Ovarian cancer (OC) is the leading cause of death from gynecological malignancies, and the fifth most common cancer death among women worldwide (1). Epithelial ovarian cancer (EOC, in this paper referred to as OC) accounts for more than 90% of all OCs, and is often referred to as ‘The Silent Killer’ due to lack of early signs and symptoms. More than 70% of patients diagnosed with OC, are diagnosed at an advanced stage [International Federation of Gynecology and Obstetrics (FIGO) stage III/IV], resulting in a 5-year overall survival <30% (2, 3). Standard treatment for advanced OC is surgery combined with neoadjuvant chemotherapy, and despite improvements in chemotherapy many patients experience chemotherapy resistance and relapses. The overall survival has only improved modestly over the last decade, therefore there is an urgent need to identify new diagnostic and prognostic biomarkers that can be used in the treatment of OC (3).

Diagnostic methods for OC today mainly include pelvic examination, transvaginal ultrasound, and serum cancer antigen 125 (CA125) measurement. However, these methods often fail to diagnose OC at an early stage. CA125 is the only serum biomarker used routinely for OC, and although quite specific (99%), the sensitivity for early stage disease in postmenopausal women is only 50–60% (4). Therefore, finding a reliable marker with increased sensitivity to
the early stages of OC remains a major clinical challenge. Recently, it has been shown that serum HE4 may play a future role in early diagnosis of OC (5).

MicroRNA (miRNAs) are small, non-coding RNA molecules of 20–22 nucleotides in length, that function in transcriptional and post-transcriptional regulation of gene expression by targeting complementary sequences of DNA and mRNA (6). miRNAs have shown to affect virtually all cellular functions, including proliferation, apoptosis, cell cycle, and differentiation (7), and have been associated with numerous kinds of cancers (8, 9). In OC, several studies have now shown miRNAs to be abnormally expressed. Ten years ago only a few hundred miRNAs were identified, today more than two and a half thousand have been identified, but the specific function of the vast majority is still unknown (10).

A major problem for determining the prognosis of advanced OC is the emergence of chemotherapy resistance, which develops in approximately 80% of patients during treatment (11). Even though improvements in platinum-based chemotherapy have been made, the first-line treatment in OC, usually consisting of platinum-based drugs combined with paclitaxel, still has a relative poor response rate, and upon emergence of resistance no alternative curative treatment is available (12). To be able to discern between patients who will benefit from chemotherapy and patients who will not, would be of great value for determining the appropriate treatment for OC.

The present review summarizes the current status and knowledge of miRNA expression in OC and the potential clinical relevance in diagnosis, prognosis, and treatment of OC.

SEARCH STRATEGY AND INCLUSION CRITERIA

This review has been performed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The following search criteria: ‘microRNA/miRNA/miRs’ and ‘epithelial ovarian cancer/ovarian cancer’ were used in the search of published literature through the Medline, EMBASE, Cochrane, and Scopus databases. Additional articles were identified by a review of references in relevant studies. We aimed to cover a wide spectrum of published literature, however, newer and large studies were favored, and the searches were restricted to articles in English. Studies on non-epithelial ovarian tumors and miRNA in urine were excluded due to the scope of

---

**Fig. 1.** Review and selection of articles.
MICRONAS AND OVARIAN CANCER

The first miRNA was discovered in 1993 in the nematode *Caenorhabditis elegans*; and for nearly a decade thought to be a curiosum of this organism. Today miRNAs are known to be expressed in a wide range of species (13).

MicroRNA are transcribed in the nucleus by RNA polymerase II as a long precursor molecule called primary-miRNA (pri-miRNA) that are able to create short ‘hairpin’ structures, which contain the miRNA sequence. These are further processed by the enzymes Drosha, member of the RNase III family, and Pasha into the hairpin precursor miRNAs (pre-miRNAs) of approximately 70 nucleotides, which are then exported to the cytoplasm by the Exportin 5 protein. In the cytoplasm the pre-miRNA is cleaved by Dicer, also an RNase III enzyme, to create a 22-nucleotide long, double-stranded RNA (14). Upon incorporation of this product into the RNA-induced silencing complex (RISC) that mediates RNA interference, one of the strands is degraded while the other is ultimately the mature miRNA used to identify complementary targets. Through the RISC, miRNAs generally inhibit mRNA translation and promote mRNA degradation (Fig. 2) (15). Additionally, miRNAs have been shown to be able to activate gene expression by interacting with complementary regions found in the promoter and coding region, as well as the 3′ untranslated region (3′UTR) of mRNA targets (16). Since mammalian miRNAs primarily use small 6–8-bp long ‘seed-sequences’ at the 5′ end of the miRNA to determine target complementarity, complementary sites to each miRNA can be found in many mRNAs, and consequently a single

