

Review Article

MicroRNA profile of cisplatin resistant ovarian carcinoma

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Abstract. Chemotherapy is a major part of cancer therapy with DNA-damaging drugs being effective in clinical practice. One such drug, cisplatin, has significant anti-tumor activity against a wide variety of solid tumors and is currently approved to treat metastatic ovarian cancer and testicular cancers. Resistance to cisplatin is a major barrier to successful treatment. On the other hand, microRNAs have potential involvement in drug resistance. Here, we briefly review the concept of drug resistance particularly as related to the cisplatin and the microRNA profiles associated with development of cisplatin resistance in ovarian carcinoma based on the available literature including our own data. As for our experiments, a panel of cisplatin-resistant ovarian carcinoma cell lines A2780/CP, 2008/C13, and IGROV-1/CP and their corresponding parental cell lines were cultured in growth medium in the presence of 1 μmol cisplatin as stimulant. Small RNAs were isolated using mirVana Kit and subjected to a high throughput microRNA profile analysis. Statistical and clustering analyses were performed using ANOVA and paired *t*-tests. Validation of the microRNA results was by real time qRT-PCR. Previously, differential microRNA microarray profiles have demonstrated the involvement of a number of microRNAs in platinum-based drug resistant ovarian cancer cell lines. These included upregulation of miR-130a, miR-27a, miR-451, miR-214 and downregulation of let-7i in the drug-resistant cell lines. In addition, higher expression of miR-27a and miR-23a was observed in drug-resistant ovarian cancer tissues which were significantly correlated with prognosis. The results of our own experiments revealed upregulation of miR-23a and miR-23b in cisplatin resistant A2780/CP and 2008/C13 ovarian cancer cell line. Taken together, these studies demonstrate that overexpression of distinct microRNAs may provide new targets for the development of novel therapeutics to overcome cisplatin resistance in ovarian cancer and prompt further in-depth studies on miR-23 family and miR-27a in relation to cisplatin resistance.

Keywords: Ovarian carcinoma cells, cisplatin resistance, microRNA profile

Introduction and background

Ovarian cancer is one of the 10 leading causes of death in women and chemotherapy remains as a major approach in the management of this disease. In 2018, approximately 22,240 new cases of ovarian cancer will be diagnosed leading to 14,070 ovarian cancer deaths in the United States [1]. The overall ovarian cancer incidence rate is estimated at 11.8 per 100,000 and the mortality rate is estimated at 6.7 per 100,000 [1]. A main obstacle to the successful treatment of ovarian cancer is the development of resistance by the cancer cells to the available chemotherapeutic agents.

In general, cancer drug resistance is considered as a multifunctional phenomenon that involves several different mechanisms [2, 3]. These mechanism(s) include the decreased uptake or influx of drugs which reduces its efficacy and thus subsequently reduces apoptosis or cell death; increased efflux of drugs out of cells mediated by transporters which diminishes the ability of drugs to kill the cancer cells; increased repair of DNA damage which occurs in tolerance to DNA damage caused by the drug; increased metabolism of drugs leading to rapid biochemical

modification or degradation of the drugs; and increase in glutathione S-transferases expression which detoxicates and inactivates the drug and topoisomerase II enzymes which catalyze the formation of transient single or double-stranded DNA and promotes re-joining of the DNA strands, thus promoting DNA replication, transcription and recombination. Additional mechanisms are: altered drug targets and mutation of drug targets which can compromise drug efficacy.

In particular, cisplatin is a DNA-damaging drug used in the treatment of a variety of cancers [4]. Cisplatin and its analogs are heavy metal complexes containing a central atom of platinum (Pt) surrounded by two chloride atoms and two ammonia molecules in the cis position. It has biochemical properties similar to that of bifunctional alkylating agents. Its anti-cancer toxicity is attributed to the ability to form intra-strand DNA adducts or cross-linkages most commonly that inhibit polymerases involved in DNA repair.

