European Review for Medical and Pharmacological Sciences 2016; 20: 2526-2531

Evidence for miR-17-92 and miR-134 gene cluster regulation of ovarian cancer drug resistance

H. ZHU, S.-Y. YANG, J. WANG, L. WANG, S.-Y. HAN

Department of Obstetrics and Gynecology, The Forth Affiliated Hospital of Harbin Medical University, Harbin, Heilongjiang Province, P. R. China

Abstract. – OBJECTIVE: Ovarian cancer treatments are often impeded by drug resistance. It has been proposed that microRNA (miRNA) expression patterns may play a role in drug resistance among ovarian cancers. The present study investigated the relationship between resistance to the cancer drug paclitaxel and miRNA expression.

MATERIALS AND METHODS: We compared the expression patterns of miRNA genes in paclitaxel-sensitive (SKOV3) and paclitaxel-resistant (SKOV3-TR30) cell lines.

RESULTS: Expression of the miR-134 gene cluster was found to be significantly lower in the paclitaxel-resistant cell line than in the paclitaxel-sensitive cell line, while the expression of the miR-17-92 gene cluster was significantly higher in the paclitaxel-resistant cells. An analysis of miRNA target gene protein expression revealed that several targets of miR-17-92 were significantly altered between the two cell types.

CONCLUSIONS: The higher expression of miR-17-92 and lower expression of mi-134 and the associated alterations of target gene expression may be associated with the drug-resistant nature of some ovarian cancers.

Key Words:
Ovarian cancer, Chemoresistance, miR-17-92, miR-134, Polymorphisms.

Introduction

Ovarian cancer is an increasingly common malignant tumor of the female reproductive tract. The incidence of ovarian cancer is second only to cervical and uterine body cancers, making it the third most prevalent cancer[1]. The mortality rate of epithelial ovarian cancer ranks highest among gynecologic tumors and, in 2007, approximately 15,000 people died from ovarian cancer in the United States[2,3]. There is mounting evidence that the high mortality rate may be due to cancer drug resistance[4-6].

Cancer drug resistance has been linked to the deletion or abnormal expression of microRNAs (miRNAs) in multiple types of tumors[7-9]. For example, miR-155 takes part in a sequence of bioprocesses that contribute to the development of drug resistance of breast cancer, including repression of FOXO3a, enhancement of epithelial-to-mesenchymal transition (EMT) and mitogen- activated protein kinase (MAPK) signaling, reduction of RhoA, and affecting the length of telomeres[10]. The lower expression of miR-340 was demonstrated to be involved in the development of cisplatin (CDDP) resistance in a hepatocellular carcinoma cell line, at least partly due to regulating the NRF2-dependent antioxidant pathway[11]. Similarly, miR-22 inhibited autophagy and promoted apoptosis to increase the sensitivity of colorectal cancer (CRC) cells to 5-fluorouracil (5-FU) treatment both *in vitro* and *in vivo*, so it was considered as both a predictor of 5-FU sensitivity for personalized treatment and a therapeutic target for colorectal cancer[12]. Here, we investigated the relationship between miRNA expression and paclitaxel resistance in ovarian cancer cells by comparing the expression patterns of miRNA gene clusters and assessing predicted target gene expression in paclitaxel-sensitive and paclitaxel-resistant cell lines.

Materials and Methods

Cell Culture and Total RNA Extraction

SKOV3 (Cell Bank, Shanghai Institute for Biological Science, Chinese Academy of Science, Shanghai, China) and SKOV3-TR30 ovarian cancer cells were cultured in medium composed of 10% fetal bovine serum (FBS), 100 μg/mL strep-

Corresponding Author: Shi-yu Han, MD; e-mail: hsy0451@126.com

tomycin, and 100 μg/mL penicillin. The drug resistance of SKOV3-TR30 ovarian cancer cells was treated with 30 nmol/L paclitaxel (Beijing SL Pharmaceutical Co., Ltd, China). Cells were cultured at 37°C, 5% CO_2, and in saturated humidity. Cells in the logarithmic phase were harvested for RNA using an RNA isolation kit (Merck, White House Station, NJ) and ion exchange adsorption column. The concentration and purity of total RNA were then analyzed by OD (Optical Density) values, as well as integrity by agarose gel electrophoresis.