![Fig. 2. MiRNA synthesis and biogenesis. A primary miRNA (pri-miRNA) transcript is encoded in the cell's DNA and transcribed in the nucleus by RNA polymerase II. The pri-miRNA contains a stem-loop structure that is cleaved in the nucleus by the endonuclease Dosha together with its RNA-binding protein DGCR8. The resulting precursor miRNA (pre-miRNA) is exported from the nucleus by exportin5 and then further cleaved by the endonuclease Dicer together with its RNA-binding protein TRBP (transactivation-response RNA-binding protein). After strand separation, the mature miRNA is incorporated in the RNA-induced silencing complex (RISC) initiating the regulatory actions of the miRNAs.](image-url)
miRNA may regulate multiple targets (17). And vice versa, as each mRNA often contain extensive 3'UTR sequences, they can be targeted by many different miRNAs.

MIRNA IN CANCER

MicroRNAs are found to be aberrantly expressed in cancer, indicating that they may function as either oncogenes or tumor suppressor genes. The linkage between miRNA and cancer was first observed in chronic lymphocytic leukemia where two miRNAs, miR-15 and miR-16, were found to be deleted or downregulated in a majority of chronic lymphocytic leukemias (18). Since then several studies have found abnormal expression of various miRNAs in different human cancers (8).

MIRNA AND OVARIAN CANCER

In 2007, Iorio et al. (19) were the first to analyze the global miRNA expression in human OC and compare the differential expression in carcinomas vs normal ovarian tissue. They found miR-141, miR-200a, miR-200b, and miR-200c to be overexpressed in carcinomas, and miR-125b1, miR-140, miR-145, and miR-199a to be downregulated. MiR-140 is located on chromosome 6q22, which is often deleted in ovarian tumors, and this miRNA is thought to target genes associated with invasion, including matrix metalloproteinase 13, fibroblast growth factor 2, and angiogenic VEGFA (19). Later, Zhang et al. (20) did one of the seminal studies on miRNA expression in OC in 2008. In this study, the authors investigated deregulated miRNAs in EOC, by comparing miRNA expression in 18 EOC cell lines to the expression in four immortalized, non-neoplastic cell lines derived from normal ovarian surface epithelium. They found 35 miRNAs to be differentially expressed between the two groups. Only four of these were upregulated in the EOC cell lines (miR-26a, miR-26b, miR-182, and miR-103) while the rest were downregulated, including the tumor suppressor miRNAs let-7d and miR-127.

In 2011, The Cancer Genome Atlas (TCGA) Research Network analyzed messenger RNA expression, miRNA expression, promoter methylation, and DNA copy number in 489 high-grade serous OC and additionally performed exon sequencing in 316 of these tumors. Transcriptomic analyses identified four different expression subtypes of high-grade OC designated: immunoreactive, differentiated, proliferative, and mesenchymal, based on the basis of the specific gene content in the subtypes. The miRNA expression on the other hand, identified three tumor subtypes, where miRNA subtype 1 overlapped with the mRNA proliferative subtype and miRNA subtype 2 overlapped the mRNA mesenchymal subtype. The miRNA subtype 1 was associated with significantly better survival, compared to the others (21). TCGA has provided basis for many other studies investigating the prognosis for high-grade OC (22–24). A recent study by Yang et al. (22) using the TCGA database showed that integrated analyses of miRNA and transcriptome expression could group the transcriptional subtypes into two more clinical relevant subtypes, one mesenchymal and one epithelial. Furthermore, they identified eight miRNAs (miR-25, miR-506, miR-29c, miR-182, miR-128, miR-101, miR-141, and miR-200a) that together were predicted to regulate 89% of the miRNA-associated genes.

Since then, several studies have investigated the differences between the miRNA profiles of ovarian surface epithelium and OC, and the potential role of miRNAs in OC diagnosis, prognosis, and treatment. Two of the most frequently identified, deregulated miRNAs in OC are the miR-200 and the let-7 families. The miR-200 family is formed by five microRNAs (miR-200a-miR-200b, miR-200c, miR-429) and expressed as two separate pri-miRNA transcripts. miR-200a, miR-200b, and miR-429 are located on chromosome 1, while miR-200c and miR-141 are located at chromosome 12 (25). Although several studies have attempted to investigate the prognostic importance of the miR-200 family in OC, no definitive conclusions have been drawn due to diverging results.