The mechanism by which cells develop resistance to cisplatin is an area of intense research because it is one of

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TABLE 1
DIFFERENTIALLY EXPRESSED MICRORNAS ASSOCIATED WITH DRUG RESISTANCE IN
OVARIAN CARCINOMA CELL LINES OR TISSUES

Cell line/tissue	MicroRNA (target)	Drug	Reference
A2780	↓ miR-130a (M-CSF)	Paclitaxel/cisplatin	Sorrentino A et al, 2008
A2780	↑ miR-27a, miR-451 (MDR1)	Doxorubicin/vinblastine	Zhu H et al, 2008
A2780	↑ miR-214 (PTEN)	Cisplatin	Yang H et al, 2008
SKOV3, 2008, OVCAR10	↓ let-7i (K-RAS, HMGA2)	Cisplatin	Yang N et al, 2008
Tumor tissues	↑ miR-27a, miR-23a	Cyclophosphamide+cisplatin or Paclitaxel+carboplatin	Eitan R et al, 2009
A2780	↑ miR-130b	Paclitaxel/cisplatin	Zong C et al, 2014
A2780	↑ miR-23a	Cisplatin	Jin AH et al, 2015

the major impediments to the clinical success of this drug. These mechanisms can be arbitrary grouped into two broad categories:

- 1) Mechanism(s) that affect the amount of damage in the DNA such as inactivation of cisplatin by glutathione S-transferase and increase in DNA repair.
- 2) Mechanism(s) that affect the response of cells to this damage such as changes in cell cycle check-points or apoptosis through alterations in the key regulatory proteins, such as p53.

Platinum-induced apoptosis is believed to be the primary mechanism by which platinum exerts its anti-tumor effects. However, it is believed that cisplatin resistance arises through multiple mechanisms, but the relationships and interactions between the various pathways leading to resistance in cancer cells are not completely understood.

Currently extensive efforts have been directed toward research on coping with drug resistance through various aspects. In this context, regulation of gene function certainly plays an important role. Thus, the regulatory modulators of genes are of critical concern.

The discovery of small non-coding RNAs called microRNAs as gene regulatory modulators has provided ample ground for research into their functions [5]. MicroRNAs are 18 to 24 nucleotide sequences that have transcriptional inhibitory functions. At present, around 2693 mature microRNAs have been identified in humans (latest miRBase version 22).

The involvement of microRNAs in cancer is an area of intense research. MicroRNAs play a critical role in the initiation and progression of human cancer. They are involved in cell proliferation, cell death and may act as oncogenes and tumor suppressors. MicroRNAs have been implicated as important players in drug resistance as well.

In this review, we summarize the current knowledge about the microRNA profiles associated with development of cisplatin resistance in ovarian carcinoma and signify

potential microRNAs deserving further in-depth investigation to overcome cisplatin-resistance.

Review

A review of the existing literature on microRNA and drug resistance in ovarian carcinoma cell lines or tissues points to specific microRNAs associated with anti-cancer drug resistance (Table 1). For instance, microRNA profile analysis in a panel of paclitaxel- (A2780TAX, A2780TC1 and A2780TC3) and cisplatin-resistant (A2780CIS) cells showed that six microRNAs (let-7e, miR-30c, miR-125b, miR-130a and miR-335) were diversely expressed in all the resistant cell lines [6]. Let-7e was upregulated in A2780TAX cells and downregulated in the other resistant cell lines. The opposite phenomenon was obtained for miR-125b, which was downregulated in A2780TAX and upregulated in the other cell lines. The miR-30c, miR-130a and miR-335 were downregulated in all the resistant cell lines, thereby suggesting a direct involvement in the development of chemoresistance. Finally downstream target validation was confirmed for the miR-130a, whose downregulation was linked to the translational activation of the M-CSF gene, a known resistance factor for ovarian cancer. In another study, miR-27a and miR-451 were upregulated in multi-drug resistant variant of A2780 ovarian carcinoma cell line (A2780DX5) [7]. Inhibition of miR-27a and miR-451 decreased the expression of P-glycoprotein and MDR1 mRNA while introduction of miR-27a and miR-451 increased MDR1 expression, suggesting a role in the regulation of MDR1/P-glycoprotein expression. In other study, upregulation of miR-214 was found in cisplatin resistant A2780 cells [8]. It was shown that miR-214 induces cell survival and cisplatin resistance by targeting PTEN which leads to down-regulation of PTEN protein and activation of Akt (survival) pathway. Further study in other ovarian carcinoma cell lines (SKOV3, 2008, OVCAR10) showed significantly downregulated let-7i in cisplatin resistant cells [9]. Let-7 inhibits several well-characterized oncogenic proteins such as K-RAS, HMGA2, c-Myc, and

