Modulation of chemoresponsiveness to platinum-based agents by microRNAs in cancer

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Abstract: Ovarian cancer accounts for the highest mortality among all gynecologic cancers. Cytoreductive surgery followed by chemotherapy with a platinum-based agent (cisplatin or carboplatin) plus paclitaxel is the first-line option for treatment of epithelial ovarian cancer. However, primary or acquired resistance to platinum-based agents is a major clinical challenge. MicroRNAs are a group of small non-coding RNAs that regulate gene expression post-transcriptionally and may function as oncogenes or tumor-suppressor genes through extensive crosstalk with intracellular signaling pathways. Importantly, their dysregulation has been implicated in ovarian tumorigenesis. Pertinent to chemotherapy, increasing evidence has revealed that miRNAs can be directly linked to chemosensitivity to platinum-based agents in ovarian cancer. In this review, we summarize current evidence concerning the role of miRNAs in prediction and modulation of cellular responses to cisplatin and carboplatin in ovarian cancer.

Keywords: Cisplatin, carboplatin, chemoresistance, microRNA, ovarian cancer, melanoma

Introduction

Ovarian cancer accounts for approximately 3% of all cancers in the Western world and has the highest mortality rate of all gynecologic cancers with the 5-year survival rate of approximately 30% [1-3]. Platinum-based chemotherapeutics, including cisplatin and carboplatin, are used in the treatment of a variety of cancers, including ovarian, bladder, lung, head and neck, and gastric cancers [4-9]. Platinum compounds mediate their cytotoxic effects through binding to DNA molecules, thereby interfering with DNA repair mechanisms in cancer cells [10-12]. Standard treatment for epithelial ovarian cancer is primarily cytoreductive surgery followed by treatment with platinum-containing compound (i.e. cisplatin or carboplatin) in combination with paclitaxel [13-15]. However, primary or acquired drug resistance is a major challenge and decreases the treatment efficiency. Patients with platinum resistance experienced progression during chemotherapy or recurrence within six months of completed chemotherapy [16-18]. Nonetheless, the exact underlying mechanisms of chemoresistance to platinum agents are still unknown. Recently, growing number of studies have investigated the mechanisms underlying chemoresistance in ovarian cancer [4, 19-21]. Understanding the molecular basis of chemoresistance is crucial to improving the effectiveness of ovarian cancer treatment.

MicroRNAs (miRNAs) are small (19-25 nucleotides in length), non-coding and evolutionarily conserved RNAs that regulate gene expression post-transcriptionally through induction of translation blockage or mRNA degradation via binding to their target mRNAs [22-25]. miRNAs play important roles in various biological and pathological processes, including development, immune response, inflammation and tumorigenesis [26-29]. Most importantly, aberrant expression of miRNAs has been documented in virtually all types of malignancies, including ovarian cancer [30-33]. Differentially expressed miRNAs exert their oncogenic or tumour-suppressive effects through regulating many cancer-pertinent cellular processes, such as proliferation, migration, apoptosis, stemness and chemoresistance [34-36]. miRNAs may also be
used as biomarkers for predicting chemoresponsiveness to maximize therapeutic effect and minimize treatment toxicity [37-39].

In this review, we summarize the literature concerning the role of miRNAs in predicting and modifying response to platinum-based chemotherapy in ovarian cancer. We also discuss the associated molecular targets and intracellular pathways involved in these processes.

Cisplatin

Cisplatin is a major landmark in the history of successful anticancer drugs since its introduction to clinical trials in 1971 [40, 41]. It is widely used in treatment of solid tumors, such as ovarian, lung, head and neck, and breast tumors [42-45]. Cisplatin is the backbone drug used in combination with other chemotherapeutic agents in the management of ovarian cancer in the clinical setting [46-48]. It is noteworthy that patients resistant to cisplatin very often exhibit cross-resistance to carboplatin [47, 49].