Microarrays and Real-Time PCR

The Affymetrix GeneChip® miRNA 3.0 version (Affymetrix, Inc., Santa Clara, CA, USA), which contains 2999 probes for detection of precursor miRNA hairpins, was used. Following RNA isolation, fluorescent labels were added to the poly-A tails and microarrays were hybridized, washed, and stained. A relative value <1 indicated low expression of miRNA in the SKOV3-TR30 cells, while a relative value >1 indicated high expression of miRNA in the SKOV3-TR30 cells compared to the SKOV3 cells. Microarray analysis was conducted by the Shanghai Generay Biotech Company (Shanghai, China). Microarray chip results were verified by real-time PCR (PCR primers and TaqMan microRNA kits were purchased from Promega Corporation, Madison, WI, USA). β-actin served as an internal control for each sample.

miRNA Analysis and Western Blotting

Bioinformatics software (TargetScan, miRBaseTargets, miRanda, and Pictar) was used to predict at least 3 possible target genes of the miRNAs highlighted by the microarray results. Protein was isolated from cells in the logarithmic growth phase and stored at -80°C. Proteins were electrophoresed and transferred to a membrane, then visualized after the membrane was blocked and washed. The internal control was β-actin.

Statistical Analysis

Double data entry was performed using EpiData version 3.1 (EpiData Software, Denmark). SAS 9.2 (SAS Institute, Cary, NC, USA) was used to perform the analysis of variance and *t*-tests. A p value <0.05 was considered to be statistically significant.

Results

miRNA Microarray

In comparison with SKOV3, a total of 103 miRNAs, including miR-134, miR-196b, and miR-34 were lowly expressed and 70 miRNAs including miR-19b, miR-17, miR-92-1, and miR-210 were highly expressed in SKOV3-TR30 ovarian cancer cells. The altered expression of the miR-17-92 and miR-134 clusters appeared to be tied to ovarian cancer drug sensitivity (Tables I-II).

Table I. Expression of miR-134 gene clusters in SKOV3-TR30 and SKOV3 ovarian cancer cell lines.

Probe ID	SKOV3-TR30		SKOV3		
	Relative expression levels	Reliability	Relative expression levels	Reliability	Ratio
hsa-miR-382	14.205	0.684	79.531	1.990 E-8	0.179
hsa-miR-409-3p	22.610	0.305	223.107	1.037 E-6	0.101
hsa-miR-134	39.857	0.011	124.912	8.014E-6	0.319
hsa-miR-376c	23.591	0.248	59.020	5.001 E-6	0.400
hsa-miR-379	29.013	0.180	106.933	2.041 E-8	0.271
hsa-miR-381	10.356	0.990	53.691	5.762 E-7	0.193
hsa-miR-487b	38.140	0.017	154.973	2.036 E-8	0.246
hsa-miR-654-3p	17.980	0.426	47.491	3.801 E-5	0.379
hsa-miR-409-5p	14.230	0.734	85.715	2.016 E-8	0.166
hsa-miR-299-3p	27.014	0.249	64.890	1.891 E-7	0.416
hsa-miR-487a	16.054	0.524	59.761	9.251 E-7	0.269
hsa-miR-485-3p	10.287	0.857	31.146	0.046	0.330
hsa-miR-154	9.976	0.925	44.893	0.0001	0.222

Note: Ratio = Sample test values of SKOV3-TR30/Sample test values of SKOV3.

Table II. Expression of miR-17-92 gene clusters in SKOV3-TR30 and SKOV3 ovarian cancer cell lines.

Probe ID	SKOV3-TR30	SKOV3	SKOV3-TR30	SKOV3	
	Relative expression levels	Reliability	Relative expression levels	Reliability	Ratio
hsa-miR-17	131.625	2.401 E-6	57.310	3.504 E-7	2.297
hsa-miR-92-1	50.017	0.003	23.472	0.098	2.131
hsa-miR-19b	2135.021	2.101E-8	624.871	2.101 E-8	3.417
hsa-miR-20a	317.751	2.110 E-8	814.972	2.110 E-8	0.390

Note: Ratio = Sample test values of SKOV3-TR30/Sample test values of SKOV3.

miR-17-92 and miR-134 Expression

Real-time PCR was used to detect miR-17-92 and miR-134 expression levels. miR-17-92 levels in SKOV3-TR30 cells were set to 1 and served as a control group. The relative expression of miR-17-92 in SKOV3 cells was 0.561 ($p<0.05$). When miR-134 levels in SKOV3-TR30 cells were set to 1 and served as a control group, the relative miR-134 expression in SKOV3 cells was 1.297 ($p<0.05$).

miRNA Target Gene Prediction and Protein Expression

Bioinformatics software was used to predict the target genes of miR-17-92 and miR-134. The software identified 30 target genes for miR-17-92, including PTEN, ABCA1, and BIM, and 128 target genes for miR-134, including Cdc42, MRP1/ABCC1, and c-Myc.