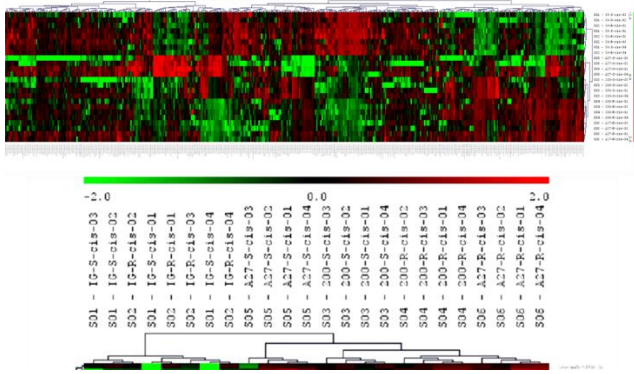


Figure 1 Clustering graph of all miRNAs with signal intensity >32

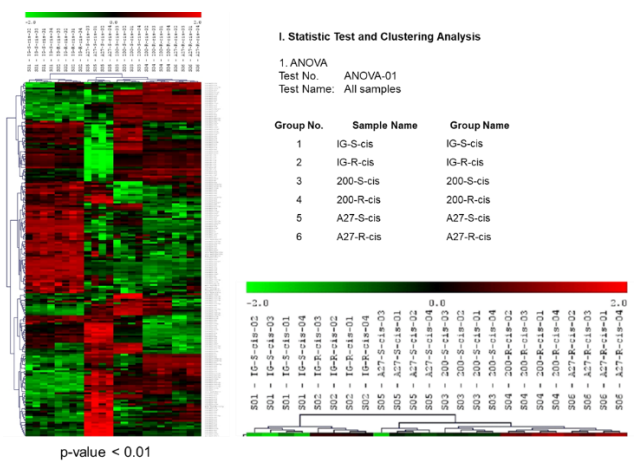


Figure 2. Statistic test and clustering of all cisplatin resistant group (R) with their parental group (S). IG: IGROV-1/CP; 200: 2008/C13; A27: A2780/CP; cis: cisplatin.

NF2 in addition to several cell cycle associated genes such as CDC25A, CDK6, CDK4, Cyclin A and Cyclin D.

A study on microRNA expression in ovarian tumor patterns associated with resistance to platinum based chemotherapy showed that miR-27a and miR-23a were upregulated in drug resistant tumors and also correlated with poorer prognosis [10]. Using the human ovarian carcinoma cell line A2780 and paclitaxel-resistant A2780/Taxol cells, the mRNA expression levels of MDR1 and GST- π ($p < 0.05$) and the protein expression levels of P-gp and GST- π were downregulated following miR-130b transfection in comparison to mock-transfected and negative control cancer cells [11]. It was suggested that miRNA-130b may be involved in the development of drug resistance in ovarian cancer. To investigate the changes in cisplatin sensitivity of resistant ovarian cancer A2780 cells after inhibition of miR-23a expression and explore the molecular mechanisms, it was found that inhibition of miR-23a expression increased the sensitivity of A2780 cells to cisplatin possibly by inhibiting the negative regulation by miR-23a target genes that causes inhibition of P-gp protein expression [12].

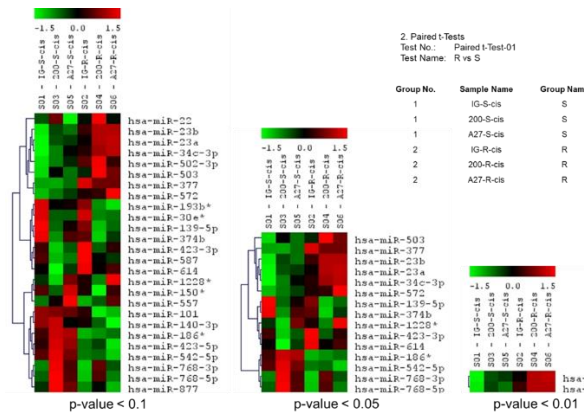


Figure 3. Cluster analysis of expression of the microRNAs differentially expressed between all cisplatin-resistant (R) and their parental cells (S). IG: IGROV-1/CP; 200: 2008/C13; A27: A2780/CP; cis: cisplatin.

