Tropical Journal of Pharmaceutical Research April 2020; 19 (4): 691-698
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v19i4.3

Original Research Article

MiR-624-5p enhances cell resistance against cisplatin via PDGFRA/Stat3/PI3K axis in ovarian cancer

Lin I-Ju[1]，Tian YongJie*
Department of Obstetrics and Gynecology, Shandong Provincial Hospital Affiliated to Shandong University, Jinan, Shandong, China

For correspondence:* ***Email:* tianyongjie@sdu.edu.cn

Sent for review: 23 Feb 2020 *Revised accepted: 26 Mar 2020*

Abstract

Purpose: *The purpose of this study was to evaluate the role of miR-624-5p in ovarian cancer.*
Methods: *MiR-624-5p expression in ovarian cancer {OC) cell lines and normal cells (NCs) was evaluated and compared the differential miR-624-5p in OC A2780 cells and cisplatin-resistant OC cell line (A2780/DDP). CCK-8 was used to evaluate changes in cell viability of the A2780 and A2780/DDP cell lines as well as silenced miR-624-5p. Western Blot examined the Stat3 and phosphorylated Pi3k. The binding between PDGFRA and miR-624-5p was predicted on Targetscan and verified through Luciferase Reporter Assay. The role of PDGFRA in A2780/DDP by overexpressing PDGFRA was evaluated by RT-qPCR and CCK-8 assays. RT-qPCR assay also measured miR-624-5p expression responsive to different dosages of cisplatin and CCK8 examined viability levels correspondingly. In addition, the interplay of PDGFRA and miR-624-5p by combined downregulation of both miR-624-5p and PDGFRA were evaluated.*
Results: *OC cells had higher miR-624-5p expression than NCs but lower compared to cisplatin-resistant A2780/DDP cells. A2780/DDP cells had higher viability than OC cell line A2780. Stat3 and phosphorylated PI3K were activated in A2780/DDP cells. Silencing miR-624-5p led to lower viability in A2780/DDP cells. miR-624-5p expression dropped as the cisplatin concentration increased, resulting in decreasing viability respectively. Luciferase Reporter assay validated the binding of miR-624-5p and PDGFRA in A2780/DDP cells. Overexpressed PDGFRA induced lower cell viability in A2780/DDP cells. Downregulation of PDGFRA partially restored the lowered viability and inhibited Stat3 as well as phosphorylated Pi3k induced by miR-624-5p inhibitor.*
Conclusion: *MiR-624-5p could add to the cellular resistance to cisplatin in OC in-vitro model, which indicated that it might help unveil the mystery of drug-resistance in clinical stage of ovarian cancer.*

Keywords: *MiR-624-5p, resistance, cisplatin, PDGFRA/Stat3/PI3K, ovarian cancer*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Ovarian cancer severely affects women's health all over the world. Given its insidious onset and rapid development, mortality of ovarian cancer ranks the first in gynecologic oncology [1]. However, very few ovarian cancer patients can be detected in early stage, which is the main reason for the high death rate of the disease. The first choice of treatment of ovarian cancer is

surgery with chemotherapy, in which platinum and paclitaxel are the common chemotherapeutics agents [2,3]. In chemotherapy, however, most patients develop recurrence problem with tolerance to platinum, resulting in 5-year survival rate lower than 30% [4]. Unfortunately, the mechanism and factors related to resistance of ovarian cancer o treatment with cisplatin and how new targets agents can be developed for the disease have not been fully investigated.

In recent years, microRNAs have become key targets and hot spots in reversing chemical resistance of cancer cells. These are small molecules as well as non-coding RNAs with 20-24 nucleotides in length, which were first discovered in *Caenorhabditis elegans* [5]. Until now, more than 2500 mature microRNAs have been detected in human genome but unfortunately, the functions of most microRNAs have not been identified [6]. MicroRNAs can bind 3'UTR of targeted mRNAs to degrade or block translation process of mRNA so that microRNAs can suppress expression levels of target genes in cells [7]. Therefore, it is essential to determine the relationships between microRNAs and tumors, especially connections between cancers and resistance to drugs. Although studies about microRNAs regulating drug resistance in ovarian cancer are common, it is rare to find articles about miR-624-5p related to drug resistance in ovarian cancer.

Therefore, the purpose of this study was to evaluate the role of miR-624-5p and the functions of microRNA in the resistance of ovarian cancer to drugs.