Drug effects on miRNA expression

Boren et al. identified a total of 7 miRNAs that were significantly associated with OVCA cell responsiveness to cisplatin [50]. These miRNAs included miR-23b, miR-381, miR-340, miR-520, miR-331, miR-185 and miR-106a. They also investigated the molecular mechanisms underlying chemoresistance in which miRNA expression levels were measured on paired mother/daughter and cisplatin-sensitive/resistant ovarian cancer cell lines. Three of 7 differentially expressed miRNAs (i.e. miR-340, miR-381, and miR-520f) in inherent cisplatin-resistant cell lines also showed significantly differential expression in paired sensitive/resistant and mother/daughter cell lines. These results indicate common molecular mechanisms in inherent and acquired cisplatin resistance.

Another study compared the differentially expressed miRNAs in platinum-sensitive and -resistant ovarian tumor cell lines using miRNA array [51]. A total of 4 downregulated miRNAs and 13 upregulated miRNAs were identified. Among them, miR-141-3p was the most deregulated miRNA. Li et al. also screened for differentially expressed miRNAs in the cisplatin-resistant human ovarian cancer cell line A2780/DDP using microarray [52]. A total of 32 miRNAs were found to be differentially expressed in A2780/DDP cells compared with its parental A2780 cells. Four abnormally expressed miRNAs (i.e. miR-146a, miR-130a, miR-374a and miR-182) were further verified by quantitative reverse transcription-PCR. miR-146a was upregulated whereas miR-130a, miR-374a, and miR-182 were downregulated in A2780/DDP cells when compared with A2780 cells.

Yang et al. found that a total of 89 miRNAs differentially expressed in cisplatin-resistant cell line SKOV3/CIS as compared with SKOV3 cells. [53]. Among them, 35 miRNAs were downregulated and 54 miRNAs were upregulated. These results suggested that differentially miRNA expression might contribute to acquisition of cisplatin resistance in ovarian cancer. In particular, the expression of miR-130a was increased in cisplatin-resistant SKOV3/CIS cells as compared with the parental SKOV3 cells. PTEN was found to be the potential target of miR-130a.

Jaarsveld et al. showed that 27 miRNAs were differentially expressed in an isogenic cisplatin-sensitive cell line as compared with a cisplatin-resistant ovarian cancer cell line using miRNA

<table>
<thead>
<tr>
<th>Num</th>
<th>Method</th>
<th>Drug</th>
<th>Deregulated</th>
<th>Upregulated</th>
<th>Downregulated</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
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<td>miR-23b, miR-381, miR-340, miR-520, miR-331, miR-185, miR-106a</td>
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<td>[50]</td>
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<td>miR-141-3p</td>
<td>13 miRNAs</td>
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<td>3</td>
<td>Microarray qRT-PCR</td>
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<td>miR-146a</td>
<td>miR-130a, miR-374a, miR-182</td>
<td>[52]</td>
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<td>4</td>
<td>Microarray qRT-PCR</td>
<td>Cisplatin</td>
<td>miR-146a</td>
<td>miR-130a</td>
<td>35 miRNAs</td>
<td>[53]</td>
</tr>
<tr>
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<td>miR-141, miR-200c, miR-215, miR-421</td>
<td>miR-493-5p</td>
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<td>miR-21</td>
<td>miR-21, miR-214</td>
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<tr>
<td>7</td>
<td>Microarray qRT-PCR</td>
<td>carboplatin</td>
<td>miR-21</td>
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</tbody>
</table>
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expression profiles [54]. Five of these deregulated miRNAs, including the family members of miR-141/200c, were correlated with cisplatin sensitivity (Table 1).

