Dysregulation of miR-106a and miR-591 confers paclitaxel resistance to ovarian cancer

J H Huh 1, T H Kim 1,2, K Kim 1,2, J-A Song 2, Y J Jung 2, J-Y Jeong 1,2, M J Lee 2, Y K Kim 2, D H Lee 3 and H J An *,1,2

1Department of Pathology, College of Medicine, CHA University, 351 Yatap-dong, Seongnam, Gyeonggi-Do, South Korea; 2Institute for Clinical Research, College of Medicine, CHA University, Seongnam, South Korea and 3Department of Physiology, CHA University, Seongnam, South Korea

Background: MicroRNAs are noncoding regulatory RNAs strongly implicated in carcinogenesis, cell survival, and chemosensitivity. Here, microRNAs associated with chemoresistance in ovarian carcinoma, the most lethal of gynaecological malignancies, were identified and their functional effects in chemoresistant ovarian cancer cells were assessed.

Methods: MicroRNA expression in paclitaxel (PTX)-resistant SKpac sublines was compared with that of the PTX-sensitive, parental SKOV3 ovarian cancer cell line using microarray and qRT–PCR. The function of differentially expressed microRNAs in chemoresistant ovarian cancer was further evaluated by apoptosis, cell proliferation, and migration assays.

Results: Upregulation of miR-106a and downregulation of miR-591 were associated with PTX resistance in ovarian cancer cells and human tumour samples. Transfection with anti-miR-106a or pre-miR-591 resensitized PTX-resistant SKpac cells to PTX by enhancing apoptosis (23 and 42% increase), and inhibited their cell migration (43 and 56% decrease) and proliferation (64 and 65% decrease). Furthermore, ZEB1 was identified as a novel target gene of miR-591, and BCL10 and caspase-7 were target genes of miR-106a, as identified by immunoblotting and luciferase assay.

Conclusion: MiR-106a and miR-591 have important roles in conferring PTX resistance to ovarian cancer cells. Modulation of these microRNAs resensitizes PTX-resistant cancer cells by targeting BCL10, caspase-7, and ZEB1.

Keywords: ovarian cancer; paclitaxel resistance; microRNAs; miR-106a; miR-591
expression of up to 70% of human genes, implying a potential role for miRNAs in the regulation of nearly every genetic pathway. Previous studies have revealed that miR-21 and miR-16 could have a role in the chemotherapeutic response of cholangiocarcinoma and gastric carcinoma cells, respectively. Therefore, understanding the regulatory role of miRNAs may lead to a better understanding of the molecular events involved in diverse biological processes (Hwang and Mendel, 2007), and could lead to the development of drug targets for therapeutic intervention.

Recent evidence has indicated that altered miRNA levels are related to the response to chemotherapeutic agents as well as oncogenesis. Previous studies have revealed that the PTX-resistant SKpac cells (SKpac-8, -11, -12, -16, and -17), and 39 ovarian tumour samples, including 6 serous benign tumours and 33 malignant serous carcinomas. Total RNA was extracted from fresh tissues and cell lines using TRIzol reagent (Invitrogen). The stem-loop qRT–PCR for mature miRNAs was conducted using a Bio-Rad (Redmond, WA, USA) CFX96 Real-Time PCR Detection System. All PCR reactions were run in triplicate and gene expression relative to 18S rRNA was normalised using the 2-△△Ct method (2-△△Ct).

Transfection of anti-miR inhibitors and pre-miR precursors. Chemically modified RNA-based anti-miR miRNA inhibitors (miR-106a and -96) and pre-miR miRNA precursors (miR-512, -591, -200c, and -203) were purchased from Ambion (Applied Biosystems, Foster City, CA, USA). To transfect cells, 60 pmol of miRNA precursor or 100 pmol of miRNA inhibitor was diluted in 250 μl of serum-free McCoy’s 5A media with 5 μl of lipofectamine 2000 (Invitrogen). We used mirVana miRNA inhibitor (for anti-miR transfection) or mirVana miRNA mimic (for pre-miR transfection) (Ambion) for the negative control. To validate the miRNA transfection, we used mirVana miRNA inhibitor (for anti-miR transfection) or mirVana miRNA mimic (for pre-miR transfection) (Ambion) for the negative control. To validate the