EXPERIMENTAL

Cell culture

Human ovarian cancer cell lines (A2780, OVCAR-3 and 3AO), human normal ovarian epithelial cell line IOSE80 (NC) and cisplatin-resistant cell line A2780/DDP were purchased from ATCC (USA). The cell lines, A2780, OVCAR-3, 3AO, IOSE80 and A2780/DDP were incubated with RPMI1640 medium, 10% fetal bovine serum (FBS) (Thermo Fisher, USA), 100U/ml penicillin and streptomycin in humidified incubators at 37 ^{o}C, 5% CO_2 for 2 hr. Cell in log phase were selected for next experiments.

Transfection

The A2780/DDP cells were planted in 96-well plate and cultured until 60% confluence was obtained. MiR-624-5p inhibitor or NC inhibitor (100pmol) was transfected into A2780/DDP cells with Lipofectamine™ 3000 Transfection Reagent (Invitrogen, USA) following manufacturer's instructions so as to form two different groups, NC inhibitor and miR-624-5p inhibitor groups of A2780/DDP cells. pcDNA3.1 NC or oe-PDGFRA was transfected into A2780/DDP cells forming two sub-groups, oe-NC and oe-PDGFRA. Finally, siRNA NC and NC inhibitor, siRNA NC and miR-624-5p inhibitor, si-PDGFRA and miR-624-5p inhibitor were co-transfected in the A2780/DDP cells to form a third group.

Cisplatin treatment

Before transfection, cisplatin-resistant cell line (A2780/DDP) was selected and 0, 3,5 µmol/L cisplatin was added into the complete medium respectively. Besides, three sub-groups of A2780/DDP cells, which were co-transfected with siRNA NC and NC inhibitor, siRNA NC and miR-624-5p inhibitor or si-PDGFRA and miR-624-5p inhibitor were incubated with 0, 3, 5 µmol/L cisplatin. These were subjected to RT-qPCR and CCK8 assays:

RT-qPCR

Total RNAs were extracted from the cells using Trizol reagent (Thermo Fisher, USA) according to manufacturer's instructions. In reverse transcription, primer of miR-624-5p was stem-loop reverse transcription primer using U6 as internal reference. Condition of reverse transcription were 42 ^{o}C, 15 min; 95 ^{o}C, 3 min. Then, products in reverse transcription were evaluated using PCR. Condition for denaturation were 95 ^{o}C, 10s and extension was 60 ^{o}C, 30s, 40 cycles. Sequences of primers of PDGFRA: sense, 5'-GAGACAGGAGTACCGTGGAG-3', antisense, 5'-CACAATCACCAACAGCACCA-3'. Sequences about primers of U6 were displayed: forward, 5'-ATTGGAACGATACAGAGAAGATT-3', reverse, 5'-GGAACGCTTCACGAATTTG-3'. Data were calculated through $2^{-\triangle\triangle Ct}$ methods.

CCK-8 assay

The cells were seeded into 96-well plate with 5×10^4 cells per well. Next day, cisplatin (0, 3 and 5 µmol/l) were added into the well plate and cells were incubated at 37 ^{o}C, 5% CO_2. Then, 10µl CCK-8 was added into each well at 24 hr, 48 hr and 72 hr and the optical density values were determined using microplate reader (Thermo Fisher, USA) at 490 nm 3 hr after adding CCK-8.

Luciferase report assay

Cells were planted into 96-well plate and incubated until 70% confluence was attained. Then, pCMV-Cypridina Luc Vector for Luciferase Assays (Thermo

Fisher, USA) and NC inhibitor, miR-624-5p inhibitor and PDGFRA wt/mut were co-transfected into cells using Lipofectamine 3000. After transfection for 6 hr, medium was replaced and after 48 hr incubation, cells were rinsed with 1×PLB (Promega, USA) to lyse the cells. Pierce™ Gaussia-Firefly Luciferase Dual Assay Kit (Thermo Fisher, USA) was applied to detect luciferase activities

