Am J Clin Exp Obstet Gynecol 2017;4(1):1-10
www.ajceog.us /ISSN:2330-1899/AJCE0G0042548

Original Article

The association of miR-let-7i with platinum resistance of a sensitive/resistant ovarian cancer parent/daughter cell line

Janiel M Cragun[1,2], Cynthia TenEyk[1,2], Haiyan Cui[2], Setsuko K Chambers[1,2]

[1]Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, University of Arizona, Tucson, AZ, USA; [2]University of Arizona Cancer Center, Tucson 85724, AZ, USA

Received October 21, 2016; Accepted January 7, 2017; Epub January 15, 2017; Published January 30, 2017

Abstract: Ovarian cancer is the most deadly of the gynecologic malignancies. Most epithelial ovarian cancers respond to initial treatments with platinum agents. However, once the tumor develops platinum resistance, the prognosis for the patient is poor. We investigated the role of microRNA as a potential marker for platinum resistance in a platinum sensitive parent and platinum resistant daughter ovarian cancer cell line (2780S and 2780/CP70). We performed quantitative reverse transcriptase-polymerase chain reaction on 20 microRNA targets in both lines. We identified miR-let-7i and miR-125b as the most differentially over- and under-expressed, respectively, in the resistant line compared to the sensitive line. Down-regulation of miR-let-7i levels in the resistant line suggested improved response to platinum ($P<0.0001$). Our results may show an association between miR-let-7i and platinum resistance and that manipulation of microRNA levels may change the platinum resistant phenotype of a cell line. Additional research of the miR-let-7i pathway is needed to further characterize this association.

Keywords: miR-let-7i, microRNA, platinum resistance, ovarian cancer

Introduction

Epithelial ovarian cancer has the highest mortality of the gynecologic malignancies. It is responsible for over 14,240 deaths annually [1]. Initially, ovarian cancer is highly sensitive to platinum therapy responding in over 70 percent of cases [2]. Platinum is a chelating agent, widely used in ovarian cancer since the mid-1980s [3]. Unfortunately, most ovarian cancers recur and eventually become resistant to platinum therapy. Once resistant, the chance of an ovarian cancer responding to any chemotherapeutic agent decreases to near 20% [3]. No current methodology improves our ability to predict a patient's response to one salvage therapy over another. Many patients suffer side-effects from ineffective salvage agents without a compensatory benefit. If we could elucidate the molecular mechanisms of platinum resistance, we may be able to better direct therapy to either improve chance of response or decrease risk of side-effects.

Our understanding of cellular mechanisms is evolving. MicroRNA (miRNA) are non-coding RNA that regulate an estimated 60% of mammalian mRNAs [4]. MiRNA are thought to inhibit translation and/or mediate regulation of messenger RNA decay by binding to a target messenger RNA [5]. In rarer cases, miRNA may initiate translation [5-7].

MiRNAs have been implicated in both tumor suppressor and oncogenic functions with examples of individual miRNAs leading to either up-regulation or down-regulation of cancer associated targets [5, 6]. Few individual miRNA appear to play consistent roles across cancer types with miRNA profiling in cancers showing widespread deregulation [5]. Examples of miRNAs which have been studied in association with ovarian cancer include miR-9, miR-10a, miR-21, miR-22, miR-25, miR-26a, miR-30, miR-31, miR-991, miR-100, miR-100, miR-105, miR-122, miR-124, miR-125b, miR-126, miR-127, miR-128, miR-132, miR-133, miR-134, miR-140, miR-141, miR-143, miR-145, miR-146, miR-147, miR-149, miR-152, miR-181, miR-183, miR-192, miR-196a, miR-199a, miR-199b, miR-200a, miR-200b, miR-211, miR-214, miR-223,

miR-224, miR-374, miR-375, miR-379, miR-382, miR-483, miR-503, miR-584, miR-625, miR-877, and the Let-7 family [5, 8-18]. MiRNAs which have been studied in association with platinum resistance include miR-9, miR-17, miR-20b, miR-21, miR-26a, miR-130a, miR-136, miR-138, miR-141, miR-145, miR-193b, miR-194, miR 214, miR-300, miR-429, miR-542-3p, miR-625, miR-642, miR-1299, miR-let-7c, and miR-let-7i [10, 16, 19-21]. In recent studies, roles of miRNA have been associated with proliferation, progression, survival and drug resistance [6, 8, 19, 22-26].

Studies to date suggest that miRNA are integral to regulatory mechanisms associated with chemotherapy resistance and therefore, altered expression could lead to a change in chemotherapy sensitivity. In this report, we hypothesized that miRNA influences the sensitivity of ovarian cancer cells to cisplatin. This study examined the relative expression in a cisplatin resistant daughter compared to a cisplatin-sensitive parent cell line of key microRNA, including many known to be associated with cancer chemoresistance. We then assessed the effect of modulating miRNA levels on the cisplatin dose-response curves.

Methods

Cell culture

We used parent-daughter ovarian cancer cell lines, 2780S and 2780/CP70. 2780S is the parent and platinum sensitive. 2780/CP70 is the daughter with in vitro acquisition of cisplatin resistance. The cells were obtained from stock owned by the University of Arizona Cancer Center. We subjected our cell lines to genetic analysis to confirm their identity. Both cell lines were grown in 1640 MEM with 10% FBS at 37 degrees Celsius, and were tested for mycoplasma and confirmed mycoplasma free. We did not use antibiotic or platinum additives to maintain the cell lines.

