-invasive cells were wiped off, while the invasive cells were stained with 10% crystal violet upon completion of the fixation step with 4% paraformaldehyde. Finally, the number of invasive cells was observed under an inverted microscope.

Wound healing assay

Cells were plated in 12-well plates after 24-hour incubation at 37°C. A wound gap was created in the center of the plate by using a sterile 10 ul pipette tip. Prior to addition of a serum-free medium, PBS was employed to gently rinse the cells for three times. Cell migration was observed by an inverted microscope at 0 hour and 24 hours, respectively. The scratched area was measured by the Image J software.

Flow cytometry

OVCAR3 cells were plated into 12-well plates overnight for cell adhesion. Subsequently, the cells were dissociated into single cells by digestion with EDTA-free trypsin and were collected. PBS was then used to rinse the collected cells to prepare single-cell suspensions (1×10^6 cells/ml). PE Annexin V Apoptosis Detection Kit with 7-ADD (BD biosciences, USA) was employed to stain the cells. Eventually, a flow cytometer (BD FACSVerse, USA) and the FlowJo software were adopted for apoptosis detection.

Western blot

Total proteins were extracted from the cells lysed with the RIPA lysis buffer and quantified by BCA assay. After separation using 12% SDS-PAGE, the proteins were then electrophoretically transferred onto nitrocellulose membranes. Upon completion of the blocking step with skim milk powder for 2 hours at room temperature, the membranes were incubated overnight with rabbit anti-p-PI3K (ab182651, 1:1000), anti-PI3K (ab32089, 1:2000), anti-p-Akt (ab38449, 1:2000), anti-Akt (ab8805, 1:2000), or anti-GAPDH (ab9485, 1:2000) antibodies at 4°C. After three subsequent washes with PBST, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies for 1 hour at room temperature. At last, the membranes were washed again with PBST, and immunolabeling was detected with the ECL chemiluminescence kit. ImageJ software was used to determine the gray values of the protein bands.

Statistical analysis

Data processing was performed *via* SPSS 22.0 software. The results were expressed as the mean ± standard deviation (S.D.). Comparisons between two groups were performed using *t*-test, while multiple comparison testing was done by using one-way analysis of variance (ANOVA) with LSD post-hoc test. $P<0.05$ indicates a significant difference.

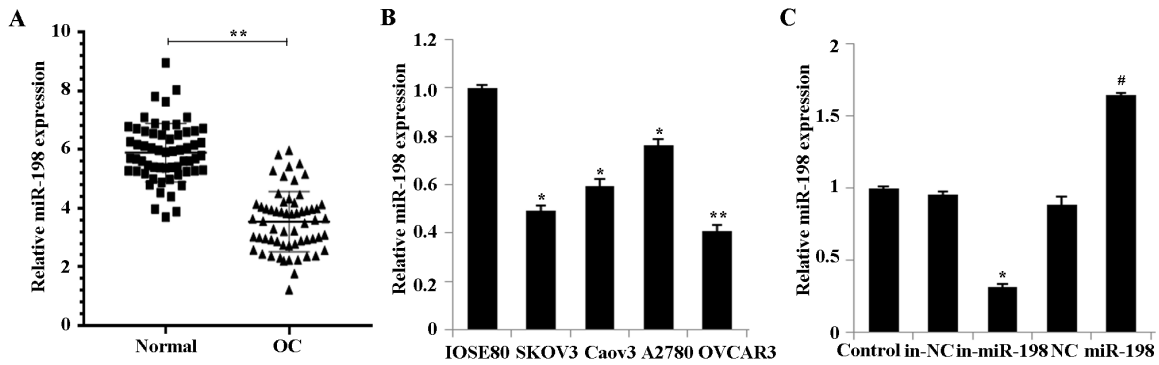

Figure 1. MiR-198 expression in ovarian cancer tissues and cells.
(**A**) RT-qPCR-based detection of miR-198 expression in ovarian cancer (OC) tissues and non-tumor adjacent tissues. (**B**) RT-qPCR-based detection of miR-198 expression in human normal ovarian epithelial cell line IOSE80 and OC cell lines SKOV3, Caov3, A2780 and OVCAR3; **P*<0.05 and ***P*<0.01 *vs*. IOSE80 (**C**) MiR-198 expression in OVCAR3 cells in each group; **P*<0.05 *vs*. in-NC group; #*P*<0.05 *vs*. NC group.