The expression of BIM was lower in SKOV3-TR30 cells than in SKOV3 cells, as shown by Western blot, while the expression of c-Myc in SKOV3-TR30 cells was higher than in SKOV3 cells. Both differences were statistically significant (p values <0.05). The expression of PTEN in SKOV3-TR30 cells appeared to be higher than in SKOV3 cells, but the difference was not statistically significant (Table III).

Discussion

Altered expression of microRNAs has been seen in multiple types of tumors, and has been implicated in chemotherapeutic drug resistance[7-9]. For example, miR-200 expression has been shown to increase sensitivity to paclitaxel chemotherapy[13], while miR-125b expression produced drug resistance in ovarian cancer cells (C13*)[14]. Altered miR-182 expression has also been shown to lead to drug resistance in ovarian cancer cells[15].

In this study, microarray analysis was used to compare the expression profiles of miRNAs in paclitaxel-resistant and paclitaxel-sensitive ovarian cancer cell lines, SKOV3-TR30 and SKOV3. 103 miRNAs, including miR-134, miR-196b, and miR-34 were lowly expressed, while 70 types of miRNAs, including miR-19b, miR-17, miR-92-1, and miR-210 were highly expressed in SKOV3-TR30. We chose to further investigate the miR-17-92 and miR-134 clusters given their correlation with drug resistance. Real-time PCR showed that miR-17-92 expression was increased, while miR-134 expression was decreased in drug resistant ovarian cancer cells, suggesting that both clusters may be involved in drug resistance.

To further investigate the mechanism underlying cancer drug resistance, we used bioinforma-

Table III. The c-Myc, BIM, and PTEN protein levels (relative grayscale).

c-Myc/GAPDH	BIM/actin	PTEN/actin	
SKOV3-TR30 cells	0.135±0.003	0.212±0.089	0.430±0.141
SKOV3 cells	0.103±0.001	0.269±0.122	0.411±0.127
t	16.81	4.021	2.745
p	0.003	0.023	0.073

Note: Ratio = Sample test values of SKOV3-TR30/Sample test values of SKOV3.

tics analysis software to predict the target genes of miR-17-92 and miR-134 clusters. Thirty target genes for the miR-17-92 cluster were identified, including PTEN, ABCA1, and BIM. 128 target genes for the miR-134 cluster were identified, including CDC42, MRP1/ABCC1, and c-Myc. The expression of BIM in SKOV3-TR30 cells was found to be significantly lower than in SKOV3 cells, while the expression of c-Myc in SKOV3-TR30 cells was significantly higher than in SKOV3 cells.

miR-17-92 is a highly conserved gene cluster, located in the 3rd intron on human chromosome 13[16]. miR-17-92 is highly expressed in breast and ovarian cancers and has been shown to be involved in cell proliferation in multiple types of tumors. miR-17-92 expression has also been shown to improve the activity of the estrogen receptor (ER) and some transcription factors[17]. The miR-17-92 cluster is composed of two paralogues, miR-106b-25 and miR-106a-363, and the carcinogenic effects of the miR-17-92 cluster may be due to an interaction between the two. We detected the protein expression levels of the predicted target genes, PTEN and BIM. Expression of BIM in SKOV3-TR30 cells was significantly lower than in SKOV3 cells, while the expression of PTEN in SKOV3-TR30 cells appeared higher than in SKOV3 cells; however, the difference was not statistically significant.

miR-134, located at human chromosome 14q32.31, has been previously shown to decrease in chemoresistant ovarian cancer[18,19]. In our study, the predicted target genes of the miR-134 cluster included c-Myc, an oncogene, whose proliferation rate in ovarian cancer reaches over 50% [20,21]. We detected the protein expression of c-Myc, and found that it was significantly increased in SKOV3-TR30 cells compared to SKOV3 cells, implicating miR-134 in drug resistance.

Conclusions

Taken together, these results suggest that the differential expression of mi-R-17-92 and miR-134 in a paclitaxel-resistant cell line may underlie the drug-resistant nature of some ovarian cancers. An analysis of target gene expression suggested that the microRNAs may act to promote drug resistance by regulating BMI and c-Myc, which are both involved in cell proliferation. Future studies will further investigate the mechanisms by which miRNAs control cancer drug resistance and explore opportunities to improve ovarian cancer treatment options.

Conflicts of interest

The authors declare no conflicts of interest.

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