Platinum resistance

We tested the cisplatin sensitivity of each line by performing clonogenic assays. Each plate was seeded and treated with doses ranging from 0 to 200 uM for 24 hours at 37 degrees Celsius. A second evaluation was performed using doses ranging from 0 to 5 uM for 72 hours at 37 degrees Celsius. A cisplatin dose-response curve was generated for each condition. We evaluated the IC_{50} of each curve to confirm that the 2780/CP70 line was platinum resistant compared to the 2780S line. The difference in IC_{50} between the resistant and sensitive cell lines was approximately 3 fold in each condition in our hands.

RNA isolation

We Isolated RNA using the miRVana™ miRNA isolation Kit, using the alternative method of lysing the cells directly in the flask. After harvesting the miRNA, A260/A280 ratios were calculated to assess for RNA purity.

Expression of miRNA

The relative expression of 20 key miRNA in our sensitive versus resistant cell line was determined by the University of Arizona Genomics Shared Resource by quantitative RT-PCR. Per protocol from the miRCURY LNA™ Universal RT microRNA PCR manual, the RNA sample was prepared. This was followed by cDNA synthesis. Real-time PCR amplification was achieved by resuspending and mixing the PCR primers. The primers, cDNA, SYBR® Green were added to the PCR plates. SNORD44 (has) was used as the housekeeping miRNA. Relative Gene Expression was determined using the $2^{-\Delta\Delta CT}$ method. Relative expression was presented as the mean of 3 independent assays using primer sets from Exiqon. MiRNA evaluated included the Let 7 family (miR-let-7a, miR-let-7b, miR-let-7c, miR-let-7d, miR-let-7e, miR-let-7f, miR-let-7g, miR-let-7i), mir-200c, miR-610, miR-199a-3p, miR-199a-5p, miR-214, miR-130b, miR-200b, miR-130a, miR-27a, miR-27b, miR-199b-5p, miR-125b.

Transfection

Studies of effect of either overexpressing miRNA or their inhibitors were performed by transient transfection of cells for 24 hours followed by treatment with cisplatin. The oligomers used for overexpression were mirVana™ miRNA mimics hsa-miR-125b-5p (UCCCUGAGACCCUAACUUGUGA) and has-let-7i-5p (sequence not provided) obtained from Ambion® by Life Technologies™. The oligomers used to inhibit expression were Anti-miR™ miRNA inhibitors hsa-miR-125b-5p (UCCCUGAGACCCUAACUUGUGA) and hsa-let-7i-5p (UGAGGUAGUAGUUUGUGCUGUU) obtained from Ambion® by

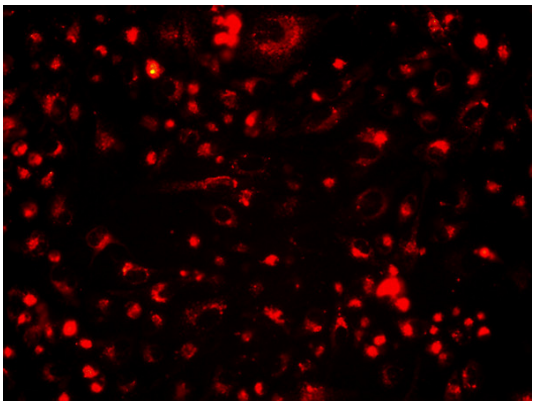

Figure 1. Transfection of 2780/CP70 cells with fluorescently labeled miR-125b.

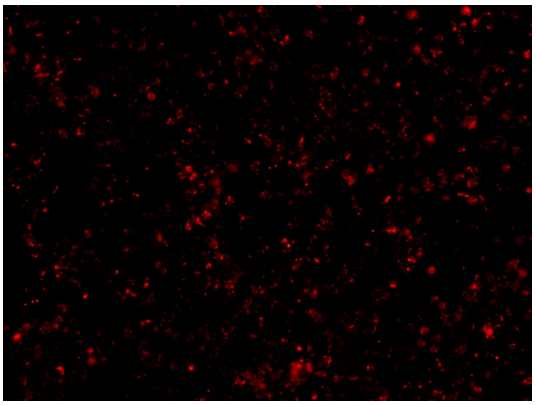

Figure 2. Transfection 2780S cells with fluorescently labeled miR-let-7i.

Life Technologies™. The control cell lines were without transfection. The dose of oligomer was 25 pMol for the majority of the experiments.

The experiments were performed multiple times, sometimes varying conditions or doses slightly to ascertain the effects. For each cell line, cells were treated with cisplatin doses ranging from 0-31.25 uM for 72 hours. The control cell lines remained untreated. The level of expression was recorded. The primary goal of the first set of experiments was to assess if the cell viability was significantly different between transfected cell lines and the control cell line after treatment with cisplatin. A separate set of experiments compared transfected cell lines to a control with transfection reagents without oligomers, called a "no-oligo" control.

Transfection for both overexpression and inhibition was performed with Lipofectamine® RNAiMAX Reagent by Invitrogen™ by Life Technologies™. A 96 well plate was used. Transfection of miRNA mimics and Anti-miRs was performed according to Lipofectamine® protocol. MiRNA-Lipofectamine® RNAiMAX complexes (both miRNA mimics and Anti-miRs) were prepared diluting the miRNA duplex in Opti-MEM® I Medium without serum and added to the base of each well of 96 well plate. Lipofectamine® RNAiMAX was then added to the diluted miRNA molecules, mixed, and incubated for 20 minutes at room temperature. The cells of either the parent or resistant daughter were added to regular 1640 MEM with 10% FBS media. After 20 minutes, the diluted cells were added to each well with the RNAi complexes already at the base. The reaction was incubated at 37 degrees Celsius in a CO_2 incubator for 24 hours.