In our own experiments, a panel of cisplatin-resistant ovarian carcinoma cell lines A2780/CP, 2008/C13, and IGROV-1/CP and their corresponding parental cell lines were used. The cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), and 1% antibiotics in the presence of 1 μ mol cisplatin as stimulant. Small RNAs were isolated using mirVana Kit and subjected to a high throughput microRNA profile analysis. Statistical and clustering analyses were performed using ANOVA and paired t-tests. Validation of the microRNA results was by real time qPCR.

Clustering graph of all microRNAs with signal intensity >32 is shown in Figure 1. Statistic test and clustering of all cisplatin resistant group and their parental group using ANOVA at $p < 0.01$ is depicted in Figure 2. Upregulated microRNAs are indicated in red and downregulated microRNAs in green color.

The cluster analysis of expression of the microRNAs differentially expressed between all cisplatin-resistant cells and their parental cells revealed a cluster of microRNAs (miR-503, miR-377, miR-23a, miR-23b, miR-34c-3p, miR-572) that was significantly upregulated in cisplatin-resistant A2780/CP and 2008/C13 cells at a p value < 0.05 by ANOVA (Fig. 3) and when it was narrowed down to a p value < 0.01 , only two microRNAs (miR-23a and miR-23b) remained to be significant in A2780/CP and 2008/C13 cisplatin-resistant cells (Fig. 3 and Table 2). Validation of miR-23a and miR-23b by real time RT-PCR showed 1.49 and 1.40-fold upregulation respectively. The overall results showed that miR-23a and miR-23b were upregulated and miR-768-5p and -3p, 374b, 423-5p, 877 and 30e were downregulated in A2780 cisplatin-resistant cells.

Using microRNA target prediction and functional study database (miRDB), several target genes common for both miR-23a and miR-23b were predicted. Among the high target rank and target score genes were SEMA6D or semaphorin 6D which is an inhibitor of chemorepellents or chemodetractant factors and ZNF138 which is a putative candidate genes for both developmental and malignant disorders. PDE7A is upregulated in CLL, and induction of the cAMP signaling pathway has been shown to induce

TABLE 2
MICRO-RNA PROFILE OF OVARIAN CARCINOMA CELL LINES VS. THEIR CISPLATIN-RESISTANT VARIANT.

No.	Reporter Name	p-value	Group 1	Group 2	Log2 (G2/G1)
			S	R	
288	hsa-miR-23a	0.000188	12324.74	19207.33	0.64082196
290	hsa-miR-23b	0.002775	11589.24	18296.17	0.661925872
721	hsa-miR-768-5p	0.040687	537.44	351.24	-0.58890803
403	hsa-miR-374b	0.044821	636.8355	379.3843	-0.65733495
720	hsa-miR-768-3p	0.046468	360.8766	235.0911	-0.57332184
431	hsa-miR-423-5p	0.050442	2257.003	1554.485	-0.53732536
733	hsa-miR-877	0.077451	362.5775	192.8371	-0.85431699
342	hsa-miR-30e*	0.082806	540.3332	462.1975	-0.4346395
Following transcripts are statistically significant but have low signals (signal < 500)					
489	hsa-miR-503	0.010957	35.32462	65.59647	0.859123512
146	hsa-miR-139-5p	0.011093	94.12867	32.24301	-1.43164864
103	hsa-miR-1228*	0.024965	33.2942	38.39276	0.196607913
645	hsa-miR-614	0.037587	10.76311	27.16869	1.374857003
430	hsa-miR-423-3p	0.038694	17.28901	23.51016	0.413390471
598	hsa-miR-572	0.040827	9.796677	22.00641	1.089519354
147	hsa-miR-140-3p	0.050042	198.4253	122.2028	-0.72161923
616	hsa-miR-587	0.069793	18.45047	32.66911	0.733098822
79	hsa-miR-101	0.080367	33.8376	24.17854	-0.5054079
223	hsa-miR-193b*	0.083185	93.76746	66.44275	-0.56597928
172	hsa-miR-150*	0.083417	35.8757	27.7818	-0.38972365
276	hsa-miR-22	0.085854	258.4984	317.5045	0.30385215
585	hsa-miR-557	0.091478	25.11438	20.56701	-0.26156677
487	hsa-miR-502-3p	0.094198	33.50893	43.36629	0.371115747