Modulation of chemosensitivity

miR-21 is a well-known oncogene promoting cell proliferation, migration and invasion in various types of tumor [8, 55, 56]. Importantly, miR-21 overexpression is associated with drug resistance. miR-21 expression was higher in cisplatin-resistant than sensitive ovarian cancer cells [57]. Enforced expression of miR-21 promoted cell proliferation in cisplatin-sensitive cells. Furthermore, downregulating miR-21 significantly reduced cell proliferation and invasion in cisplatin-resistant ovarian cancer cells. In addition, the tumor suppressor gene programmed cell death 4 (PDCD4) was identified as a target of miR-21 and c-Jun N-terminal kinase (JNK)-1/c-Jun/miR-21 pathway was involved in miR-21-mediated regulation of cisplatin resistance. Generally, the passenger strand, or the non-incorporated strand, is considered non-functional. However, miR-21-3p, the passenger strand of miR-21, was also increased in cisplatin-resistant ovarian cancer cells [58]. Moreover, miR-21-3p conferred cisplatin resistance to many ovarian cancer cell lines while miR-21-5p increased cisplatin sensitivity. NAV3 was identified to be a potential target of miR-21-3p. In summary, miR-21-3p could induce cisplatin resistance in ovarian cancer through targeting the NAV3 gene.

miR-103/107 overexpression sensitizes ovarian cancer cells to cisplatin and reduced the percentage of RAD51 foci-positive cells in response to chemotherapy [59]. Expression of miR-130a was increased in cisplatin-resistant cell lines [60], in which inhibition of miR-130a could overcome the cisplatin resistance and inhibit MDR1 mRNA and P-glycoprotein (P-gp) expression [53]. miR-130a played a role in both MDR1/P-gp- and phosphoinositide 3-kinase (PI3K)/Akt/PTEN/mammalian target of rapamycin (mTOR)-mediated drug-resistance pathways in SKOV3/CIS cells, indicating a key role of miR-130a in the modulation of platinum-based chemotherapy [53]. miR-130b decreased sensitivity to cisplatin in ovarian cancer line compared with mock-transfected and negative control cancer cells [61]. In addition, the expression of MDR1, GST-π, P-gp and GST-π were decreased following miR-130b transfection. Expression levels of miR-141 were higher in non-serous ovarian tumors resistant to therapy and KEAP1 was identified to be its direct target [54]. Overexpression of KEAP1 increased cisplatin sensitivity through regulating the nuclear factor (NF)-κB pathway. These findings suggested that miR-141-mediated modulation of KEAP1 played a significant role in the response of ovarian cancer to cisplatin [54]. Let-7i was downregulated in chemotherapy-resistant ovarian cancer in which suppressing let-7i expression enhanced the resistance to cisplatin [62].

miR-130a and miR-374a overexpression decreased the cisplatin sensitivity of A2780 cells while their inhibitors re-sensitized A2780/DDP cells [52]. Ziliak et al. found that miR-193b increased cisplatin resistance in 7 ovarian cancer cell lines [63]. Akt is a crucial cell survival
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Table 2. Modulation of chemosensitivity

<table>
<thead>
<tr>
<th>miRNAs</th>
<th>Drug</th>
<th>Effect</th>
<th>Target gene</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-21</td>
<td>Cisplatin</td>
<td>Sensitivity</td>
<td>PDCD4</td>
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<tr>
<td>miR-21-3p</td>
<td>Cisplatin</td>
<td>Resistance</td>
<td>NAV3</td>
<td>[58]</td>
</tr>
<tr>
<td>miR-21-5p</td>
<td>Cisplatin</td>
<td>Sensitivity</td>
<td></td>
<td>[58]</td>
</tr>
<tr>
<td>miR-103/107</td>
<td>Cisplatin</td>
<td>Sensitivity</td>
<td></td>
<td>[59]</td>
</tr>
<tr>
<td>miR-130a</td>
<td>Cisplatin</td>
<td>Sensitivity</td>
<td>MDR1</td>
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<tr>
<td>miR-130b</td>
<td>Cisplatin</td>
<td>Resistance</td>
<td></td>
<td>[61]</td>
</tr>
<tr>
<td>miR-141</td>
<td>Cisplatin</td>
<td>Resistance</td>
<td>KEAP1</td>
<td>[54]</td>
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<tr>
<td>Let-7i</td>
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<td>Resistance</td>
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<td>[62]</td>
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<td>miR-130a</td>
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<td>Sensitivity</td>
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<td>[52]</td>
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<tr>
<td>miR-374a</td>
<td>Cisplatin</td>
<td>Sensitivity</td>
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<td>[52]</td>
</tr>
<tr>
<td>miR-193b</td>
<td>Cisplatin</td>
<td>Resistance</td>
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<td>PTEN</td>
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<tr>
<td>miR-141</td>
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<td>chrXq27.3 miRNAs</td>
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<td>Sensitivity</td>
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<td>[85]</td>
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</table>