Western blot

Cells were first washed with phosphate buffered saline (PBS) three times, and then lysed with RIPA lysis buffer (Thermo Scientific™, USA) and 10μl PMSF (100 mmol/l) (Beyotime, Shanghai, China) on ice for 30 min and the mixture was centrifuged at 12000 rpm, 4 °C for 5 min. The proteins (40 μg) were extracted from the supernatant and BCA kit (Beyotime, Shanghai, China) was applied for quantification. The proteins were split with SDS-PAGE gel and transferred into PVDF membranes (Beyotime, Shanghai, China). After that, the membranes were rinsed with Pierce™ 20× TBS Buffer (Thermo Fisher, USA) and incubated with primary antibodies: Anti-Stat3 (1:1000; ab76315, Abcam, Cambridge, UK), anti-PI3K (1:1000; ab70912) and GAPDH (1:2000; ab181602) overnight at 4 °C. Next, membranes were washed with Tris buffered saline (TBS) three times and incubated with secondary antibodies marked by HRP: goat anti-mouse IgG (1:800; ab205719) and goat anti-rabbit IgG (1:800; ab205718) at room temperature for 2 hr and washed with TBS three times. Finally, gray bands of proteins were analyzed by BeyoECL Plus（Beyotime, Shanghai, China）with GAPDH as the internal reference.

Data analysis

Data were shown as mean ± SD using SPSS 19.0 (IBM, USA). Each experiment was repeated three times. Students' t-test and one-way ANOVA were applied. P<0.05 was considered to be significant.

RESULTS

MiR-614-5p was upregulated in cisplatin-resistant OC cells and promoted cell viability and activated STAT3/Pi3K signaling

Compared to IOSE80 cell line, miR-624-5p was expressed higher in tumor cell lines, especially in A2780 cell line (Figure 1A). Between OC cell line A2780 and drug-resistance OC cell line A2780/DDP, the expression of miR-624-5p was higher in A2780/DDP (Figure 1B). Moreover, cell viabilities in were also evaluated in A2780 and A2780/DDP cells. Compared to tumor cell line, drug resistant cell line had higher cell viabilities (Figure 1C). Then Stat3 activation and phosphorylated Pi3k were detected in both A2780 and A2780/DDP cell lines, which supported that Stat3 activity and PI3K phosphorylation were significantly increased (Figure 1D).

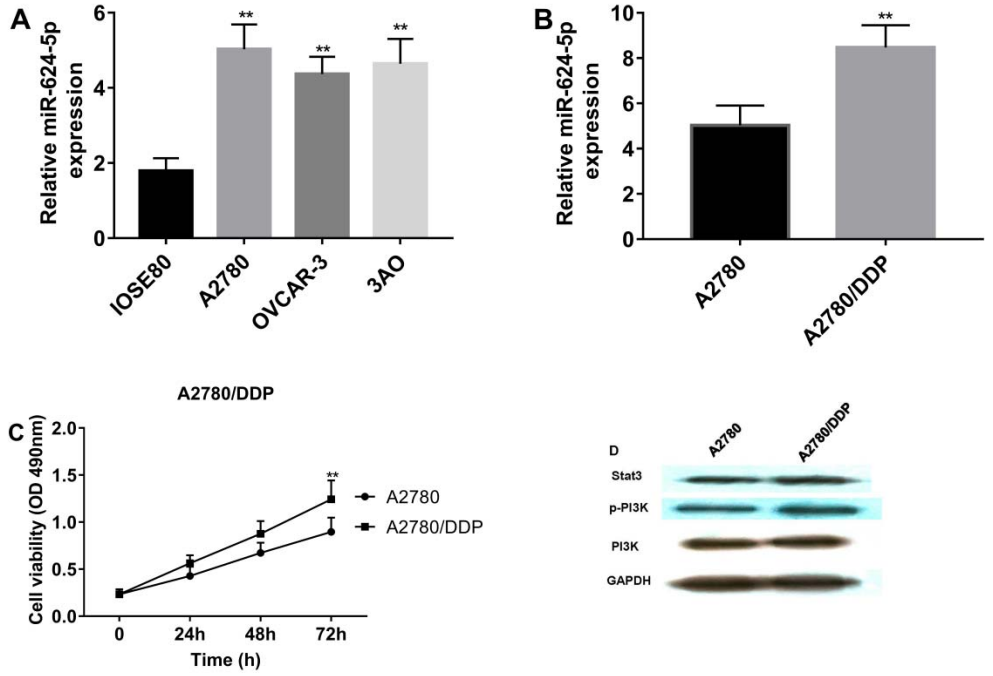

Figure 1: MiR-624-5p expressed higher in ovarian cancer cell lines and enhance cell viabilities. A. RT-qPCR was used to detect expressions of miR-624-5p in normal ovarian cell line (IOSE80) and tumor cell lines (A2780, OVCAR-3 and 3AO), P<0.05. B. MiR-624-5p expressions were validated in A2780 cell line and A2780/DDP cell line using RT-qPCR, P<0.05. C. cell viabilities were measured by CCK-8, P<0.05. D. Western blot was used to evaluate expressions of proteins, P<0.05.