RESULTS

Low miR-198 expression in ovarian cancer

It could be seen from the results of RT-qPCR in Fig. 1A that there is a significantly lower expression of miR-198 in the OC tissues when compared to the normal tissues ($P<0.01$). At the cellular level, a markedly decreased miR-198 expression was found in the SKOV3, Caov3, and A2780 cell lines when compared to the IOSE80 cell line ($P<0.05$) (Fig. 1B). Among these, OVCAR3 cells with the lowest expression of miR-198 were selected for subsequent studies. Furthermore, miR-198 expression level in OVCAR3 was intervened through transfection of miR-198 mimics or inhibitors. According to the RT-qPCR results, a markedly lower miR-198 expression was shown in the in-miR-198 group when compared to the in-NC group ($P<0.05$), while the miR-198 group had a significantly higher level of miR-198 than the NC group ($P<0.05$) (Fig. 1C).

MiR-198 inhibits OVCAR3 cell proliferation, migration and invasion, and promotes apoptosis

To further investigate the functional impact of miR-198 on OC cells, cell proliferation, migration and invasion were examined after intervention with miR-198 expression. Figure 2A–B shows that down-regulation of miR-198 expression led to a marked promotion of

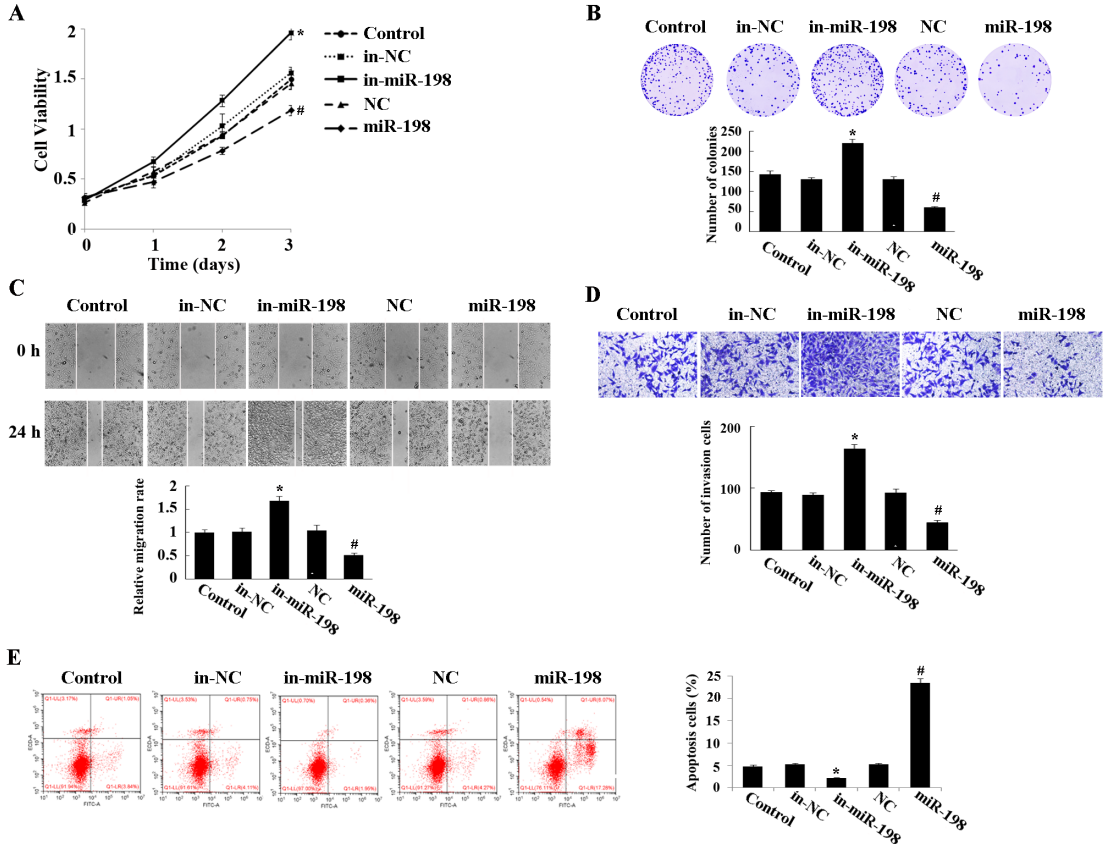

Figure 2. MiR-198 affects OVCAR3 cell proliferation, migration and invasion.
(**A, B**) MTT assay-based detection of OVCAR3 cell proliferation (**C, D**) Wound healing and transwell assay-based detection of OVCAR3 cell migration and invasion (**E**) Flow cytometry-based detection of OVCAR3 cell apoptosis. **P*<0.05 *vs*. in-NC group, #*P*<0.05 *vs*. NC group.