pathway and its activation plays a key role in cisplatin resistance. miR-214 was elevated in ovarian cancer and enhanced ovarian cancer cell survival and cisplatin resistance through targeting PTEN to activate the PTEN/Akt pathway. Moreover, inhibition of miR-214 expression sensitized ovarian cancer cells to cisplatin-induced apoptosis. miR-214 serves as an anti-apoptotic factor to mediate cisplatin resistance [64].

miR-506 increased ovarian cancer cell sensitivity to DNA damage through directly targeting DNA damage repair gene RAD51. Systemic delivery of miR-506 in nude mice significantly increased the cisplatin response [65]. Lysophosphatidic acid, epidermal growth factor and platelet-derived growth factor enhanced cell proliferation and increased miR-30c-2 expression in ovarian cancer. Ovarian cancer cells transfected with miR-30c-2 nearly eliminated cisplatin-induced cytotoxicity [66].

Dicer belongs to the RNase III family and controls maturation of miRNAs. Dicer downregulation results in a global decrease in miRNA expression and plays a significant role in cellular transformation and tumorigenesis. Kuang et al. demonstrated that Dicer downregulation increased cell proliferation, migration and cell cycle progression in ovarian cancer cells [67]. In addition, Dicer expression was lower in cisplatin-resistant ovarian cancer cells than parental cells. Knockdown of Dicer inhibited the sensitivity of ovarian cancer cells to cisplatin. These findings suggest that Dicer is involved in cisplatin resistance in ovarian cancer. DGCR8 binds to Drosha, an RNase III enzyme, to form the Microprocessor complex that cleaves a primary transcript of miRNA. The expression of DGCR8 was higher in ovarian cancer. Knockdown of DGCR8 sensitized ovarian cancer cells to cisplatin-induced apoptosis. In addition, deregulation of miRNA expression was observed in DGCR8-knockdown ovarian cancer cells, where miR-27b was the most highly downregulated miRNAs [68].

Aside from host miRNAs, viral miRNA also influenced cisplatin resistance. miR-BART7, a herpetic viral miRNAs from Epstein-Barr virus, induced cisplatin-resistance directly [69] (Figure 1, Table 2).
Response prediction

Expression levels of miR-141 were higher in patients with non-serous ovarian tumors that are resistant to platinum-based chemotherapy (platinum-free interval < 6 months) [54]. Elevated miR-506 expression was associated with better response to platinum chemotherapy, longer progression-free and overall survival in epithelial ovarian cancer patients [65]. Reduced let-7i expression was associated with the shorter progression-free survival in ovarian cancer [62]. Therefore, miR-141, miR-506 and let-7i might be used to predict chemotherapy response in patients with ovarian cancer.

Gu et al. showed that deregulation of miRNAs played a role in the favorable prognosis of patients with wild-type BRCA1/2 [70]. Ovarian cancer patients with alterations of BRCA1/2 have a better prognosis than non-carriers. Three miRNAs (i.e. miR-146a, miR-148a and miR-545) that target BRCA1/2 were associated with better survival outcomes in patients with wild-type BRCA1/2 treated with platinum-based chemotherapy. Therefore, patients who could benefit from platinum-based chemotherapy could be predicted from BRCA1/2-directed miRNA signature (Table 3).