**Table 1. Significantly altered miRNAs by microRNA microarray in PTX-resistant SKpac cells compared with parent SKOV3 cells**

| miRNA   | Fold     | s.d.   | P-value | Chromosome | Putative target genes               |
|---------|----------|--------|---------|------------|-------------------------------------|
| miR-96  | 20.69    | 0.25   | 0.00    | 7q32.2     | CCNG1, RAB35, FOXO1, CASP2, SOX5, ABCD1, PPP1R9B, ATG7, RASA1, CCND2 |
| miR-106a| 16.65    | 0.54   | 0.02    | Xq26.2     | RB1, VEGFA, HIP3, CDKN1A, p21, ARID4B, Myb, BCL10, CASP7 |
| miR-629 | 10.6     | 1.25   | >0.05   | 15q23      | TP53INP2, PERP, CFLAR, RASSF8, TNIISF10D, PTPRB, CARD8, RAB12, ZEB1, AKTIP |
| miR-591 | ~372     | 0.25   | 0.00    | 7q21.3     | ZEB1, MOXD1, Cxcrf41, RANBP9, PGIT18, SPARC |
| miR-624 | ~41.22   | 6.07   | >0.05   | 14q12      | PPP6R3, CASP10, RRA52, IL20, RAB22A, SMAD2, PTN22, JAG2, CADM2, KLF4, APAF1 |
| miR-512-5p| ~37.22  | 0.67   | 0.00    | 19q13.42   | BCCL2L2, TERT, CTNMB1, CD6, TAB3, CEND1, CDK3, NOTCH3, CCNL2 |
| miR-155 | ~40.76   | 21.31  | >0.05   | 21q13.1    | MYB, FOS, MAPK14, FGF7, WEE1, RAPH1, RAB38, TP53IN1, KLF9, CARD11, RBHE |
| miR-203 | ~25.13   | 1.51   | 0.05    | 14q32.33   | RASAL2, MAP3K13, NFYJ, MYJ, TCP4, SRC, ZEB1, SMAD9, BCL7A, BCL11B, FOXK1 |
| miR-452 | ~16.97   | 2.97   | >0.05   | Xq28       | BAG4, ITGA9, RAB38, CDK6, TBK1, ROH1, NOTCH2NL, TNF, IL3, TNFAIP8, CASD1, SMAD4 |
| miR-200c| ~12.8    | 0.69   | 0.02    | 12p13.11   | ZEB1, ZEB2, LRPIB, WIF1, PTPIB, ELAVL2, DUSP1, USP25 |

The patient’s samples were divided into two groups, chemosensitive and chemoresistant, according to the responsiveness to the first-line chemotherapy based on the NCCN guidelines. Informed consent was obtained from each patient prior to surgery and this study was approved by the Ethical Committee of Bundang CHA Medical Center.

**MicroRNA extraction and miRNA microarray.** For the selection of the candidate miRNAs, the miRNA expression profiles for four PTX-resistant SKpac cell lines (SKpac-8, -11, -12, and -17) and the parent ovarian cancer cell line (SKOV3) were evaluated and compared using a bead-based miRNA microarray (Human-miRNA-V1 Bead Chips; Illumina, San Diego, CA, USA) containing 739 human precursor and mature miRNA oligonucleotide probes. MicroRNA was extracted using TRIzol reagent (Invitrogen) according to the manufacturer’s instructions. Microarray analysis was conducted following the manufacturer’s recommendations. After hybridisation, the arrays were imaged at a resolution of 0.8 μm using a BeadArray Reader. Raw data were extracted using software provided by the manufacturer (Illumina BeadStudio v3.1.3, Gene Expression Module v3.3.8). The selected gene signal values were log-transformed and normalised via the quintile method. Comparative gene expression between SKpac cells and SKOV3 cells was expressed as the fold change. All data analyses and visualisations of differentially expressed genes were conducted using ArrayAssist (Stratagene, La Jolla, CA, USA).
efficiency of transfection, miRNA expression was examined by qRT–PCR.

**TUNEL assay.** Cells (2 × 10⁵) were fixed with 75% ethanol for 2 h at −20°C. They were incubated in 0.1% Triton X-100 and 0.1% sodium citrate for 2 min on ice. Apoptotic cells were analysed using the In Situ Cell Death Detection kit (Roche, Mannheim, Germany) and detected by fluorescence-activated cell sorting (FACS) using a flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA).