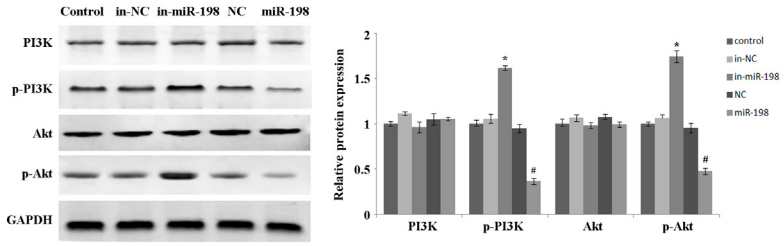

Figure 3. MiR-198 affects the activation of PI3K/Akt pathway.
The protein content of PI3K/Akt pathway was detected by Western blot. *P<0.05 *vs*. in-NC group, #P<0.05 *vs*. NC group.

OVCAR3 cell proliferation and colony formation when compared to the in-NC or NC groups (P<0.05), while miR-198 overexpression led to an inhibition of those processes (P<0.05). The results of wound healing assay (Fig. 2C) and transwell assay (Fig. 2D) showed that down-regulation of miR-198 expression has significantly promoted migration and invasion of the OVCAR3 cells when compared to the in-NC or NC groups (P<0.05), while miR-198 overexpression inhibited OVCAR3 cell migration and invasion (P<0.05). In addition, a decrease in apoptosis was found in the in-miR-198 group (P<0.05), while an increase in the miR-198 group (P<0.05). Taken together, OC cell proliferation, migration, as well as invasion could be inhibited, while apoptosis was promoted by miR-198. Therefore, we reasoned that miR-198 could act as a tumor suppressor gene of OC.

MiR-198 inhibits activation of the PI3K/Akt pathway in OVCAR3 cells

The PI3K/Akt pathway has a pivotal role in OC metastasis and recurrence, which is considered to be an important target for the OC treatment (Rodon *et al*., 2013). As can be seen in the KEGG pathway enrichment analysis based on the RNA sequencing data in the Cancer Genome Atlas (TCGA), the PI3K/Akt pathway shows a high activation in advanced OC (stage III and IV) when compared to early OC (stage II) (Xiao *et al*., 2020). Impact of miR-198 upon this pathway in OVCAR3 cells was further examined. Figure 3 shows upregulation of the p-P13K and p-Akt expression in the in-miR-198 group (P<0.05), while there is inhibition of these genes in the miR-198 group (P<0.05). Collectively, miR-198 plays an inhibitory role in OC by inhibiting activation of the PI3K/Akt pathway.

DISCUSSION

Ovarian cancer, particularly epithelial OC, is one of the most deadly gynecological malignancies. Identifying the molecular mechanism involved in OC progression contributes to providing better treatment options. Targeted therapy is effective in improving the overall survival of OC patients (Chen *et al*., 2019). MiR-198 is a therapeutic miRNA with effective therapeutic effects in lung cancer, colorectal cancer, hepatocellular carcinoma, as well as breast cancer (Gu *et al*., 2019). MiR-198 presents low expression in papillary thyroid carcinoma (Marotta *et al*., 2016), osteosarcoma (Lulla *et al*., 2011) and hepatocellular carcinoma (Tan *et al*., 2011). In addition, miR-198 is a novel inhibitor that negatively regulates invasion of hepatocellular carcinoma cells via the HGF/c-MET pathway (Tan *et al*., 2011). Chonglei and others (????) have found an under-expression of miR-198 in multiple myelomas, and a significant inhibition of multiple myeloma cell colony formation, proliferation, and migration by ectopic restoration of miR-198 (Bi *et al*., 2015). MiR-198 down-regulates the central signaling of the proliferative pathway in liver cancer. In contrast, genes mediating cell adhesion are significantly up-regulated by miR-198 (Elfimova *et al*., 2013). What's more, miR-198 promotes apoptosis in human prostate cancer, as well as in lung cancer cell lines (Li *et al*., 2018; Zhu *et al*., 2018). In conclusion, miR-198 exhibits inhibitory effects in cell proliferation, invasion, and migration, and it can also induce apoptosis, thus suppressing cancer cell growth. In our study, up-regulation of miR-198 expression caused inhibition of OC cell proliferation, migration and invasion and promoted apoptosis, suggesting that miR-198 has the potential to inhibit OC progression.