Carboplatin

Since its introduction to clinical usage in 1992, carboplatin has become a commonly preferred agent over cisplatin because of its distinct toxicity profile. The advantage of carboplatin is its simple pharmacokinetics and a predictable toxicity profile [71-73]. The comparative therapeutic efficacy of cisplatin and carboplatin remains controversial [74, 75]. However, carboplatin is one of the most effective chemotherapeutic drugs for the treatment of ovarian cancer [76, 77]. Recently, it was proved that carboplatin plus paclitaxel is not inferior, when compared with cisplatin plus paclitaxel in patients with advanced ovarian cancer [78]. Carboplatin plus paclitaxel has less toxicity and is easier to administer [79, 80].

Drug effects on miRNA expression

miRNA profiling was evaluated among ovarian cancer cells in ascites and matched omental metastasis in patients with epithelial ovarian cancer. After being treated with carboplatin, malignant ovarian cancer cells in ascites demonstrated higher cell viability compared to omental metastasis. In addition, the expression levels of miR-21 and miR-214 were significantly higher in malignant cells of ascites [81]. This finding implicated that miR-21 and miR-214 might contribute to intrinsic carboplatin resistance in ovarian cancer (Table 1).

Modulation of chemosensitivity

Inhibition of miR-200c or miR-141 conferred ovarian cancer cells with resistance to paclitaxel and carboplatin [82]. The miR-200 family plays crucial roles in modulating chemosensitivity to carboplatin and paclitaxel in ovarian cancer. Prislei et al. demonstrated that expression of miR-200c was higher in the cisplatin-sensitive isogenic cells [83]. In addition, enforced expression of miR-200c increased cisplatin activity in ovarian cancer cells. A decrease of the total colony area was also observed in the miR-200c-overexpressing cells treated with cisplatin. Cittelly et al. showed that restoration of miR-200c in ovarian cancer cells, alone or in combination with paclitaxel, significantly decreased tumor burden in established tumors [84]. miR-193b, which is located on the opposite arm of miR-193b, contributed to resistance to both carboplatin and cisplatin in ovarian cancer cell lines through decreasing CRIM1 expression (Table 2).

Response prediction

Leskela et al. found that miR-200 was correlated with treatment response to the paclitaxel–carboplatin regimen [82]. Patients with higher miR-200c levels demonstrated lower relapse/progression rates. Bagnoli et al. demonstrated that low expression of chrXq27.3 miRNAs was
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associated with early relapse in ovarian cancer patients [85] (Figure 1, Table 2).

Concluding remarks and future perspectives

miRNAs are a class of small, non-coding RNA which regulate gene expression at post-transcriptional levels. Recent studies have demonstrated that miRNAs could be used to predict clinical outcomes of chemotherapy in various cancers. More importantly, miRNAs can modulate efficacy of chemotherapy. In ovarian cancer, cancer cells resistant to platinum-based agents often showed altered miRNA expression profiles. Furthermore, many of these deregulated miRNAs were found to modulate cellular sensitivity to cisplatin and carboplatin. It is therefore hopeful that targeted delivery of chemosensitizing miRNAs might help to maximize therapeutic effect of platinum-based agents, thereby improving clinical outcomes in patients with metastatic ovarian cancer. However, the mechanisms underlying miRNA regulation of chemosensitivity in ovarian cancer remain largely uninvestigated. In addition, studies in mouse xenograft models are limited. More importantly, evidence on safety and efficacy of miRNA-based treatment are lacking in humans. Further investigations with systematic identification and functional characterization of miRNAs are thus required. Future studies should also reveal the targets and signaling pathways in the regulation of chemosensitivity.

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Disclosure of conflict of interest

None.

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