The human let-7 family consists of 13 discrete genetic loci that resolve into 10 mature miRNA sequences (Let-7a, Let-7b, Let-7c, Let-7d, Let-7e, Let-7f, Let-7g, Let-7i, miR-98, and miR-202), and are reported to be frequently downregulated in several different cancer types, including OC (26, 27). Let-7 is further known to suppress multiple oncogenes in OC such as KRAS, HRAS, c-MYC, and HMGA-2, and to inhibit cell cycle activators such as CD25, CDK6 as well as Cyclin A, D1, D2, and D3 (27–30). And as a result of these studies the let-7 family is believed to function as a tumor suppresor.

The number of studies investigating potential miRNAs related to OC diagnosis, prognosis, prediction of chemotherapy, and treatment has been immense, and the majority has been published within the last 3 years indicating that miRNAs are a suspected key element in the understanding of the mechanisms regulating OC.

MIRNAS AS PROGNOSTIC MARKERS OF OC

Since miRNAs are frequently reported deregulated in cancer and often have tissue-specific expression
Low expression of the let-7 family has also been identified as a potential marker for early diagnosis, and associated with a decreased overall survival in several studies of OC (36–38). An early study identified let-7 as a regulator of the gene HMGA2 (an early embryonic gene) in an OC cell line. High HMGA2/let-7 ratio was found to be associated with decreased progression-free survival (PFS), and loss of let-7 expression could be used as a marker for less differentiated cancer (39). Even though most let-7 members are reported to act as tumorsuppressors, the let-7a-3 isoform has been suggested to promote tumorigenesis and silencing of the let-7a-3 locus seemed to be associated with a better prognosis in OC, and therefore more likely to act as an oncogene (40, 41).

Correspondingly the miR-200 family has been investigated as a prognostic marker in several studies of OC. In a study by Hu et al. (42), 55 patients with stage III and IV OC, including all histologic subtypes, were investigated and they found expression of the miR-200 family cluster, including miR-200a/b/c and miR-429 to be significantly lower in patients with recurrent disease compared to recurrence free patients. Similar results were seen in a study by Eitan et al. (43) where the relation of miRNA expression to prognosis was investigated in 57 stage I or stage III OC patients with either serous or endometroid histology, and found miR-200a, miR-34, and miR-449b to be the most down-regulated miRNAs in advanced OC (stage III), and high miR-200a expression to be associated with early stage disease (stage I) and a more favorable outcome. Again in another large study of 107 OC patients, including all histologic subtypes and stages, it was shown that patients with high-grade OC combined with high miR-200a levels have an improved survival compared to patients with low miR-200a levels (44). And finally in line with all the above studies Marchini et al. (45) showed that loss of miR-200c in stage I EOC was associated with a poor PFS and OS. However, diverging results were observed in a smaller study of 20 patients with OC. Here Nam et al. (38) identified 23 miRNAs that were differentially expressed in OC compared to normal ovarian tissue, and further observed that high expression of miR-200a/b/c, miR-18, miR-93, miR-141, and miR-429 and low expression of let-7b and miR-199a significantly correlated with decreased PFS and OS.

Interestingly Prislei et al. recently demonstrated that overexpression of miR-200c in a panel of OC cell lines correlated with either poor or good prognosis depending on the cellular location of the RNA-binding protein HuR. HuR is known to modulate class III β-tubulin, a factor associated with

(31), they appear to be new and attractive potential biomarkers for cancer. However, the different studies are often not concordant with regard to which miRNAs are identified, and how they correlate with the clinical information, thus hampering our understanding. This could be explained by variations in the evolving new techniques used for miRNA expression analyses, the different subtypes of cancers used, differences between study cohorts, or caused by the heterogeneous nature of the tumor biology. The most notably studies that have identified potential prognostic miRNAs in OC are listed in Table 1.