Inhibition of miR-624-5p suppressed cell viabilities of cisplatin-resistant OC cells

RT-qPCR showed that miR-624-5p inhibitor could reduce expression of miR-624-5p in A2780/DDP cells (Figure 2A). CCK-8 found that miR-624-5p inhibitor could repress cell viabilities in A2780/DDP cells (Figure 2B). Further RT-qPCR discovered that miR-624-5p expression was reduced in a cisplatin dosage-dependent way (Figure 2C). It was displayed by CCK8 that as cisplatin dosage added, cell viabilities were reduced in A2780/DDP (Figure 2D).

PDGFRA was a target gene of miR-624-5p and inhibited miR-624-5p and viabilities of cisplatin-resistant OC cells

Through Targetscan (http://www.targetscan.org/vert_72/), PDGFRA was predicated to bind miR-624-5p (Figure 3A). Thus, in order to verify this, inhibited NC or miR-624-5p inhibitor and PDGFRA mutant type or PDGFRA wild type were co-transfected into A2780/DDP . Luciferase activity was significantly higher in miR-624-5p inhibitor with wild type PDGFRA than NC inhibitor with wild type PDGFRA. In mutant type of PDGFRA, luciferase activities had no significant differences (Figure 3B). Compared to parent cell line A2780, expression of PDGFRA was significantly decreased in A2780/DDP cell line (Figure 3C). Moreover, CCK8 assays indicated that overexpressed PDGFRA inhibited cell viabilities in A2780/DDP cells (Figure 3D).

MiR-624-5p inhibitor could reduce cellular resistance to cisplatin and downregulation of PDGFRA reversed partially the effect brought by miR-624-5p through Stat3/PI3K signaling pathway in ovarian cancer

Since miR-624-5p was highly expressed in A2780/DDP cells and expression of PDGFRA was low compared to OC cells, correlation between miR-624-5p and PDGFRA was analyzed. In A2780/DDP cell line, miR-624-5p inhibitor suppressed miR-624-5p while downregulation of PDGFRA restored miR-624-5p expression (Figure 4A). Moreover, Stat3 and p-PI3K were inhibited by down-regulated miR-624-5p and reactivated through suppressed in A2780 cell line (Figure 4C). As concentrations of cisplatin grew, the cell viability induced by 0,3 to 5 umol/L cisplatin, was reduced in a dosage-dependent manner (Figure 4B,D,E). Among the three groups inside each subfigure, miR-624-5p inhibitor decreased cell viability while si-PDGFRA recovered the viability in A2780/DDP cells, suggesting that the resistance to cisplatin was decreased by miR-625-5p inhibition and increased by PDGFRA downregulation (Figure 4B,D,E).