**Colony-forming assay.** Cells were seeded at 1 × 10⁵ cells per well in six-well plates. The next day, cells were transfected with miRNA inhibitors or precursors and incubated for 48 h. Transfected cells were then replated at 300 cells per well in a gelatin-coated six-well culture dish. After 14 days, colonies were fixed with 4% paraformaldehyde for 10 min and then visualised using hematoxylin and counted. Groups of >50 cells were scored as colonies.

**Cell migration assay.** Cell migration was evaluated using the Oris Cell Migration Assay kit (Platypus Technologies, Madison, WI, USA). Cells were plated (2.5 × 10⁵ cells per well) in six-well plates. Twenty-four hours later, cells were transfected with miRNA inhibitors or precursors and incubated for an additional 48 h. Transfected cells were then replated at 2.5 × 10⁴ cells per well in a collagen-coated migration well. The next day, the stoppers were removed to create a detection zone. After 20 h, cells were visualised using hematoxylin, and were counted under a microscope.

**Immunoblotting.** Cells were lysed in RIPA buffer (Biotech, Seoul, Korea) and immunoblotting was performed. Primary antibodies were incubated overnight at 4°C as follows: β-actin, 1:10 000 (Cell Signaling Technologies, Danvers, MA, USA); ARID4B, 1:100 (Abnova, Taipei, Taiwan); RB1, 1:500 (Abnova); HIPK3, 1:500 (Abcam, Cambridge, UK); ZEB1, 1:500 (Abcam); MYLIP, 1:1000 (Abcam, Taipei, Taiwan); ZEB1, 1:500 (Abcam); RB1, 1:500 (Abnova); HIPK3, 1:3000 (Abcam); p21, 1:1000 (Epitomics, Burlingame, CA, USA); Caspase-7, 1:1000 (Abcam), and BCL10, 1:1000 (Epitomics).

**qRT–PCR array for apoptosis gene.** An apoptosis qRT–PCR array (Real-Time Primers, Elkins Park, PA, USA) was used to analyse the expression of apoptosis-related genes. For first-strand cDNA synthesis, 1 μg of total RNA was reverse-transcribed in a final reaction mix of 20 μl using the iScript cDNA synthesis kit (Bio-Rad), according to the manufacturer’s instructions. The qRT–PCR was performed on a CFX96 (Bio-Rad) using SYBR Green PCR Master Mix (Bio-Rad) and universal cycling conditions (10 min at 95°C, 15 s at 95°C, and 1 min 60°C for 49 cycles). The fold change in gene expression for all the genes was calculated using the Ct method (2−DDCt).
Luciferase assay. The luciferase assay was performed to assess whether miR-591 could directly repress ZEB1, and whether miR-106a directly represses caspase-7 or BCL10 in ovarian cancer cells. The 3' UTRs of ZEB1, caspase-7, and BCL10 were amplified by PCR from genomic DNA of SKpac cells. The putative binding sites for miR-591 within the 3' UTR of ZEB1, and for miR-106a within 3' UTRs of caspase-7 or BCL10 were identified using the Targetscan algorithm (targetscan.org). The wild-type or mutated binding sites were cloned separately into the NheI and XhoI sites of the pGL3-control vector (Ambion). The pGL3-control (100 ng) and pRL-TK plasmids (5 ng; for normalisation) were transfected into SKpac cells seeded in 24-well plates (3 × 10⁴ cells per well). Synthetic pre-miR-591 or pre-miR-106a (Ambion) of 20–60 nM was added to the above reactions. Luciferase activity was measured after 48 h on an Infinite 200pro series luminometer (Tecan Group, Zurich, Switzerland) using the Dual-Luciferase reporter assay system (Promega, Mannheim, Germany) according to the manufacturer’s instructions. All experiments were performed in triplicate and normalised to Renilla luciferase activity.

Statistical analysis. The significant differences between groups were determined using the Student’s t-test. Statistical analysis to determine the significant differences in the expression of each miRNA between groups of chemosensitive and chemoresistant ovarian carcinomas was conducted using the non-parametric Kruskal–Wallis test. The survival curves of ovarian cancer patients...
were estimated via the Kaplan–Meier method, and the resulting curves were compared using the log-rank test. \( P \)-values of < 0.05 were considered statistically significant. Statistical analysis was performed using the SAS statistics software package (SAS Enterprise Guide 4.1).