Investigations of the miRNA processing enzymes Dicer and Drosha have indirectly implicated miRNA function in OC cancer. Merritt et al. investigated the protein levels of these enzymes immunohistochemically and found them to be decreased in respectively 60% and 51% of tissue samples from 111 patients with OC. Furthermore, tumor tissue with high levels of Dicer and Drosha were associated with increased median survival (32). These results were confirmed in a study by Faggad et al. (33) where they found a significant association between lower Dicer expression and poor overall survival, and additionally that tumors with low Dicer content were shown to be associated with decreased levels of mature miRNAs, further suggesting that miRNAs are involved in progression of OC. An unrelated study in lung cancer patients also identified a correlation between reduced Dicer expression and poor prognosis, indicating that this may be a general cancer phenomenon (34). The study by Faggad et al. also measured miRNA expression in relation to Dicer expression, and found as in the Merrit study that the miRNA expression was significantly lower in Dicer-negative patients compared to Dicer-positive patients, indicating that the effect observed due to altered Dicer and Drosha expression could be caused by loss of miRNA content.

A third study by Flavin et al. (35) could not detect an association between Dicer expression and survival in an analysis of 50 OC. All of the three studies examine patients of similar distribution of FIGO stages, but of different histologic subtypes. Faggad et al. investigated only patients with serous histology, whereas Flavin et al. included all histologic subtypes. Merritt et al. did not include information on histology. Additionally different scoring methods for describing protein levels were used. Thus, the negative result in the study by Flavin et al. could be due to a less sensitive scoring method combined with a smaller sample size and more heterogeneous tissue samples. However, Flavin et al. did find a significant relationship between Dicer expression levels and the presence of lymph node metastases.
Table 1. Potential prognostic miRNAs identified in ovarian cancer tissue

<table>
<thead>
<tr>
<th>miRNAs</th>
<th>Discovery cohort</th>
<th>Platform</th>
<th>Alteration</th>
<th>Endpoint/ clinical outcome</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Let-7 family</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Let-7i</td>
<td>72 OC, FIGO III-IV (serous, endometrioid, mucinous, clear cell, others), Ovarian cell lines (SKOV3, 2008, OVCAR10, OVCAR3)</td>
<td>miRNA microarray and Real-time RT-PCR</td>
<td>Downregulated</td>
<td>Decreased OS</td>
<td>(37)</td>
</tr>
<tr>
<td>Let-7b</td>
<td>20 OSC</td>
<td>miRNA microarray and Northern blot</td>
<td>Downregulated</td>
<td>Decreased OS</td>
<td>(38)</td>
</tr>
<tr>
<td>Let-7d, f, g, miR-98</td>
<td>107 OC, FIGO III-IV, (serous, endometrioid, mucinous, clear cell) NCI60 Cell lines</td>
<td>miRNA microarray and Real-time PCR</td>
<td>Downregulated</td>
<td>Decreased OS</td>
<td>(39)</td>
</tr>
<tr>
<td>Let-7a-3</td>
<td>214 OC, FIGO I-IV (serous, endometrioid, mucinous, clear cell, undifferentiated)</td>
<td>Real-time methylation-specific PCR and RT-PCR</td>
<td></td>
<td>Let-7a-3 methylation</td>
<td>Decreased risk of death (41)</td>
</tr>
<tr>
<td><strong>miR-200 family</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-200a, b, c, miR-141 and miR-429</td>
<td>20 OSC</td>
<td>DNA microarray (GenePix 4000B) and Northern blot</td>
<td>Upregulated</td>
<td>Decreased PFS and OS</td>
<td>(38)</td>
</tr>
<tr>
<td>miR-200a, b, c and miR-429</td>
<td>48 OC, 7 PPC FIGO III-IV OC SKOV-3 cells</td>
<td>RT-PCR</td>
<td>Downregulated</td>
<td>Decreased PFS and OS</td>
<td>(42)</td>
</tr>
<tr>
<td>miR-200a</td>
<td>57 OC patients, FIGO I or III (serous and endometrioid)</td>
<td>DNA microarray (Agilent Microarray Scanner)</td>
<td>Upregulated in early stage</td>
<td>Improved OS</td>
<td>(43)</td>
</tr>
<tr>
<td>miR-200a</td>
<td>107 OC patients, FIGO I-IV (serous, endometrioid, mucinous, clear-cell, carcinosarcomas, Brenner tumor)</td>
<td>DNA microarray (Human Genome U133 Plus 2.0 array, affymetrix) and qRT-PCR</td>
<td>Upregulated in late stage</td>
<td>Improved OS</td>
<td>(44)</td>
</tr>
<tr>
<td>miR-200c</td>
<td>144 OC patients, all FIGO I (serous, endometrioid, mucinous, clear cell)</td>
<td>qRT-PCR</td>
<td>Downregulated in early stage</td>
<td>Decreased PFS and OS</td>
<td>(45)</td>
</tr>
<tr>
<td>miR-200c</td>
<td>Cell lines: A2780, OVCAR-3, A278-CIS, A2780-ADR (differing sensitivities to paclitaxel)</td>
<td>qRT-PCR</td>
<td>Upregulated</td>
<td>Poor or good prognosis depending on cellular location</td>
<td>(46)</td>
</tr>
<tr>
<td>miR-18</td>
<td>20 OSC</td>
<td>DNA microarrays</td>
<td>Downregulated</td>
<td>Decreased PFS and OS</td>
<td>(38)</td>
</tr>
<tr>
<td>miR-30c, d and miR30e-3p</td>
<td>109 OC, FIGO I-IV (serous, mucinous, endometrioid, clear cell) 23 Borderline tumors 17 benign tumors 22 normal ovaries 6 normal HOSE cell lines (controls)</td>
<td>miRNA microarrays and qRT-PCR</td>
<td>Downregulated</td>
<td>Decreased disease-specific survival</td>
<td>(48, 49)</td>
</tr>
<tr>
<td>miR-34b, c</td>
<td>33 high-grade OSC 2 low grade OSC 2 serous borderline tumors 3 normal fallopian tube samples (FIGO I-IV)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Thus, the effect of the miR-200 expression could be dependent upon the localization of HuR, indicating that information upon this protein might be beneficial when interpreting the miR-200 data.