The PI3K/Akt signaling pathway participates in various cellular processes, such as cell metabolism, growth, and apoptotic death. Dysregulation of this pathway is linked to cancer occurrence and progression (Thorpe *et al*., 2015; Noorolyai *et al*., 2019). PI3K mainly phosphorylates the phosphatidylinositol secondary messenger through cell surface receptor activation and acts as an important regulator of macrophage phagocytosis. Akt binds to the PIP product of PI3K via its pleckstrin homology domain for recruitment to the plasma membrane. Activated Akt translocates into the cytoplasm or nucleus, which phosphorylates the mammalian target of rapamycin (mTOR) to promote cell growth and protein synthesis (Chaisuparat *et al*., 2016). It has been demonstrated in previous studies that the OC cell proliferation, invasion, and metastasis are promoted, while apoptosis is suppressed by abnormal activation of the PI3K/Akt pathway (Zhang *et al*., 2019). Therefore, in our study, p-PI3K and p-Akt expression in OC cells was assessed, so as to confirm the involvement of this pathway in the anticancer mechanism of miR-198 in OC. The results revealed that miR-198 overexpression has significantly inhibited the p-PI3K and p-Akt expression, while down-regulation of miR-198 had an opposite effect. According to the existing studies, down-regulated miR-198 inhibits the PI3K/AKT pathway activation via targeting PTEN, thus eventually suppressing proliferation and invasion of retinoblastoma cells (Wei *et al*., 2018). The above results suggest a regulatory role of miR-198 in OC *via* inhibition of the PI3K/Akt pathway activation.

In summary, our findings show that miR-198 is expressed at a low level in the OC tissues, and confirm that miR-198 is crucial for inhibition of the OC cell proliferation, migration, and invasion, as well as for promoting apoptosis. Therefore, we further propose that miR-198 may act as a tumor suppressor by down-regulation of the PI3K/Akt pathway activity. The current study will provide a theoretical basis and new therapeutic strategies for targeted therapy of OC. This study also has short-

comings. The number of ovarian cancer tissues is small, and the correlation between the expression of miR-198 and clinicopathological information needs to be further explored.

Declarations

Ethics approval and consent to participate: This study was approved by the Ethics Committee of Changzhou No.2 People's Hospital.

Consent for publication: Not Applicable

Competing interests: The authors have no conflicts of interest to declare.

Funding Support: No funding received.

Authors' Contribution

Study concept and design: HX, HS; Acquisition of data: YZ, JC; Analysis and interpretation of data: YZ, HX; Drafting of the manuscript: HX, HS; Critical revision of the manuscript for important intellectual content: JC, HS; Statistical analysis: HX, HS. All authors have read and approved the final version of the manuscript.

Availability of data and materials: Research data are not shared.

REFERENCES

Almeida MI, Reis RM, Calin GA (2011) MicroRNA history: discovery, recent applications, and next frontiers. *Mutat Res* **717**: 1–8. https://doi.org/10.1016/j.mrfmmm.2011.03.009

Bi C, Chung TH, Huang G, Zhou J, Yan J, Ahmann GJ, Fonseca R, Chng WJ (2015) Genome-wide pharmacologic unmasking identifies tumor suppressive microRNAs in multiple myeloma. *Oncotarget* **6**: 26508–26518. https://doi.org/10.18632/oncotarget.4769

Chaisuparat R, Limpiwatana S, Kongpanitkul S, Yodsanga S, Jham BC. (2016) The Akt/mTOR pathway is activated in verrucous carcinoma of the oral cavity. *J Oral Pathol Med* **45**: 581–585. https://doi.org/10.1111/jop.12422