RESULTS

MiRNA expression patterns by microarray in PTX-resistant ovarian cancer cells. MiRNA expression in four PTX-resistant cell lines (SKpac-8, -10, -11, and -12) was compared with that of the parent SKOV3 ovarian cancer cell line. The expression profiles were determined using a bead-based miRNA microarray containing 739 human precursor and mature miRNA oligonucleotide probes. Ten miRNAs (three upregulated and seven downregulated) were determined to be deregulated by 10-fold in PTX-resistant SKpac (as compared with that of SKOV3 cells, whereas miR-591 (0.32-fold), miR-512 (0.53-fold), miR-203 (0.64-fold), and miR-200c (0.58-fold) were downregulated (Figure 1B).

To further investigate whether the above-identified miRNAs are associated with chemoresistant ovarian cancers ex vivo, qRT–PCR was performed on 6 benign serous tumours and 33 serous carcinomas (of which 20 were chemosensitive and 13 were chemoresistant). The miRNA expression in both chemoresistant and chemosensitive carcinomas was compared with that of benign tumours. Of the six miRNAs examined, miR-106a was clearly increased in 69% (9 out of 13 cases) of chemoresistant carcinomas (mean value: 3.2-fold), whereas it was increased only in 25% (5 out of 20 cases) of chemosensitive carcinomas (mean value: 1.12-fold) (\( P = 0.032; \) Figure 1C). Expression of the remaining miRNAs did not significantly differ between these two groups (Supplementary Figure S1).

We also performed the correlation of patient’s survival and miRNAs expression to evaluate their clinical impact. The patients were divided into two groups, the higher-expressing group and the lower-expressing group, by the cutoff value of two-fold expression relative to that in the benign serous tumours. Follow-up was available for all 33 patients with serous carcinomas and the mean follow-up of the study population was 29 months (range: 2–80 months). Eight patients (24%) died of disease during the follow-up period. A statistically significant difference in OS was noted (Figure 1D) between the higher-expressing and lower-expressing groups of miR-106a (OS: 50% vs 87%, \( P = 0.007 \)).

Manipulation of miR-106a and miR-591 increased PTX-induced apoptosis in SKpac cells. To assess whether miRNA modulation would affect the chemosensitivity of PTX-resistant SKpac cells,
PTX-induced apoptosis was examined by TUNEL assay. SKpac cells (SKpac-10, -16 and -17) were transfected with precursors or inhibitors of the six significantly deregulated miRNAs and treated with 80 nM PTX. Apoptosis was evaluated by flow cytometry and compared with that of PTX-treated control miRNA-transfected cells. More than 90% of the endogenous miRNA expression was downregulated by the inhibitor, and a 4×20 000-fold increase in miRNA expression was induced by the precursors (Supplementary Figure S2). PTX-induced apoptosis increased by 15% and 23% at 24 h, and 42% and 15% at 48 h after transfection with anti-miR-106a and pre-miR-591, respectively (\( P < 0.05 \); Student’s t-test), compared with that of control miRNA-transfected SKpac cells (Figure 2A and B). No significant differences were observed in response to transfection with the other miRNA precursors (miR-512 and miR-203) or inhibitor (miR-96), except with pre-miR-200c at 48 h. To confirm whether miR-106a and miR-591 have a direct function in the development of PTX resistance, a gain-of-function approach was used in PTX-sensitive parental SKOV3 cells, which express relatively low levels of miR-106a and high level of miR-591. A TUNEL assay revealed that SKOV3 cells transfected with pre-miR-106a and anti-miR-591 was markedly increased (4–4.5-fold) compared with PTX-treated, control miRNA-transfected cells (\( P < 0.05 \)).