All studies on the miR-200 family and OC were performed with different microarray platforms or RT-PCR, and part of the discrepancy could perhaps be attributed to this (Table 1). Additionally, differences in study design, such as size and type of cohort, reference group, varying FIGO stages, and histologic subtypes, could likewise affect result interpretation. Also lack of information about study methods makes it difficult to compare and interpret the results. For example, the study by Nam et al. was small and did not include information on stage at diagnosis. However, over all, the majority of the studies show that high miR-200 expression is linked to a favorable prognosis (42–45), but may depend on the cellular location of various factors (46).

Several additional studies have identified other miRNAs that could be potential prognostic markers for OC. In a study by Lee et al. (47) investigating miRNA expression in patients with OC compared to benign ovarian tumors and borderline tumors they showed that higher miR-30d expression was an independent prognostic marker of longer OS and higher expression of miR-30c, miR-30d, miR30e-3p, and miR-181d were independent

<table>
<thead>
<tr>
<th>miRNAs</th>
<th>Discovery cohort</th>
<th>Platform</th>
<th>Alteration</th>
<th>Endpoint/ clinical outcome</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-93</td>
<td>83 EOC 6 wild type OSE cell samples (serous, mucinous, endometrioid, clear cell, mixed) FIGO I-IV</td>
<td>qRT-PCR</td>
<td>Upregulated</td>
<td>Decreased PFS and OS</td>
<td>(38)</td>
</tr>
<tr>
<td>miR-181d</td>
<td>109 OC, FIGO I-IV (serous, mucinous, endometrioid, clear cell) 23 Borderline tumors 17 benign tumors 22 normal ovaries 6 normal HOSE cell lines (controls)</td>
<td>RT-qPCR</td>
<td>Upregulated</td>
<td>Improved OS and disease-free survival</td>
<td>(47)</td>
</tr>
<tr>
<td>miR-199a</td>
<td>20 OSC patients 8 healthy controls</td>
<td>DNA microarray (GenePix 4000B) and Northern blot</td>
<td>Downregulated</td>
<td>Decreased PFS and OS</td>
<td>(38)</td>
</tr>
<tr>
<td>miR-422b</td>
<td>33 high-grade OSC 2 low-grade OSC 2 serous borderline tumors 3 normal fallopian tube samples (FIGO I-IV)</td>
<td>miRNA microarrays and qRT-PCR</td>
<td>Downregulated</td>
<td>Decreased disease-specific survival</td>
<td>(48)</td>
</tr>
<tr>
<td>miR-509-5p, miR-510</td>
<td>Microarray: 20 OSC (FIGO I and III) Validation (qRT-PCR): 51 OSC (FIGO I and II)</td>
<td>miRNA microarray and qRT-PCR</td>
<td>Downregulated</td>
<td>Decreased OS</td>
<td>(50)</td>
</tr>
<tr>
<td>miR-519a</td>
<td>54 OC, FIGO I-IV (Serous, mucinous, endometrioid, clear cell) 5 benign serous ovarian tumors 14 ovarian borderline tumors</td>
<td>miRNA microarray and qRT-PCR</td>
<td>Upregulated</td>
<td>Decreased OS</td>
<td>(51)</td>
</tr>
</tbody>
</table>