Transfection efficiency was demonstrated in representative experiment after 24 hours transfection using the same conditions described above with fluorescent labeled microRNA (miR-125b and miR-let-7i), showing near 100% intranuclear transfection (**Figures 1** and **2**).

Cisplatin dose-response curves

Cisplatin dose-response curves were generated, using an MTT assay to determine cell viability, to determine the effect of miRNA overexpression or silencing compared to control after treatment with cisplatin. The cells were exposed to cisplatin doses ranging from 0 to 31.25 uM of cisplatin, with the specific range varying between experiments. This range was chosen to include the IC_{50}s of both sensitive and resistant cell lines. Each cell line and experimental condition (miRNA transfected or untreated control) was treated with each dose of cisplatin in four adjacent wells on a 96-well plate (i.e., 4 technical replicates for each combination of cell-line type and dose). Cell viability was measured after cisplatin exposure of 72 hours for each well using an MTT assay.

Statistical methods

Each experiment was analyzed with Graph Pad Prism software (La Jolla, CA). A dose-response curve was created using a log of the dose of oligonucleotide used. The data was normalized using the y axis as a common scale. A nonlinear regression was performed and the IC_{50} of each curve was evaluated.

Table 1. Median delta-delta CT levels and associated Standard deviations of 3 replicates

miRNA	Median	Standard deviation
Let-7i	293.23626	16.41582
Let-7d	205.49406	42.00302
Let-7f	77.85892	14.72001
Let-7g	37.30644	1.60631
Let-7b	24.95034	10.23678
Let-7e	3.07531	1.47765
Let-7a	1.94749	0.44423
610	1.72059	0.26897
199a-3p	1.43740	0.07335
Let-7c	1.41337	0.03878
199a-5p	1.32318	0.11090
214	0.84446	0.01100
130b	0.62209	0.01750
200b	0.53458	0.32826
130a	0.51860	0.00989
27a	0.34856	0.00367
27b	0.28634	0.18483
199b-5p	0.09329	0.00456
125b	0.00021	0.00014

Because the conditions varied between experiments, the mixed effects analysis of variance model (referred to as the mixed effects model in this paper) was used to assess the difference between cisplatin dose response curves, where transfection and cisplatin doses were fixed effects and time was a random effect. The *p*-value for multiple comparisons was adjusted by Dunnett adjustment. The mixed effects model used data from 9 independent experiments, with the conditions for each experiment summarized in **Table 2**. The results shown below (**Tables 3**, **4**) are the comparisons of cell viability by MTT assay in the transfected versus control cells after adjusting for the cisplatin and time effects. *P*-value <0.05 was considered significant. The mixed analyses were conducted using SAS V9.1.2 software (Cary, NC).

Results

Relative expression of 20 miRNA was studied in our cell lines (**Figure 3**). Levels of miR-let-7i and miR-125b showed the most discrepancy between sensitive and resistant cell lines. Thus, we chose to focus on study of these miRNAs. MiR-let-7i was overexpressed in the resistant line with relative expression of miR-let-7i in the resistant line compared to the sensitive line of 293.2363 fold. In contrast, miR-125b has decreased expression in the resistant line, with relative expression of miR-125b in the resistant line compared to the sensitive line of 0.000214. **Table 1** shows estimated standard deviation from raw data of 3 different runs.

Because it appeared that miR-125b was associated with cisplatin sensitivity, we asked if inhibition of miR-125b expression would confer cisplatin resistance in the sensitive cell line. We were unable to demonstrate any significant effect on the cisplatin dose-response curve through inhibition of miR-125b (representative experiment shown in **Figure 4**), a result confirmed by mixed effects model (**Tables 3** and **4**).

We next studied whether there was an effect of overexpression of miR-125b on the cisplatin resistant cell line. We found that miR-125b overexpression appeared to improve cisplatin dose response of the 2780/CP70 cells in individual experiments (representative experiment shown in **Figure 5**). However, significance was not consistent across multiple experiments, and the level of shift of the cisplatin dose response curve to the left was minimal. While the mixed effects model showed significance when the control condition contained no transfection reagents (**Table 3**), it failed to show a significant effect of miR-125b overexpression on platinum response in the resistant cell line when the control conditions were more stringent including the transfection reagents (**Table 4**). In addition, we cannot rule out the possibility that a non-specific effect of the oligomer could have contributed to some of the minor changes in cisplatin dose response seen in the mixed effects models (**Tables 3** and **4**). Collectively, we interpret these results that there is no significant effect of manipulation of miR-125b in either resistant or sensitive cell line on cisplatin dose response.