Chen L, Cheng X, Tu W, Qi Z, Li H, Liu F, Yang Y, Zhang Z, Wang Z (2019) Apatinib inhibits glycolysis by suppressing the VEGFR2/AKT1/SOX5/GLUT4 signaling pathway in ovarian cancer cells. *Cell Oncol (Dordrecht)* **42**: 679–690. https://doi.org/10.1007/s13402-019-00455-x

Elfimova N, Sievers E, Eischeid H, Kwiecinski M, Noetel A, Hunt H, Becker D, Frommolt P, Quasdorff M, Steffen HM, Nürnberg P, Büttner R, Teufel A, Dienes HP, Drebber U, Odenthal M (2013) Control of mitogenic and motogenic pathways by miR-198, diminishing hepatoma cell growth and migration. *Biochim Biophys Acta* **1833**: 1190–1198. https://doi.org/10.1016/j.bbamcr.2013.01.023

Frixa T, Donzelli S, Blandino G. (2015) Oncogenic MicroRNAs: key players in malignant transformation. *Cancers* **7**: 2466–2485. https://doi.org/10.3390/cancers7040904

Gu J, Li X, Li H, Jin Z, Jin J (2019) MicroRNA-198 inhibits proliferation and induces apoptosis by directly suppressing FGFR1 in gastric cancer. *Biosci Rep* **39**. https://doi.org/10.1042/bsr20181258

Jin M, Yang Z, Ye W, Xu H, Hua X (2014) MicroRNA-150 predicts a favorable prognosis in patients with epithelial ovarian cancer, and inhibits cell invasion and metastasis by suppressing transcriptional repressor ZEB1. *PloS One* **9**: e103965. https://doi.org/10.1371/journal.pone.0103965

Köbel M, Kalloger SE, Huntsman DG, Santos JL, Swenerton KD, Seidman JD, Gilks CB; Cheryl Brown Ovarian Cancer Outcomes Unit of the British Columbia Cancer Agency, Vancouver BC (2010) Differences in tumor type in low-stage *versus* high-stage ovarian carcinomas. *Int J Gynecol Pathol* **29**: 203–211. https://doi.org/10.1097/PGP.0b013e3181c042b6

Li Y, Luo H, Xiao N, Duan J, Wang Z, Wang S (2018) Long noncoding RNA SChLAP1 Accelerates the proliferation and metastasis of prostate cancer *via* targeting miR-198 and promoting the MAPK1 pathway. *Oncol Res* **26**: 131–143. https://doi.org/10.3727/096504017x14944585873631

Loganathan S, Kandala PK, Gupta P, Srivastava SK (2012) Inhibition of EGFR-AKT axis results in the suppression of ovarian tumors in vitro and in preclinical mouse model. *PloS One* **7**: e43577. https://doi.org/10.1371/journal.pone.0043577

Lulla RR, Costa FF, Bischof JM, Chou PM, de F Bonaldo M, Vanin EF, Soares MB (2011) Identification of differentially expressed MicroRNAs in osteosarcoma. *Sarcoma* **2011**: 732690. https://doi.org/10.1155/2011/732690

Marotta V, Sciammarella C, Colao A, Faggiano A (2016) Application of molecular biology of differentiated thyroid cancer for clinical prognostication. *Endocrine-Related Cancer* **23**: R499-R515. https://doi.org/10.1530/erc-16-0372

Niu L, Zhou Y, Zhang W, Ren Y (2019) Long noncoding RNA LINC00473 functions as a competing endogenous RNA to regulate MAPK1 expression by sponging miR-198 in breast cancer. *Pathol Res Practice* **215**: 152470. https://doi.org/10.1016/j.prp.2019.152470

Noorolyai S, Shajari N, Baghbani E, Sadreddini S, Baradaran B (2019) The relation between PI3K/AKT signalling pathway and cancer. *Gene* **698**: 120–128. https://doi.org/10.1016/j.gene.2019.02.076

Odunsi K (2017) Immunotherapy in ovarian cancer. *Ann Oncol* **28**: viii1–viii7. https://doi.org/10.1093/annonc/mdx444

Program ER (2005) Surveillance epidemiology and end results (SEER) program: An overview. *National Cancer Institute*

Ramalingam P (2016) Morphologic, immunophenotypic, and molecular features of epithelial ovarian cancer. *Oncology (Williston Park NY)* **30**: 166–176. PMID: 26892153