OC, ovarian cancer; OS, overall survival; PFS, progression-free survival; PPC, primary peritoneal cancers; HOSE, human ovarian surface epithelial; OSE, ovarian serous epithelial; OSC, ovarian serous carcinomas; FIGO, International Federation of Gynecology and Obstetrics; qRT-PCR, quantitative reverse transcription polymerase chain reaction.
prognostic markers for disease-free survival. Others have identified low expression of miR-34c and miR-422b in high-grade OC to be associated with decreased disease-specific survival (48). Corney et al. found downregulation of miR-34b/c in late-stage OC and associated with mesenchymal-to-epithelial transition (49). Also low expression of miR-510 and miR-509-5p was shown to be significantly associated with poor OS (50). Kim et al. (51) found a significant upregulation of miR-519a to be associated with poor survival outcome.

The most notable studies that have identified potential prognostic miRNAs are listed in Table 1, and although a number of miRNAs are interesting as potential prognostic markers, the underlying mechanisms resulting in altered miRNA still remains to be resolved, and most of the mentioned miRNAs require further validation in independent series.

**MIRNAS IN PREDICTION OF CHEMOTHERAPY SENSITIVITY**

One of the major challenges in OC treatment is the development of chemotherapy resistance. Approximately 20% of all primary OC patients are resistant to the standard platinum-based chemotherapy resulting in a fast, most often catastrophic, disease course. Out of the 80% initially sensitive to chemotherapy nearly all patients will eventually experience relapse and develop drug resistance, and the acquired resistance often leaves no other available curative treatment (3, 12).

One of the first miRNAs identified to be significantly deregulated in chemotherapy-resistant EOC is the let-7i, and it was further observed that decreased levels of let-7i also was correlated with shorter survival (37). Sorrentini et al. were likewise some of the first to identify miRNAs to be altered in drug-resistant OC tumors cells. They identified five miRNAs (let-7e, miR-30c, miR-125b, miR-130a, and miR-335) that were diversely expressed in resistant OC cell lines. Let-7e was upregulated in A2780TAX cells, but downregulated in other resistant cell lines. On the contrary, miR-125b was downregulated in A2780TAX, and upregulated in other cell lines. MiR-30c, miR-130a, and miR-335 were found to be downregulated in all resistant cell lines (52). The following year, in the before mentioned study by Eitan et al., they identified an array of miRNAs that could predict patients’ response to platinum-based chemotherapy. They identified 18 miRNAs that were differentially expressed between stage I and stage III OC, and seven miRNAs that were significantly, differentially, expressed between chemo-sensitive and chemo-resistant patients (miR-27a, miR-23a, miR-30c, let-7g, miR-199a-3p, miR-378, and miR-625). A high expression of five miRNAs (miR-27a, miR-23a, miR-449b, miR-21, miR-24-2) showed a correlation with poorer survival (43). Let-7a expression has also been linked to the chemotherapeutic response, where the beneficial impact of the addition of paclitaxel to platinum therapy was better in patients with low let-7a levels (53). Bagnoli et al. (54) identified a cluster of eight miRNAs (miR-506, miR-509-5p, miR-509-3p, miR-508-3p, miR-514, miR-507, miR-513a-5p, miR-513b) located on chrXq27.3 locus that were down modulated in early relapsing, advanced-stage OC patients, and associated with a reduced sensitivity to chemotherapeutic.