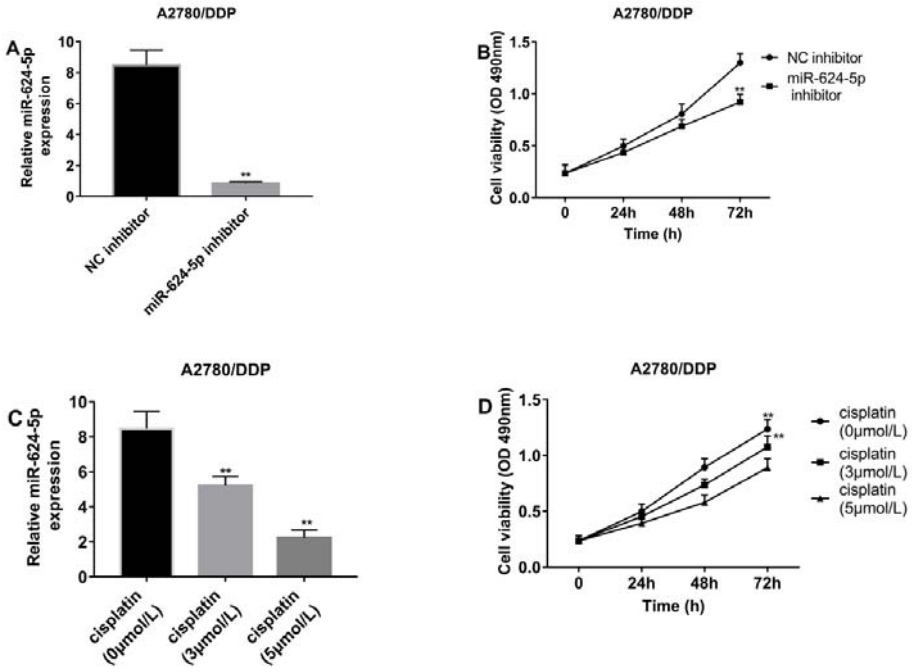

Figure 2: Cisplatin suppressed expression of miR-624-5p and inhibited cell viabilities of drug resistant cell line. A. Expressions of miR-624-5p were evaluated by RT-qPCR, P<0.05. B. cell viability was analyzed by CCK-8, P<0.05. C. Expressions of miR-624-5p with different cisplatin were examined by RT-qPCR, P<0.05. D. CCK-8 was used to detect cell viabilities with growing concentrations of cisplatin, P<0.05.

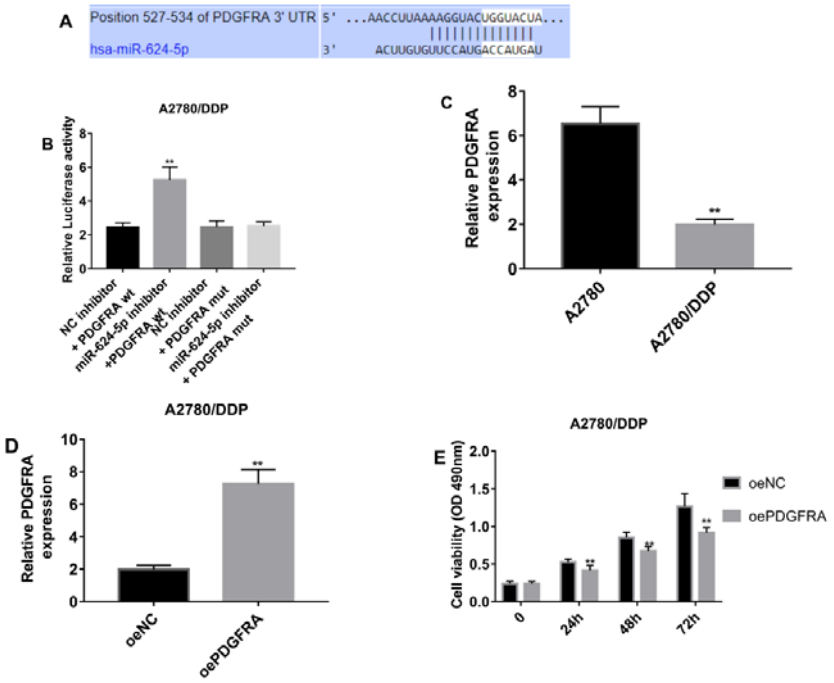

Figure 3: PDGFRA was the target gene of miR-624-5p and inhibited cell viabilities. A. Bioinformatic tool predicted binding site between miR-624-5p and PDGFRA. B. Luciferase activities showed binding conditions between wild type and mutant type PDGFRA with miR-624-5p inhibitor and NC inhibitor, P<0.05. C. Expressions of PDGFRA were evaluated by RT-qPCR, P<0.05. D. Expressions of overexpressed PDGFRA were validated by RT-qPCR, P<0.05. E. cell viabilities were analyzed by CCK-8, P<0.05.