Since miR-let-7i appeared associated with cisplatin resistance, we wondered if silencing of miR-let-7i would confer relative cisplatin sensitivity in the resistant cell line (representative experiment shown in **Figure 6**). In this experiment the IC_{50} was significantly different between the two conditions (P<0.0001). In the platinum-resistant cell line, subsequent experiments continued to suggest silencing of miR-let-7i led to increased cisplatin sensitivity. Because dosing conditions varied between ex-

Table 2*. Conditions for the 9 cisplatin dose response experiments used in analysis

2870S				2870/CP70			
No oligo	No transfection	125b DOWN transfection	Let-7i UP transfection	No oligo	No transfection	125b UP transfection	Let-7i DOWN transfection
	1	1			1		1
	2	2			2		2
3	3	3		3	3		
4	4			4	4		
	5		5		5	5	
	6				6	6	
	7						
	8		8		8	8	
	9	9	9		9		9

*The numbers in this table represent the experiment number.

Table 3. Mixed effects analysis of variance model of cisplatin dose response using no transfection as the control condition

Cell line	Transfection	Mean (S.D.)	*p*-value
2870S	No transfection	0.768 (0.045)	
	125b transfection (125b DOWN)	0.694 (0.046)	0.1069
	Let-7i transfection (Let7i UP)	0.513 (0.055)	<0.0001
2870/CP70	No transfection	1.567 (0.053)	
	125b transfection (125b UP)	1.208 (0.058)	<0.0001
	Let-7i transfection (Let7i DOWN)	1.138 (0.061)	<0.0001

Table 4. Mixed effects analysis of variance model of cisplatin dose response using no oligo as the control condition

Cell line	Transfection	Mean (S.D.)	*p*-value
2870S	No Oligo	0.686 (0.073)	
	125b transfection (125b DOWN)	0.655 (0.053)	0.0627
	Let-7i transfection (Let7i UP)	0.458 (0.067)	<0.0001
2870/CP70	No Oligo	1.508 (0.065)	
	125b transfection (125b UP)	1.256 (0.083)	0.0738
	Let-7i transfection (Let7i DOWN)	1.033 (0.085)	0.0006

periments we also applied a mixed effects analysis of variance model to our results as described in the methods section. This model also confirmed that across experiments, as a group, there appeared to be increased sensitivity of cells to cisplatin in the platinum resistant line after silencing miR-let-7i (P<0.0001 and P=0.0006; **Tables 3** and **4**, respectively).

We next studied whether overexpression of miR-let-7i led to cisplatin resistance in the parent cisplatin sensitive cells. We were unable to demonstrate that over-expression of miR-let-7i confers cisplatin resistance in the 2780S cells in our hands (representative experiment shown in **Figure 7**). Instead, the data appear to suggest that in the 2780S cells, overexpression of miR-let-7i led to chemosensitization, confirmed by mixed effects model (**Tables 3** and **4**). This result does not appear to be a non-specific effect of oligomer, as the effect is greater than seen by the miR-125b oligomer. Thus, this finding may be real using these cells.

Discussion

MicroRNAs bring both challenges and opportunities for research in cancer biology. Techniques are improving to facilitate isolation and identification of miRNA. MiRNA appear to be more stable in paraffin-embedded tissue than their longer messenger RNA (mRNA) counterparts allowing for processing with less demanding storage conditions. Further, the ability to extend studies to paraffin-embedded tissue creates an opportunity to research archived specimens. Work by Lu et al. suggests that miRNA microarray profiles may accurately classify poorly differentiated cancer samples better than mRNA microarray profiles [5, 27]. Currently, there is a significant limitation to RNA genetic research in cancer as, traditionally, fresh frozen tissue has been needed for isolation of messenger RNA.

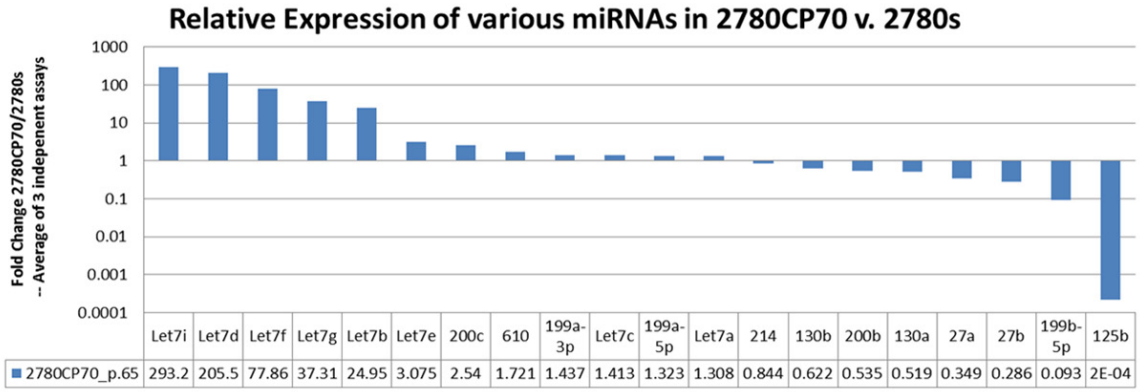

Figure 3. Relative expression of various miRNAs.

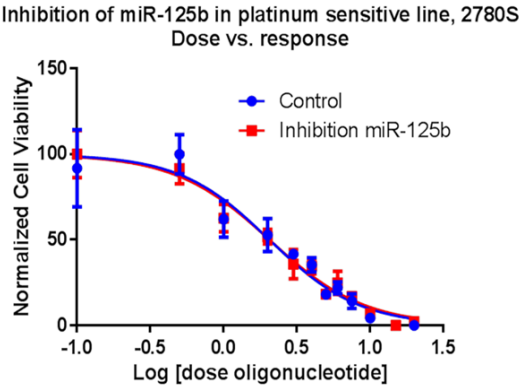

Figure 4. Inhibition of miR-125b in the sensitive line did not affect response to cisplatin. P=0.4945.