**Figure 3. Cell migration and colony-forming assay.** (A) Cell migration across collagen-coated wells was assessed using the Oris Cell Migration Assay kit. Migration of PTX-treated (80 nM) SKpac cells transfected with pre-miR-591 or anti-miR-106a was markedly decreased compared with that of control mRNA-transfected cells. The graph represents the mean ± standard error of triplicate experiments (\( * P < 0.05 \)). (B) The mean number of colonies formed by SKpac cells transfected with anti-miR-106a or pre-miR-591 decreased compared with PTX-treated, control miRNA-transfected cells. The graph represents the mean ± standard error of triplicate experiments (\( * P < 0.05 \)). (C) Colony formation by PTX-treated SKOV3 cells transfected with pre-miR-106a or anti-miR-591 was markedly increased (4–4.5-fold) compared with PTX-treated, control miRNA-transfected cells (\( * P < 0.05 \)).
Alteration of apoptosis-related gene expression by miR-106a and miR-591.

To determine which genes or pathways are involved in the regulation of apoptosis by these miRNAs, a qRT–PCR array was performed before and after transfection of anti-miR-106a and pre-miR-591 in SKpac cells (SKpac-10, -16 and -17). Of 84 apoptosis-related genes, 14 pro-apoptotic genes were significantly increased after transfection of anti-miR-106a or pre-miR-591 (Table 2), including members of the TNF ligand and receptor families, the caspase family, DNA damage-associated genes, and BCL10. All of these genes were downregulated in the PTX-resistant SKpac cells compared with the chemosensitive SKOV3 cells, and they were statistically significant except FADD and TNFSF9. The dominant pro-apoptotic genes were TNFRSF10A for anti-miR-106a (30.68-fold), and caspase-8 for pre-miR-591 (9.15-fold). Anti-apoptotic genes were not significantly altered by manipulation of these miRNAs.

Manipulation of miR-106a and miR-591 impaired cell migration and proliferation in PTX-resistant SKpac cells. To determine if modulation of the candidate miRNAs renders SKpac cells less migratory, an Oris Cell Migration Assay was performed on SKpac cells (SKpac-10,16, 17). The number of migrating cells decreased by 43% and 56% in response to PTX treatment in cells transfected with anti-miR-106a and pre-miR-591, respectively, compared with PTX-treated, control miRNA-transfected cells (Figure 3A).

To determine if these miRNAs were involved in regulating the inhibition of cell growth by PTX, a colony-forming assay was performed. Transfection with anti-miR-106a and pre-miR-591 led to a 64% and 65% decrease, respectively, in the number of colonies formed by SKpac cells at 48 h, compared with PTX-treated, control miRNA-transfected cells (Figure 3B, $P < 0.05$, t-test).

The function of these miRNAs was confirmed using a gain-of-function approach with PTX-sensitive SKOV3 cells. The number of colonies formed by PTX-treated SKOV3 cells transfected with pre-miR-106a or anti-miR-591 was increased by 4–4.5-fold compared with PTX-treated, control miRNA-transfected cells (Figure 3C).

Identification of potential targets for miR-106a and miR-591 in ovarian cancer cells. The miRNAs can regulate a large number of target genes, and several databases are available for the prediction of the targets of selected miRNAs. Predicted target genes (Landais et al, 2007; Ivanovska et al, 2008) were identified (Table 1) via Target Scan (http://www.targetscan.org) and PicTar-Vert (http://pictar.mdc-berlin.de). To assess whether these miRNAs directly regulate the putative target genes, the genes related with oncogenesis were selected, and protein expression levels were examined by immunoblotting. The protein levels of RB1, p21, BCL10, and Caspase-7 increased two-fold after treatment with anti-miR-106a. ZEB1 expression decreased to 0.2-fold after transfection of pre-miR-591.
miR-106a and miR-591 in chemoresistant ovarian cancer

DISCUSSION

Chemoresistance is one of the most important obstacles to the successful treatment of ovarian cancer (Stordal et al., 2007). However, many evidences indicate that the mechanisms responsible for chemoresistance are likely to be multifaceted and extremely intricate (Stordal et al., 2007; Tumbarello et al., 2012).