Additionally, the miR-200 family has also been reported to affect response to chemotherapy, however, the results have not been concordant. First Leskela et al. found that tumors with high level of β-tubulin III protein had significantly decreased levels of miR-200c, miR141, and miR429, and patients not responding to chemotherapy had significantly lower miR-200c levels than women who achieved complete response. In functional studies using the OC cell-lines Hey, SKOV3, OVCA 420, and OVCA 433, Cochrane et al. showed that restoration of miR-200c reduced β-tubulin III in OC and restored sensitivity to paclitaxel, a chemotherapeutic compound often given in combination with platinum-based drugs (55, 56). Corresponding results were obtained by Prislei et al. (46), who showed that overexpression of miR-200c in A2780 cells sensitized cells to cisplatin and paclitaxel. Furthermore, Mateescu et al. (44) showed that upregulation of miR-200a and miR-141 restored sensitivity to paclitaxel. However, Van Jaarsveld et al. (57) reported the opposite effect, that overexpression of miR-141 in OC A2780 cells increased resistance to the platinum-based chemotherapy Cisplatin. As previously mentioned, Prislei et al. suggested that the action of the miR-200s family could be dependent on the nuclear or cytoplasmic location of the RNA-binding protein HuR, and the conflicting results could possibly be addressed to this phenomenon.

In a recent patient cohort study, Vecchione et al. (58), analyzed miRNA expression in 198 OC tumors samples, and identified three miRNAs (miR-484, miR-642 and miR-217) that were downregulated in chemoresistant patients, while Parikh et al. identified miR-181a as a predictor of tumor recurrence and chemo-resistance in high-grade OC. They showed that miR-181a expression was significantly higher in tumor biopsies that were taken after patients have recurred, compared to the
matched-tumor biopsies taken at primary surgery (59).

**CIRCULATING miRNAs AND OVARIAN CANCER**

Within the last couple of years it has been revealed that miRNAs can be found circulating in peripheral blood, and proven to be very stable because they are bound to protein complexes or incorporated into micro vesicles, such as exosomes, that are resistant to degradation by RNAses (60, 61). Therefore, circulating miRNAs have potential as a minimal invasive, diagnostic, and prognostic marker, and has within the last years been the focus of increasing amounts of research.

One of the initial studies identifying tumor-derived miRNAs in circulation in OC patients was published in 2008 by Taylor et al. They isolated circulating tumor-derived exosomes from sera of 50 patients diagnosed with OC, compared it to their respective miRNA expression profile from tissue tumor cells and with exosomal miRNA from sera specimens of patients with benign disease (n = 10) and healthy controls (n = 10). They found 218 of 467 tested miRNAs to be expressed in both tumor cells and serum-derived exosomes, and most of them were expressed similarly. Further they showed that the exosomal miRNA profiles in OC patients were similar and significantly distinct from benign disease. There were no significant differences between different stages of OC, and interestingly exosomal miRNA were virtually absent in healthy controls, indicating that the circulating exosomal miRNAs were mainly tumor derived (62). In the same year Resnick et al. compared miRNA expression profiles in serum from 28 patients with OC compared to 15 healthy controls, and found five miRNAs (miR-21, 92, 93, 126, and 29a) to be significantly overexpressed in serum from patients with OC compared to controls, while three miRNAs (miR-155, 127, and 99b) were significantly downregulated, however, it should be confirmed in other studies if this is a true downregulation. Interestingly they also found that three miRNAs (miR-21, 92, and 93) were overexpressed in three patients with normal pre-operative CA-125. Resnick et al. (63) were the first to describe the use of RT-PCR in the serum miRNA examination, however, considering the limited sample size and lack of long-term survival data, it was unclear if the serum miRNA were actually tumor derived. Subsequently Kan et al. (64) reported the miRNA-200 family to be highly overexpressed in serum of patients with OC (n = 28) relative to healthy controls (n = 28), indicating that serum miRNAs could be promising diagnostic markers. Several other studies have subsequently identified additional specific miRNA in plasma that could distinguish patients with OC from benign tumors or healthy controls (Table 2) (65–68).

Serum miRNAs have also been reported to correlate with survival outcome. In 2013 Hong et al. identified miR-221 to be upregulated in 96 patients with OC compared to healthy controls (n = 35). Further they showed that the level of miR-221 was significantly associated with FIGO stage and tumor histologic grade. High miR-221 was an independent, unfavorable, significant prognostic factor for survival (69). Also high expression of miR-21 in plasma has been correlated with poor OS, and high expression of miR-92 as well as miR-20a has been shown to be correlated with FIGO stage in OC (70–72). Gao and Wu (73) found that miR-200c showed a descending trend from early stages to advanced stages, while the level of miR-141 exhibited an increasing trend. In a recent study, Shapira et al. (74) further identified five miRNAs that were differently expressed between patients with long OS compared to patients with short OS, however, only miR-1290 remained significant after multiple adjustments.