Ray J, Hoey C, Huang X, Jeon J, Taeb S, Downes MR, Boutros PC, Liu SK (2019) MicroRNA-198 suppresses prostate tumorigenesis by targeting MIB1. *Oncol Rep* **42**: 1047–1056. https://doi.org/10.3892/or.2019.7234

Rodon J, Dienstmann R, Serra V, Tabernero J (2013) Development of PI3K inhibitors: lessons learned from early clinical trials. *Nat Rev Clin Oncol* **10**: 143–153. https://doi.org/10.1038/nrclinonc.2013.10

Siegel RL, Miller KD, Jemal A (2018) Cancer statistics, 2018. *CA: Cancer J Clin* **68**: 7–30. https://doi.org/10.3322/caac.21442

Sopik V, Iqbal J, Rosen B, Narod SA (2015) Why have ovarian cancer mortality rates declined? Part I. Incidence. *Gynecol Oncol* **138**: 741–749. https://doi.org/10.1016/j.ygyno.2015.06.017

Svoronos AA, Engelman DM, Slack FJ (2016) OncomiR or tumor suppressor? the duplicity of MicroRNAs in cancer. *Cancer Res* **76**:3666-70. https://doi.org/10.1158/0008-5472.Can-16-0359

Tan S, Li R, Ding K, Lobie PE, Zhu T (2011) miR-198 inhibits migration and invasion of hepatocellular carcinoma cells by targeting the HGF/c-MET pathway. *FEBS Lett* **585**: 2229–2234. https://doi.org/10.1016/j.febslet.2011.05.042

Thorpe LM, Yuzugullu H, Zhao JJ (2015) PI3K in cancer: divergent roles of isoforms, modes of activation and therapeutic targeting. *Nat Rev Cancer* **15**: 7–24. https://doi.org/10.1038/nrc3860

Waldmann A, Eisemann N, Katalinic A (2013) Epidemiology of malignant cervical, corpus uteri and ovarian tumours – current data and epidemiological trends. *Geburtshilfe und Frauenheilkunde* **73**: 123–129. https://doi.org/10.1055/s-0032-1328266

Wang SS, Fang YY, Huang JC, Liang YY, Guo YN, Pan LJ, Chen G (2019) Clinical value of microRNA-198-5p downregulation in lung adenocarcinoma and its potential pathways. *Oncology letters*. **18**: 2939–2954. https://doi.org/10.3892/ol.2019.10610

Wei D, Miao Y, Yu L, Wang D, Wang Y (2018) Downregulation of microRNA-198 suppresses cell proliferation and invasion in retinoblastoma by directly targeting PTEN. *Mol Med Rep* **18**: 595–602. https://doi.org/10.3892/mmr.2018.8979

Wilczyński M, Żytko E, Szymańska B, Dzieniecka M, Nowak M, Danielska J, Stachowiak G, Wilczyński JR (2017) Expression of miR-146a in patients with ovarian cancer and its clinical significance. *Oncol Lett* **14**: 3207–3214. https://doi.org/10.3892/ol.2017.6477

Xiao Y, Yu Y, Jiang P, Li Y, Wang C, Zhang R (2020) The PI3K/mTOR dual inhibitor GSK458 potently impedes ovarian cancer tumorigenesis and metastasis. *Cell Oncol (Dordrecht)* **43**: 669–680. https://doi.org/10.1007/s13402-020-00514-8

Xu F, Ni M, Li J, Cheng J, Zhao H, Zhao J, Huang S, Wu X (2020) Circ0004390 promotes cell proliferation through sponging miR-198 in ovarian cancer. *Biochem Biophys Res Commun* **526**: 14–20. https://doi.org/10.1016/j.bbrc.2020.03.024

Zhang LQ, Yang HQ, Yang SQ, Wang Y, Chen XJ, Lu HS, Zhao LP (2019) CNDP2 Acts as an activator for human ovarian cancer growth and metastasis *via* the PI3K/AKT pathway. *Technol Cancer Res Treat* **18**: 1533033819874773. https://doi.org/10.1177/1533033819874773

Zhu L, Xue F, Xu X, Xu J, Hu S, Liu S, Cui Y, Gao C (2018) MicroRNA-198 inhibition of HGF/c-MET signaling pathway overcomes resistance to radiotherapy and induces apoptosis in human non-small-cell lung cancer. *J Cell Biochem* **119**: 7873–7886. https://doi.org/10.1002/jcb.27204