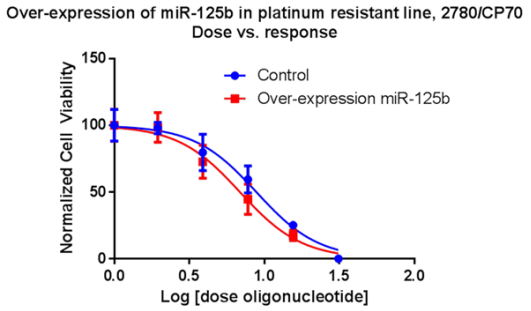

Figure 5. Over-expression of miR-125B in the resistant line initially appeared to increase response to cisplatin, P=0.009.

Our study has shown that there may be potential for manipulation of miRNA influencing response to therapy. The strengths of this study include a genetically confirmed and mycoplasma free platinum sensitive parent and resistant daughter line. IC_{50} curves confirmed that the parent was more sensitive than the daughter. However, the difference in their sensitivity was small and this may have confounded our results. Our findings were internally consistent as we were able to reproduce results; however, we were unable to demonstrate that overexpression of miR-let-7i in the sensitive line conferred resistance as was hypothesized from our findings that miR-let-7i was overexpressed in platinum resistant 2780/CP70 cells. Our results are different from others in the literature. However, many but not all of those studies used different cell lines, used tissues, or focused on other cancers.

Our study found an increase in miR-let-7i expression in platinum resistant daughter 2780/CP70 compared to parent 2780S cells. This is not consistent with findings of other studies. MiR-let-7i has been studied in ovarian cancer cell lines and found to be down regulated in platinum resistant compared sensitive lines that are different than ours [7, 28]. Yang et al. also noted that miR-let-7i was down regulated in tissues from platinum resistant patients. In another study, the same cell lines as ours were used, but miR-let-7i was not identified as being differentially expressed. In this study, Kumar et al. performed miRNA profiling of the 2780S and 2780/CP70 cell lines using miRNA chips of 1500 miRNA, in which he identified 11 differentially expressed miRNA [26]. Neither miR-let-7i nor 125b were identified as being differentially expressed using his approach. Other miRNA targets which have been identified to be differentially expressed between platinum sensitive and resistant ovarian cancer tissues or other cell lines include miR-17, miR-20b, miR-21, miR-27a, miR-378, miR-23a, miR-93, miR-138, miR-193b, miR-194, miR-300, miR-542-

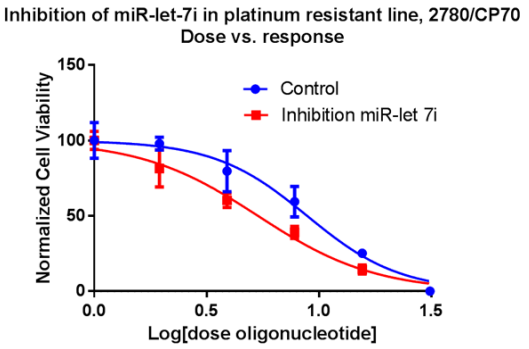

Figure 6. Inhibition of miR-let-7i in the platinum resistant line appears to increase response to cisplatin, P<0.0001.

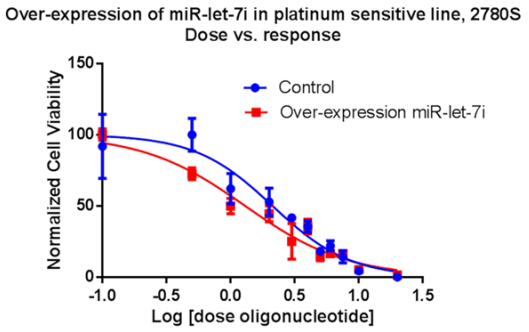

Figure 7. Over-expression of miR-let-7i in the platinum sensitive line did not confer resistance, P<0.001.

3p, miR-625, miR-642, miR-1299, and miR-let-7c [26, 28-30]. In several cell lines, Let-7i inhibition increased sensitivity to other anticancer compounds eg., NSC670500 [31]. MiR-Let-7i has been described as a modulator in different cancers. The Let-7 family is thought to be tumor suppressor [32, 33] and may be a regulator of cell proliferation pathways [34, 35]. Decreased expression has been correlated with poor response to chemotherapy in gastric cancer [36]. It is also shown decreased expression in lung squamous cell cancer and mixed epithelial mesenchymal uterine tumors [37, 38]. Further, it has been hypothesized to be involved with colorectal cancer metastatic potential [39, 40].

We also found that miR-125b was significantly down regulated in our resistant line compared to the sensitive parent line. This finding was also different from that found by Kong et al., who described that miR-125b was upregulated in C13 cells, a platinum resistant daughter line of 2008, the platinum sensitive parent [41]. They were also able to induce resistance with up regulation of miR-125b [41]. This pair of cell lines differed from ours. It is possible, if not likely, that the mechanisms for cisplatin resistance in the 2008 cell line may differ from those in the 2780/CP70 cell line. Although Sorrentino et al. also found miR-125b to be upregulated in platinum resistant line, they conversely found miR-125b to exhibit decreased expression in ovarian cell line A2780TAX, resistant to paclitaxel [42]. Elevated miR-125b has also been associated with chemo-resistance in breast cancer patient tissues and blood serum [43, 44]. Whereas Lv et al. found decreased levels of miR-125b in anthracycline resistance cell lines [45]. MiR-125b may prove to be a marker of chemo-resistance.