MicroRNAs are a recently discovered class of small noncoding RNAs encoded by the genomes of a wide range of multicellular...
organisms. Accumulating evidence suggests that miRNAs can act as regulators of chemosensitivity (Meng et al, 2006; Si et al, 2007; Blower et al, 2008) in addition to regulating oncogenes or tumour suppressors (Esquela-Kerscher and Slack, 2006; Hwang and Mendell, 2007). With respect to ovarian cancer, the upregulation of miR-214 was reported to promote the survival of ovarian cancer cells and induce resistance to cisplatin (Yang et al, 2008). A recent study using high-throughput analysis of the miRNA profile in PTX-resistant cells (Sorrentino et al, 2008) demonstrated an association between some miRNAs and resistance to specific compounds for chemotherapy. Another study using a miRNA microarray with ovarian cancer tissue identified seven miRNAs differentially expressed in platinum-resistant ovarian tumours (Eitan et al, 2009). However, these previous studies only compared the expression profiles of drug-resistant and drug-sensitive cells or tissues, without functional validation documenting the cellular consequences induced by targeted inactivation or re-expression of candidate miRNAs.

In the present study, we screened and selected putative miRNAs affecting PTX resistance in ovarian cancer using a miRNA microarray and qRT–PCR, and found that the upregulation of miR-106a and downregulation of miR-591 were associated with PTX resistance. We confirmed that the modulation of these miRNAs in PTX-resistant ovarian cancer cells re-sensitized the cells to PTX, through analyses of apoptosis, cell migration, and colony formation. This was supported by a gain-of-function approach showing that the experimental upregulation of miR-106a or downregulation of miR-591 in chemosensitive parental SKOV3 cells led to decrease sensitivity to PTX.

The oncogenic potential of miR-106a has been reported in colon, pancreas, and prostate carcinomas in a large-scale miRNA microarray analysis (Volinia et al, 2006). However, the oncogenic function of miR-106a in ovarian cancer has not yet been reported, and its downregulation has been previously connected to platinum resistance (Boren et al, 2009). In the present study, however, we observed for the first time that chemoresistance and poor survival of the patient were significantly associated with upregulation of miR-106a, and that experimental downregulation of miR-106a increased the apoptotic, anti-proliferative, and anti-migratory effects of PTX on PTX-resistant ovarian cancer cells.

A role for miR-591 in oncogenesis had not been described until recently, when it was reported that miR-591 suppresses the growth of orthotopic neuroblastoma xenografts (Shooshet et al, 2011). The present study also supports that miR-591 is a tumour suppressor, by showing that the restoration of miR-591 increased apoptosis and impaired cancer cell proliferation and migration. In addition, we found that ZEB 1 is a direct target gene of miR-591, by immunoblotting and luciferase assay. The ZEB factors are transcriptional repressors that induce the epithelial–mesenchymal transition (Nakahata et al, 2010; Ohashi et al, 2010; Takeyama et al, 2010) by suppressing the expression of many epithelial genes, including E-cadherin (Vandewalle et al, 2009). The acquisition of mesenchymal phenotype is critically associated with chemoresistance (Adam et al, 2009; Ahmed et al, 2010; Marchini et al, 2012), as well as tumour cell motility and invasion. Taken together, the restoration of miR-591 reverses the sensitization of drug-resistant ovarian cancer cells to PTX, and reduces cancer cell migration and proliferation, probably by the repression of its target genes, ZEB1. This study is the first to report that miR-591 is associated with PTX resistance, and that ZEB1 is a direct target of miR-591.

As apoptotic cell death is an important factor determining the sensitivity of cancer cells to chemotherapy drugs, we also examined which genes or pathways were involved in the regulation of apoptosis by these miRNAs. The TNF ligand/receptor and caspase families were significantly increased by modulation of these miRNAs, with the highest upregulation of TNFRSF10A (miR-106a) and caspase-8 (miR-591). Interestingly, most of these genes were significantly downregulated in PTX-resistant SKpac cells, which showed high miR-106a and low miR-591 expression compared with the chemosensitive SKOV3 cells, suggesting that these genes might be regulated by these miRNAs directly, or indirectly. Among these genes, BCL10 and caspase-7 were direct target genes of miR-106a according to the Target Scan (http://www.targetscan.org), which was confirmed by immunoblotting and luciferase assay. The other genes were likely to be indirectly regulated by these miRNAs. This result is in accordance with the previous study demonstrating that protein expressions of BCL-2 and BCL10 were significantly reduced in PTX-resistant ovarian cancer cells induced by long-term etoposide treatment (Solar et al, 2008), and that inactivation of BCL10 was not related with gene mutations in ovarian cancer (Bertoni et al, 1999). Taken together, it is postulated that BCL10 is regulated by miR-106a in ovarian cancer cells, and the chemoresistance associated with miR-106a may be induced by direct regulation of BCL10 and/or caspase-7.