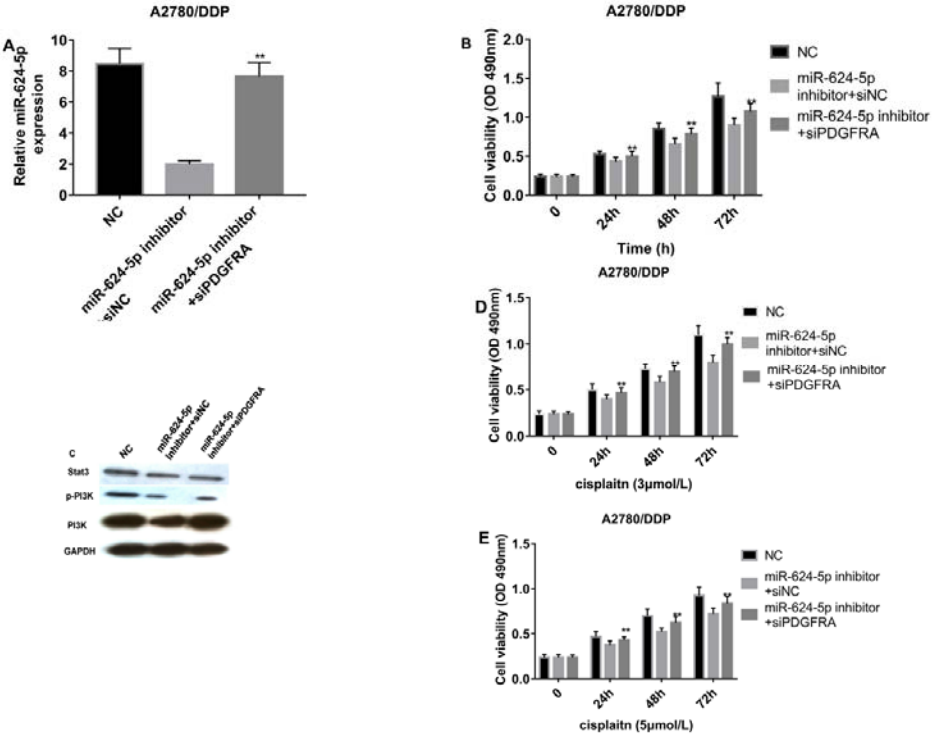

Figure 4: PDGFRA suppressed functions of miR-624-5p through Stat3/PI3K signaling pathway A. Expressions of miR-624-5p with down-regulated NC and PDGFRA were detected by RT-qPCR, P<0.05. B. cell viabilities were measured by CCK-8, P<0.05. C. Expressions of proteins were validated by western blot, P<0.05. D, E. cell viabilities with cisplatin (3μmol/L and 5μmol/L) were detected by CCK-8, P<0.05.

DISCUSSION

In the present study, the role of miR-624-5p in the cellular model in ovarian cancer was investigated by adopting cisplatin-resistant cell line to mimic the phenotype of resistance to cisplatin in ovarian patients. Results showed that miR-624-5p was upregulated in the cisplatin-resistant cell line A2780/DDP compared to normal ovarian cell line, A2780, and cell viability was higher in A2780/DDP cells than A2780. Besides, Stat3/Pi3k signaling after inhibition of miR-624-5p, lower viability in A2780/DDP cells was observed, which indicated that downregulated miR-624-5p could contain A2780/DDP cell proliferation. MiR-624-5p expression decreased as the concentration of cisplatin increased. Targetscan predicted the putative binding of miR-624-5p and PDGFRA and luciferase confirmed the binding site. Thereafter, it was observed that PDGFRA expression was lower in resistant cells than normal OC cells. Cell viability was lower in the overexpressed PDGFRA A2780/DDP cells. This suggested that PDGFRA could deter cell viability in A2780/DDP cells. When the A2780/DDP cells were co-transfected with miR-624-5p inhibitor and si-PDGFRA, it was observed that the cell viability as well as Stat3/Pi3k were recovered by PDGFRA downregulation in this co-transfected group in comparison with the miR-624-5p inhibitor group possibly indicating that PDGFRA could reverse the effect produced by miR-624-5p in the resistant cells. In other words, miR-624-5p inhibition could contribute to decrease of cisplatin resistance while PDGFRA downregulation could add to cisplatin resistance. The finding that miR-624-5p targeted and inhibited PDGFRA indicates that miR-624-5p inhibition helped to curb cellular resistance to cisplatin in ovarian cancer.