There were many limitations to our study. We performed many experiments, but with varied experimental conditions. Optimally, in order to decrease variability between experiments, we would need to perform a larger number of biological replicates while maintaining the same experimental conditions. This would increase the power to detect differences in IC_{50} values in the cisplatin dose response curves generated after manipulation of miRNA levels.

We used only one pair of platinum sensitive and resistant cell lines. Clearly, other pairs of in vitro derived cisplatin resistant cell lines sometimes yield differing data, even in our own hands, as seen in the case of study of differential expression of miR-125b. Regardless of the direction of miR-125b differential expression, neither knockdown of miR-125b in the platinum sensitive cell line, nor overexpression of miR-125b in the resistant cell line appear to change ovarian cancer cell sensitivity to cisplatin in our hands. Differences in miR-125b expression may be unique to our cell line and may not represent specifically platinum resistance. Alternative models of drug resistance including tumor xenografts have several advantages over cell line models. Further, our model was an in vitro derived platinum resistant model. Lastly, the small sample size of our cisplatin dose-response experiments may have impacted on this negative result.

Our finding which was significant and reproducible was that miR-let-7i was associated with cisplatin resistance in the cisplatin resistant cell line. Inhibition of miR-let-7i in the resistant line significantly improved cisplatin sensitivity as measured cell viability post treatment (P< 0.0001). This finding however may be confined,

to our knowledge, to this pair of cell lines under the conditions applied. Unfortunately, our study was unable to demonstrate an effect of overexpression of miR-let-7i in the sensitive line on enhancing cisplatin resistance (**Figure 7**). In fact, the reverse finding was seen, underscoring once again that the findings of this study appear to be cell line specific. Notably, as discussed above, the role of miR-let-7i in the literature is neither consistent among different cell lines nor consistent among different cancers. Future research would need to address which messenger RNAs may be targeted by the miR-let-7i miRNA studied in this resistant cell line and which pathways may be involved to initiate a drug resistant model.

There are many drug resistance mechanisms, including decreased drug accumulation, increased repair of drug induced damage; altered gene expression and drug target; and increased resistance to apoptosis [46]. Sensitivity to chemotherapy is important to patient survival. Currently, the ability to predict response remains elusive. MiRNA provide a new potential tool in the quest for predictive markers and therapeutic targets. In gynecologic tumors, current research aims to determine specific miRNA signatures [30, 42, 47, 48]. Further investigation in miRNA and their molecular targets is needed to further elucidate both their role in chemotherapy sensitivity and their potential role in chemotherapeutic treatment.

Acknowledgements

University of Arizona Cancer Center Support Grant P30 CA023074 for support of the Biostatistics and Genomics Shared Resources, and Start-up funds from the Division of Women's Cancers at the University of Arizona Cancer Center.

Disclosure of conflict of interest

None.

Address correspondence to: Janiel M Cragun, Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, University of Arizona, Tucson, AZ, USA. E-mail: jcragun@uacc.arizona.edu

References

[1] American Cancer Society. Cancer Facts & Figures 2016. Atlanta: American Cancer Society; 2016.

[2] Ozols RF, Bundy BN, Greer BE, Fowler JM, Clarke-Pearson D, Burger RA, Mannel RS, DeGeest K, Hartenbach EM, Baergen R; Gynecologic Oncology Group. Phase III trial of carboplatin and paclitaxel compared with cisplatin and paclitaxel in patients with optimally resected stage III ovarian cancer: a gynecologic oncology group study. J Clin Oncol 2003; 21: 3194-3200.

[3] M. G. Treatment of relapsed epithelial ovarian cancer. Philadelphia: Lippincott Williams and Wilkins; 2001.

[4] Friedman RC, Farh KK, Burge CB and Bartel DP. Most mammalian mRNAs are conserved targets of microRNAs. Genome Res 2009; 19: 92-105.

[5] Corney DC and Nikitin AY. MicroRNA and ovarian cancer. Histol Histopathol 2008; 23: 1161-1169.

[6] Dahiya N and Morin PJ. MicroRNAs in ovarian carcinomas. Endocr Relat Cancer 2010; 17: F77-89.

[7] Yang N, Kaur S, Volinia S, Greshock J, Lassus H, Hasegawa K, Liang S, Leminen A, Deng S, Smith L, Johnstone CN, Chen XM, Liu CG, Huang Q, Katsaros D, Calin GA, Weber BL, Butzow R, Croce CM, Coukos G and Zhang L. MicroRNA microarray identifies Let-7i as a novel biomarker and therapeutic target in human epithelial ovarian cancer. Cancer Res 2008; 68: 10307-10314.

[8] Woo HH, Laszlo CF, Greco S and Chambers SK. Regulation of colony stimulating factor-1 expression and ovarian cancer cell behavior in vitro by miR-128 and miR-152. Mol Cancer 2012; 11: 58.

[9] Tang Z, Ow GS, Thiery JP, Ivshina AV and Kuznetsov VA. Meta-analysis of transcriptome reveals let-7b as an unfavorable prognostic biomarker and predicts molecular and clinical subclasses in high-grade serous ovarian carcinoma. Int J Cancer 2014; 134: 306-318.