Overall, we found that miR-106a and miR-591 have a critical role in the development of PTX resistance in ovarian cancer cells, and that the regulation of these miRNAs can re-sensitize PTX-resistant cancer cells through the increase of many pro-apoptotic genes of TNF ligand/receptor and caspase families, and by directly targeting ZEB1, BCL10, and caspase-7. Our results suggest that the modulation of these miRNAs may be a promising therapeutic strategy for overcoming PTX resistance in ovarian cancer.
miR-106a and miR-591 in chemoresistant ovarian cancer

Bertoni F, Luminari S, Intini D, Carobbio S, Codegoni AM, Spataro V, Neri A (1999) Analysis of BCL-10 gene mutations in ovarian cancer cell lines. Br J Cancer 10(10): 1259.

Blower PE, Chung JH, Verducci JS, Lin S, Park JK, Dai Z, Liu CG, Schmittgen TD, Reinhold WC, Croce CM, Weinstein JN, Sadee W (2008) MicroRNAs modulate the chemosensitivity of tumor cells. Mol Cancer Ther 7(1): 1–9.

Boren T, Xiong Y, Hakam A, Wenham R, Apte S, Chan G, Kamath SG, Chen DT, Dressman H, Lancaster JM (2009) MicroRNAs and their target messenger RNAs associated with ovarian cancer response to chemotherapy. Gynecol Oncol 113(2): 249–255.

Cannistra SA (2004) Cancer of the ovary. N Engl J Med 351(24): 2519–2529.

Eitan R, Kushnir M, Lithwick-Yanai G, David MB, Hoshen M, Glezerman M, Cannistra SA (2004) Cancer of the ovary. N Engl J Med 351(24): 2519–2529.

Marchini S, Fruscio R, Clivio L, Beltrame L, Porcu L, Nerini IF, Cavalieri D, Landais S, Landry S, Legault P, Rassart E (2007) Oncogenic potential of the miR-106b family regulate p21/CDKN1A and promote cell cycle progression. Mol. Cell Biol 27(8): 2167–2174.

Landaiss L, Landry S, Legault P, Rassart E (2007) Oncogenic potential of the miR-106-363 cluster and its implication in human T-cell leukemia. Cancer Res 67(12): 5699–5707.

Esquela-Kerscher A, Slack FJ (2006) Oncomirs—microRNAs with a role in cancer. Nat Rev Cancer 6(4): 259–269.

Hwang HW, Mendell JT (2007) MicroRNAs in cell proliferation, cell death, and tumorigenesis. Br J Cancer 96(Suppl): R40–R44.

Ivanovskva I, Ball AS, Diaz RL, Magnus JF, Kibukawa M, Schelter JM, Kobayashi SV, Lim L, Burchard J, Jackson AL, Linsley PS, Cleary MA (2008) MicroRNAs in the miR-106b family regulate p21/CDKN1A and promote cell cycle progression. Mol. Cell Biol 28(7): 2167–2174.

Landais S, Landry S, Legault P, Rassart E (2007) Oncogenic potential of the miR-106-363 cluster and its implication in human T-cell leukemia. Cancer Res 67(12): 5699–5707.

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

Marchini S, Fruscio R, Clivio L, Beltrame L, Porcu L, Nerini IF, Cavalieri D, Chiorino G, Cattoretti G, Mangioni C, Milani R, Torri V, Romualdi C, Zambelli A, Romano M, Signorelli M, Giandomenico SD, D'Incalci M (2012) Resistance to platinum-based chemotherapy is associated with epithelial to mesenchymal transition in epithelial ovarian cancer. Eur J Cancer 48(2): 520–530.

Meng F, Henson R, Lang M, Welbe H, Maheshwari S, Mendell JT, Jiang J, Schmittgen TD, Patel T (2006) Involvement of human micro-RNA in growth and response to chemotherapy in human cholangiocarcinoma cell lines. Gastroenterology 130(7): 2113–2129.

Nakahata S, Yamazaki S, Nakauchi H, Morishita K (2010) Downregulation of ZEB1 and overexpression of Smad7 contribute to resistance to TGF-beta-mediated growth suppression in adult T-cell leukemia/lymphoma. Oncogene 29(29): 4157–4169.