Ovarian cancer has high morbidity and 5-year survival rate is only about 30% [8]. For patients with this condition, curative effects of the chemotherapy based on cisplatin that is commonly used is limited because of resistance to cisplatin which often results in recurrence, transition and high mortality of the disease [9]. Therefore, finding a potential mechanisms of cisplatin resistance in ovarian cancer is vital. MicroRNAs are endogenous single-strand non-coding RNA with 21-25 nucleotides in length, which could degrade transcription of mRNAs [10]. It was widely unveiled that miR-130a was upregulated in ovarian cancer and helped to enhance cisplatin resistance in the disease [11]. Similarly, miR-21 induce paclitaxel resistance by targeting APAF1 in cellular model of ovarian cancer [12]. Also, miR-411 is regulated by SLC27A2 and modulated chemo-resistance in ovarian cancer both *in vitro* and *in vivo* [13]. MiR-624-5p has been identified as a regulator in osteosarcoma and hepatoblastoma [14, 15]. Since there has been no in-depth study into the role of miR-624-5p in ovarian cancer, RT-qPCRwas used in this study to check the relative expression in ovarian cancer cell lines, normal epithelial cell line as well as cisplatin-resistant cell line resulting in the finding miR-624-5p was highly expressed in cisplatin-resistant cells. CCK8 assay validated that upregulated miR-624-5p could add to cellular resistance to cisplatin in ovarian cancer. The predicted potential target gene, PDGFRA, was validated and through Oncomine Database (https://www.oncomine.org/), it was identified that a familiar mRNA PDGFRA, was among the 10% top under expressed in ovarian cancer based on the Human Genome U95A-Av2 Array (8603), Human Genome U95B Array (5939), Human Genome U95C Array (4699), Human Genome U95D Array (3584) and Human Genome U95E Array (5037) from Lu. Besides, results from this study identified lower expression of PDGFRA in resistant cells than OC cells. Previously, PDGF/PDGFR has close connections with several signaling pathways such as PI3K/AKT, JAK-STAT and Ras/MAPK [16-18]. In this study, it was discovered that overexpression of PDGFRA could suppress cellular resistance to cisplatin and the inhibition of PDGFRA could restore miR-624-5p expression in resistant cells and recover cell viability inhibited by miR-624-5p inhibitor.

Expressions of Stat3 and p-PI3K were also resumed through downregulation of PDGFRA after reduced by miR-624-5p inhibitor. STAT3 has been known to play an oncogenic role in ovarian cancer. It was recently discovered that STAT3 inhibitor could alleviate the resistance to cisplatin in ovarian cancer both in cellular model and mice model, suggesting that the treatment of ovarian cancer might be improved if Stat3 inhibitor is applied together with cisplatin [19]. STAT3 is a canonical signaling which regulates cell proliferation and apoptosis as well as metastasis in various cancers including ovarian cancer [20, 21]. Pi3k signaling modulates cellular proliferation and apoptosis and is involved in platinum resistance in ovarian cancer by regulating miRNAs and mRNAs [22, 23]. Our results agreed with these findings and enriched the regulatory mechanisms beneath Stat3/Pi3k signaling pathway in ovarian cancer.

CONCLUSION

MiR-624-5p was upregulated and targeted PDGFRA while PDGFRA was downregulated in

cisplatin resistant ovarian cancer cells. MiR-624-5p inhibitor was exposed to decrease cellular resistance against cisplatin and PDGFRA inhibition could restore the cellular resistance. Stat3/Pi3k signaling was inhibited by miR-624-5p inhibition and activated by si-PDGFRA. These findings implied that downregulation of miR-624-5p might suppress the cellular resistance to cisplatin by targeting PDGFRA via Stat3/ Pi3k signaling pathway, which suggested that there might be a possibility that miR-624-5p inhibitors could be combined with cisplatin in the treatment of ovarian cancer in clinical stage.

DECLARATIONS

Acknowledgement

None declared

Conflict of interest

No conflict of interest is associated with this study.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