[10] Frederick PJ, Green HN, Huang JS, Egger ME, Frieboes HB, Grizzle WE and McNally LR. Chemoresistance in ovarian cancer linked to expression of microRNAs. Biotech Histochem 2013; 88: 403-409.

[11] Chung YW, Bae HS, Song JY, Lee JK, Lee NW, Kim T and Lee KW. Detection of microRNA as novel biomarkers of epithelial ovarian cancer from the serum of ovarian cancer patients. Int J Gynecol Cancer 2013; 23: 673-679.

[12] Kobayashi M, Salomon C, Tapia J, Illanes SE, Mitchell MD and Rice GE. Ovarian cancer cell invasiveness is associated with discordant exosomal sequestration of Let-7 miRNA and miR-200. J Transl Med 2014; 12: 4.

[13] Wang Y, Li L, Qu Z, Li R, Bi T, Jiang J and Zhao H. The expression of miR-30a* and miR-30e*

is associated with a dualistic model for grading ovarian papillary serious carcinoma. Int J Oncol 2014; 44: 1904-1914.
[14] Luo J, Zhou J, Cheng Q, Zhou C and Ding Z. Role of microRNA-133a in epithelial ovarian cancer pathogenesis and progression. Oncol Lett 2014; 7: 1043-1048.
[15] He J, Jing Y, Li W, Qian X, Xu Q, Li FS, Liu LZ, Jiang BH and Jiang Y. Roles and mechanism of miR-199a and miR-125b in tumor angiogenesis. PLoS One 2013; 8: e56647.
[16] Ying HC, Xu HY, Lv J, Ying TS and Yang Q. MicroRNA signatures of platinum-resistance in ovarian cancer. Eur J Gynaecol Oncol 2015; 36: 16-20.
[17] Zuberi M, Khan I, Mir R, Gandhi G, Ray PC and Saxena A. Utility of serum miR-125b as a diagnostic and prognostic indicator and its alliance with a panel of tumor suppressor genes in epithelial ovarian cancer. PLoS One 2016; 11: e0153902.
[18] Iorio MV, Visone R, Di Leva G, Donati V, Petrocca F, Casalini P, Taccioli C, Volinia S, Liu CG, Alder H, Calin GA, Menard S and Croce CM. MicroRNA signatures in human ovarian cancer. Cancer Res 2007; 67: 8699-8707.
[19] Yang L, Li N, Wang H, Jia X, Wang X and Luo J. Altered microRNA expression in cisplatin-resistant ovarian cancer cells and upregulation of miR-130a associated with MDR1/P-glycoprotein-mediated drug resistance. Oncol Rep 2012; 28: 592-600.
[20] Zhao HM, Wei W, Sun YH, Gao JH, Wang Q and Zheng JH. MicroRNA-9 promotes tumorigenesis and mediates sensitivity to cisplatin in primary epithelial ovarian cancer cells. Tumour Biol 2015; 36: 6867-73.
[21] Zhao H, Liu S, Wang G, Wu X, Ding Y, Guo G, Jiang J and Cui S. Expression of miR-136 is associated with the primary cisplatin resistance of human epithelial ovarian cancer. Oncol Rep 2015; 33: 591-598.
[22] Zhang L, Volinia S, Bonome T, Calin GA, Greshock J, Yang N, Liu CG, Giannakakis A, Alexiou P, Hasegawa K, Johnstone CN, Megraw MS, Adams S, Lassus H, Huang J, Kaur S, Liang S, Sethupathy P, Leminen A, Simossis VA, Sandaltzopoulos R, Naomoto Y, Katsaros D, Gimotty PA, DeMichele A, Huang Q, Butzow R, Rustgi AK, Weber BL, Birrer MJ, Hatzigeorgiou AG, Croce CM and Coukos G. Genomic and epigenetic alterations deregulate microRNA expression in human epithelial ovarian cancer. Proc Natl Acad Sci U S A 2008; 105: 7004-7009.
[23] Li Z, Hu S, Wang J, Cai J, Xiao L, Yu L and Wang Z. MiR-27a modulates MDR1/P-glycoprotein expression by targeting HIPK2 in human ovarian cancer cells. Gynecol Oncol 2010; 119: 125-130.
[24] Langhe R, Norris L, Saadeh FA, Blackshields G, Varley R, Harrison A, Gleeson N, Spillane C, Martin C, O'Donnell DM, D'Arcy T, O'Leary J and O'Toole S. A novel serum microRNA panel to discriminate benign from malignant ovarian disease. Cancer Lett 2015; 356: 628-636.
[25] Yang C, Cai J, Wang Q, Tang H, Cao J, Wu L and Wang Z. Epigenetic silencing of miR-130b in ovarian cancer promotes the development of multidrug resistance by targeting colony-stimulating factor 1. Gynecol Oncol 2012; 124: 325-334.
[26] Kumar S, Kumar A, Shah PP, Rai SN, Panguluri SK and Kakar SS. MicroRNA signature of cis-platin resistant vs. cis-platin sensitive ovarian cancer cell lines. J Ovarian Res 2011; 4: 17.
[27] Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, Mak RH, Ferrando AA, Downing JR, Jacks T, Horvitz HR and Golub TR. MicroRNA expression profiles classify human cancers. Nature 2005; 435: 834-838.
[28] Yang Y, Li H, Hou S, Hu B, Liu J and Wang J. The noncoding RNA expression profile and the effect of lncRNA AK126698 on cisplatin resistance in non-small-cell lung cancer cell. PLoS One 2013; 8: e65309.
[29] Fu X, Tian J, Zhang L, Chen Y and Hao Q. Involvement of microRNA-93, a new regulator of PTEN/Akt signaling pathway, in regulation of chemotherapeutic drug cisplatin chemosensitivity in ovarian cancer cells. FEBS Lett 2012; 586: 1279-1286.
[30] Eitan R, Kushnir M, Lithwick-Yanai G, David MB, Hoshen M, Glezerman M, Hod M, Sabah G, Rosenwald S and Levavi H. Tumor microRNA expression patterns associated with resistance to platinum based chemotherapy and survival in ovarian cancer patients. Gynecol Oncol 2009; 114: 253-259.
[31] Blower PE, Chung JH, Verducci JS, Lin S, Park JK, Dai Z, Liu CG, Schmittgen TD, Reinhold WC, Croce CM, Weinstein JN and Sadee W. MicroRNAs modulate the chemosensitivity of tumor cells. Mol Cancer Ther 2008; 7: 1-9.
[32] Takahashi M, Sung B, Shen Y, Hur K, Link A, Boland CR, Aggarwal BB and Goel A. Boswellic acid exerts antitumor effects in colorectal cancer cells by modulating expression of the let-7 and miR-200 microRNA family. Carcinogenesis 2012; 33: 2441-2449.
[33] Lee YS and Dutta A. The tumor suppressor microRNA let-7 represses the HMGA2 oncogene. Genes Dev 2007; 21: 1025-1030.
[34] Johnson CD, Esquela-Kerscher A, Stefani G, Byrom M, Kelnar K, Ovcharenko D, Wilson M, Wang X, Shelton J, Shingara J, Chin L, Brown D and Slack FJ. The let-7 microRNA represses cell proliferation pathways in human cells. Cancer Res 2007; 67: 7713-7722.