Note: Validation of miR-23a and miR-23b expression by real time RT-PCR showed 1.49 and 1.40 fold upregulation respectively. S: sensitive; R: resistant.

apoptosis and amplify the effects of glucocorticoids in inducing apoptosis in CLL cells [13]. Increasing intracellular concentrations of cAMP may arrest growth, induce apoptosis and attenuate cancer cell migration in various cancers [14, 15]. Nek6 (NIMA (Never In Mitosis Gene A)-Related-Kinase 6) is a serine-threonine protein kinase which is necessary for progression through the metaphase portion of mitosis. Nek6 is elevated in malignant tumors and human cancer cell lines as compared with normal tissue and fibroblast cells and is believed to play a role in tumorigenesis [16]. In addition, Nek6 is a factor driven by hypoxia that cooperates with Hif-1 α and the cytoskeletal gateway of drug resistance in mediating an aggressive phenotype in serous ovarian cancer patients [17]. Overall involvement of the target genes of miR-23a and miR-23b may have caused cisplatin resistance.

Several approaches are available in which microRNAs may be used as therapeutic targets; For instance, modified antisense oligonucleotides complementary to miRNAs may be used to inhibit them, or RNA analogs termed “antagomirs” to silence microRNAs. To overexpress and/or enhance the function of microRNAs, enforced expression of a short hairpin RNA from a polymerase promoter in a non-viral or viral vector (these can be further processed into mature microRNAs) may be used, or in vivo delivery of double-stranded mimics of microRNA may be employed to enhance microRNA function.

TABLE 4
TARGET GENES COMMON FOR BOTH MIR-23A AND MIR-23B MICRO-RNAS PREDICTED USING MICRORNA TARGET PREDICTION AND FUNCTIONAL STUDY DATABASE (MIR-DB).

Target	Score	Gene Symbol	Gene Description
1	100	SEMA6D	sema domain, transmembrane domain (TM), and cytoplasmic domain, (semaphorin) 6D
2	100	KIAA1467	KIAA1467
3	100	TRIL	TLR4 interactor with leucine-rich repeats
4	100	INTU	inturned planar cell polarity protein
5	100	CLCN3	chloride channel, voltage-sensitive 3
6	100	ZNF138	zinc finger protein 138
7	100	AUH	AU RNA binding protein/enoyl-CoA hydratase
8	100	PDE7A	phosphodiesterase 7A
9	100	AKAP11	A kinase (PRKA) anchor protein 11
10	100	PDE4B	phosphodiesterase 4B, cAMP-specific
11	100	NEK6	NIMA-related kinase 6
12	100	TMED5	transmembrane emp24 protein transport domain containing 5
13	100	TMPO	thymopoietin
14	100	BORA	bora, aurora kinase A activator
15	100	PPP4R4	protein phosphatase 4, regulatory subunit 4
16	100	SESN3	sestrin 3
17	100	CNOT6L	CCR4-NOT transcription complex, subunit 6-like
18	100	STX12	syntaxin 12
19	100	NUP50	nucleoporin 50kDa
20	100	TNRC6A	trinucleotide repeat containing 6A
21	100	NACC2	NACC family member 2, BEN and BTB (POZ) domain containing

Conclusion

In summary, microRNA research is rapidly advancing and the available data including our own results distinguishes several important microRNAs that deserve in-depth investigations. Among them, miR-23 family and miR-27 family appear to be good candidates as new and potent therapeutic targets or chemoresistant modulators in coping with cisplatin resistance in ovarian carcinoma. We believe these miRNAs will be important as new cancer biomarker and therapeutic targets.

Conflict of Interest

The authors declare no conflicts of interest.

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