REFERENCES

1. *Kuhlmann JD, Rasch J, Wimberger P, et al. microRNA and the pathogenesis of ovarian cancer--a new horizon for molecular diagnostics and treatment? Clinical chemistry and laboratory medicine. 2012; 50: 601-15.*
2. *Lengyel E. Ovarian cancer development and metastasis. The American journal of pathology. 2010; 177: 1053-64.*
3. *Torre LA, Trabert B, DeSantis CE, et al. Ovarian cancer statistics, 2018. CA: a cancer journal for clinicians. 2018; 68: 284-96.*
4. *Holschneider CH, Berek JS. Ovarian cancer: epidemiology, biology, and prognostic factors. Seminars in surgical oncology. 2000; 19: 3-10.*
5. *Lee RC, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell. 1993; 75: 843-54.*
6. *Kozomara A, Griffiths-Jones S. miRBase: annotating high confidence microRNAs using deep sequencing data. Nucleic acids research. 2014; 42: D68-73.*
7. *He L, Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation. Nature reviews Genetics. 2004; 5: 522-31.*
8. *Edwards SJ, Barton S, Thurgar E, et al. Topotecan, pegylated liposomal doxorubicin hydrochloride, paclitaxel, trabectedin and gemcitabine for advanced recurrent or refractory ovarian cancer: a systematic review and economic evaluation. Health technology assessment (Winchester, England). 2015; 19: 1-480.*
9. *Matsuo K, Eno ML, Im DD, et al. Clinical relevance of extent of extreme drug resistance in epithelial ovarian carcinoma. Gynecologic oncology. 2010; 116: 61-5.*
10. *Calin GA, Sevignani C, Dumitru CD, et al. Human microRNA genes are frequently located at fragile sites and genomic regions involved in cancers. Proceedings of the National Academy of Sciences of the United States of America. 2004; 101: 2999-3004.*
11. *Chen C, Hu Y, Li L. NRP1 is targeted by miR-130a and miR-130b, and is associated with multidrug resistance in epithelial ovarian cancer based on integrated gene network analysis. Molecular medicine reports. 2016; 13: 188-96.*
12. *Au Yeung CL, Co NN, Tsuruga T, et al. Exosomal transfer of stroma-derived miR21 confers paclitaxel resistance in ovarian cancer cells through targeting APAF1. Nature communications. 2016; 7: 11150.*
13. *Chen FD, Chen HH, Ke SC, et al. SLC27A2 regulates miR-411 to affect chemo-resistance in ovarian cancer. Neoplasma. 2018; 65: 915-24.*
14. *Indersie E, Lesjean S, Hooks KB, et al. MicroRNA therapy inhibits hepatoblastoma growth in vivo by targeting beta-catenin and Wnt signaling. Hepatology communications. 2017; 1: 168-83.*
15. *Luo Y, Liu W, Tang P, et al. miR-624-5p promoted tumorigenesis and metastasis by suppressing hippo signaling through targeting PTPRB in osteosarcoma cells. Journal of experimental & clinical cancer research : CR. 2019; 38: 488.*
16. *Hanada M, Feng J, Hemmings BA. Structure, regulation and function of PKB/AKT--a major therapeutic target. Biochimica et biophysica acta. 2004; 1697: 3-16.*
17. *Vignais ML, Sadowski HB, Watling D, et al. Platelet-derived growth factor induces phosphorylation of multiple JAK family kinases and STAT proteins. Molecular and cellular biology. 1996; 16: 1759-69.*
18. *Amagasaki K, Kaneto H, Heldin CH, et al. c-Jun N-terminal kinase is necessary for platelet-derived growth factor-mediated chemotaxis in primary fibroblasts. The Journal of biological chemistry. 2006; 281: 22173-9.*
19. *Dorayappan KDP, Wanner R, Wallbillich JJ, et al. Hypoxia-induced exosomes contribute to a more aggressive and chemoresistant ovarian cancer phenotype: a novel mechanism linking STAT3/Rab proteins. Oncogene. 2018; 37: 3806-21.*
20. *Chen L, Wang J, Wu J, et al. Indirubin suppresses ovarian cancer cell viabilities through the STAT3 signaling pathway. Drug design, development and therapy. 2018; 12: 3335-42.*
21. *Park GB, Kim D. MicroRNA-503-5p Inhibits the CD97-Mediated JAK2/STAT3 Pathway in Metastatic or Paclitaxel-Resistant Ovarian Cancer Cells. Neoplasia (New York, NY). 2019; 21: 206-15.*
22. *Gasparri ML, Besharat ZM, Farooqi AA, et al. MiRNAs and their interplay with PI3K/AKT/mTOR pathway in ovarian cancer cells: a potential role in platinum resistance. Journal of cancer research and clinical oncology. 2018; 144: 2313-18.*
23. *Zhang LQ, Yang SQ, Qu XD, et al. GRP137 promotes cell proliferation and metastasis through regulation of the PI3K/AKT pathway in human ovarian cancer. Tumori.*

2018; 104: 330-37.