[35] Sassen S, Miska EA and Caldas C. MicroRNA: implications for cancer. Virchows Arch 2008; 452: 1-10.
[36] Liu K, Qian T, Tang L, Wang J, Yang H and Ren J. Decreased expression of microRNA let-7i and its association with chemotherapeutic response in human gastric cancer. World J Surg Oncol 2012; 10: 225.
[37] Yang Y, Li X, Yang Q, Wang X, Zhou Y, Jiang T, Ma Q and Wang YJ. The role of microRNA in human lung squamous cell carcinoma. Cancer Genet Cytogenet 2010; 200: 127-133.
[38] Kowalewska M, Bakula-Zalewska E, Chechlinska M, Goryca K, Nasierowska-Guttmejer A, Danska-Bidzinska A and Bidzinski M. microRNAs in uterine sarcomas and mixed epithelial-mesenchymal uterine tumors: a preliminary report. Tumour Biol 2013; 34: 2153-2160.
[39] Zhang P, Ma Y, Wang F, Yang J, Liu Z, Peng J and Qin H. Comprehensive gene and microRNA expression profiling reveals the crucial role of hsa-let-7i and its target genes in colorectal cancer metastasis. Mol Biol Rep 2012; 39: 1471-1478.
[40] Hur K, Toiyama Y, Schetter AJ, Okugawa Y, Harris CC, Boland CR and Goel A. Identification of a metastasis-specific MicroRNA signature in human colorectal cancer. J Natl Cancer Inst 2015; 107.
[41] Kong F, Sun C, Wang Z, Han L, Weng D, Lu Y and Chen G. miR-125b confers resistance of ovarian cancer cells to cisplatin by targeting pro-apoptotic Bcl-2 antagonist killer 1. J Huazhong Univ Sci Technolog Med Sci 2011; 31: 543-549.
[42] Sorrentino A, Liu CG, Addario A, Peschle C, Scambia G and Ferlini C. Role of microRNAs in drug-resistant ovarian cancer cells. Gynecol Oncol 2008; 111: 478-486.
[43] Zheng Y, Li S, Boohaker RJ, Liu X, Zhu Y, Zhai L, Li H, Gu F, Fan Y, Lang R, Liu F, Qian X, Xu B and Fu L. A MicroRNA expression signature in taxane-anthracycline-based neoadjuvant chemotherapy response. J Cancer 2015; 6: 671-677.
[44] Wang H, Tan G, Dong L, Cheng L, Li K, Wang Z and Luo H. Circulating MiR-125b as a marker predicting chemoresistance in breast cancer. PLoS One 2012; 7: e34210.
[45] Lv J, Xia K, Xu P, Sun E, Ma J, Gao S, Zhou Q, Zhang M, Wang F, Chen F, Zhou P, Fu Z and Xie H. miRNA expression patterns in chemoresistant breast cancer tissues. Biomed Pharmacother 2014; 68: 935-942.
[46] Yan XD, Li M, Yuan Y, Mao N and Pan LY. Biological comparison of ovarian cancer resistant cell lines to cisplatin and Taxol by two different administrations. Oncol Rep 2007; 17: 1163-1169.
[47] Torres A, Torres K, Maciejewski R and Harvey WH. MicroRNAs and their role in gynecological tumors. Med Res Rev 2011; 31: 895-923.
[48] Boren T, Xiong Y, Hakam A, Wenham R, Apte S, Chan G, Kamath SG, Chen DT, Dressman H and Lancaster JM. MicroRNAs and their target messenger RNAs associated with ovarian cancer response to chemotherapy. Gynecol Oncol 2009; 113: 249-255.