Nam EJ, Yoon H, Kim SW, Kim H, Kim YT, Kim JH, Kim JW, Kim S (2008) MicroRNA expression profiles in serous ovarian carcinoma. Clin Cancer Res 14(9): 2690–2695.

Ohashi S, NatsuiZaka M, Wong GS, Michaylira CZ, Grugan KD, Stairs DB, Kalabis J, Vega ME, Kalman RA, Nakagawa M, Klein-Szanto AI, Heylin M, Diehl JA, Rustgi AK, Nakagawa H (2010) Epidermal growth factor receptor and mutant p53 expand an esophageal cellular subpopulation capable of epithelial-to-mesenchymal transition through ZEB transcription factors. Cancer Res 70(10): 4174–4184.

Shohet JM, Ghosh R, Coarfa C, Ludwig A, Benham AL, Chen Z, Patterson DM, Barbieri E, Mestdagh P, Sikorski DN, Milosavljevic A, Kim ES, Gunaratne PH (2011) A genome-wide search for promoters that respond to increased MYCN reveals both new oncogenic and tumor suppressor microRNAs associated with aggressive neuroblastoma. Cancer Res 71(11): 3841–3851.

Si ML, Zhu S, Wu H, Lu Z, Wu F, Mo YY (2007) miR-21-mediated tumor growth. Oncogene 26(19): 2799–2803.

Solar P, Feldman L, Jeong JY, Busingye JR, Sytkowski AJ (2008) Erythropoietin treatment of human ovarian cancer cells results in enhanced signaling and a paclitaxel-resistant phenotype. Int J Cancer 122(2): 281–288.

Sorrentino A, Liu CG, Addario A, Peschle C, Scambia G, Ferlini C (2008) Role of microRNAs in drug-resistant ovarian cancer cells. Gynecol Oncol 111(3): 478–486.

Stordal B, Pavlakis N, Davey R (2007) A systematic review of platinum and taxane resistance from bench to clinic: an inverse relationship. Cancer Treatment Rev 33(8): 688–703.

Takayama Y, Sato M, Horio M, Hase T, Yoshida K, Yokoyama T, Nakashima H, Hashimoto N, Sekido Y, Gazdar AF, Minna JD, Kondo M, Hasegawa Y (2010) Knockdown of ZEB1, a master epithelial-to-mesenchymal transition (EMT) gene, suppresses anchorage-independent cell growth of lung cancer cells. Cancer Lett 296(2): 216–224.

Tumbaradel OA, Temple J, Brunton JD (2012) s33 integrin modulates transforming growth factor beta induced (TGFBI) function and paclitaxel response in ovarian cancer cells. Mol Cancer 11: 36.

Vandewalle C, Van Roy, Berx G (2009) The role of the ZEB family of transcription factors in development and disease. Cell Mol Life Sci 66(5): 773–787.

Volina S, Calin GA, Liu CG, Amba S, Cimmino A, Petrocca F, Visone R, Iorio M, Roldo C, Ferracin M, Prueitt RL, YanaHara N, Lanza G, Scarpa A, Vecchione A, Negrini M, Harris CC, Croce CM (2006) A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci USA 103(7): 2257–2261.

Winter 3rd WE, Maxwell GL, Tian C, Carlson JW, Ozols RF, Rose PG, Markman M, Armstrong DK, Muggia AF, McGuire WP (2007) Prognostic factors for stage III epithelial ovarian cancer: a Gynecologic Oncology Group Study. J Clin Oncol 25(4): 3621–3627.

Xia L, Zhang D, Du R, Pan Y, Zhao L, Sun S, Hong L, Liu J, Fan D (2008) miR-15b and miR-16 modulate multidrug resistance by targeting BCL2 in human gastric cancer cells. Int J Cancer 122(2): 372–379.

Yan H, Kong W, He L, Zhao J, O’Donnell JD, Wang J, Wenham R, Coppola D, Kruk PA, Nicosia SV, Cheng QJ (2008) MicroRNA expression profiling in human ovarian cancer: miR-214 induces cell survival and cisplatin resistance by targeting PTEN. Cancer Res 68(2): 425–433.