In a substantial study, including 360 patients with OC and 200 healthy controls, Zheng et al. found miR-205 and let-7f to be potentially early diagnostic biomarkers for OC. The combination of high miR-205 expression, low let-7f expression and elevated serum CA-125 levels further improved the accuracy of early detection of OC in stage I disease (36), which hopefully can be applied to hospital settings to improve the accuracy of OC diagnosis, and could be a potential valuable biomarker for early diagnosis of OC. One smaller study, only investigating ovarian clear cell carcinomas, found four miRNAs to be significantly higher in preoperative blood samples compared to post operative. MiR-130a was identified as a potential biomarker for diagnosis of early, recurrent disease, where elevated expression could be detected in recurrent patients even before an elevated CA125 serum level was observed (75).

In an earlier, minor study by Hausler et al. (76) they identified upregulated miR-30e-1 and downregulated miR-342-3p, miR-181a, and miR-450b-5p to be associated with recurrent OC after multiple chemotherapy treatments. Another recent and substantial study investigating plasma miRNA expression, compared patients with endometriosis to patients with EOC and healthy controls. In this study, a panel of miRNAs (miR-15b, miR-16, miR-21, and miR-195) was shown to increase from controls to endometriosis to EOC. And the results were

© 2016 APMIS. Published by John Wiley & Sons Ltd
further supported by the fact that the identified miRNAs were closely, similarly expressed in a transgenic mouse model of epithelial-associated OC (77).

Moreover, some studies have identified circulating miRNAs as potential therapeutic targets. Shen et al. (78) showed that miR-26a in plasma could distinguish patients with OC from healthy controls, and functional experiments in cell culture and nude mice revealed that inhibition of miR-26a decreased tumor cell growth. Wei et al. demonstrated that miR-212 inhibited OC cell proliferation, migration, and invasion, and showed that the important tumor promoter HB-EGF was a target of miR-212.

Table 2. Alternated circulating miRNAs in ovarian cancer

<table>
<thead>
<tr>
<th>References</th>
<th>Year</th>
<th>Sample type</th>
<th>Sample size</th>
<th>Sample size</th>
<th>Upregulated</th>
<th>Downregulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>(62) 2008</td>
<td>Exosomal (serum) miRNA</td>
<td>50 patients 10 benign 10 controls</td>
<td>miR-21, miR-141, miR-200a, miR-200b, miR-200c, miR-203, miR-205, miR-214</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(63) 2008</td>
<td>Serum miRNA</td>
<td>28 patients 15 controls</td>
<td>miR-21, miR-92, miR-93, miR-126, miR-29a, miR-127, miR-99b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(76) 2010</td>
<td>Whole blood</td>
<td>24 patients 15 controls</td>
<td>miR-30c1 miR-342-3p, miR-181a, miR-450b-5p</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(64) 2012</td>
<td>Serum miRNA</td>
<td>28 patients 28 controls</td>
<td>miR-200a, miR-200b, miR-200c</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(69) 2013</td>
<td>Serum miRNA</td>
<td>96 patients 35 controls</td>
<td>miR-221</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(70) 2013</td>
<td>Serum miRNA</td>
<td>94 patients 40 controls</td>
<td>miR-21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(71) 2013</td>
<td>Serum miRNA</td>
<td>Patients 50 Controls 50</td>
<td>miR-92</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(77) 2013</td>
<td>Plasma miRNA</td>
<td>EAOC 33 Serous OC 21 Endometriosis 33 Controls 20</td>
<td>miR-15b, miR-16, miR-21, miR-195</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(36) 2013</td>
<td>Serum miRNA</td>
<td>Patients 360 Controls 200 Patients 18 Controls 24</td>
<td>miR-205 Let-7f</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(65) 2014</td>
<td>Serum miRNA</td>
<td>Patients 34 Benign 10 Controls 23</td>
<td>miR-20a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(74) 2014</td>
<td>Serum miRNA</td>
<td>Patients 42 Benign 36 Controls 23</td>
<td>Exclusively expressed in patients with OC: miR-1274a, 625-3p, 720. miR-1290 (elevated in patients with long overall survival). miR-106a, 126, 146a, 150, 16, 17, 19b, 20a, 223, 24, 921, 106b, 191, 193a-5p, 30b, 20a-5p, 30c, 320, 328</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(75) 2014</td>
<td>Serum miRNA</td>
<td>Patients 21</td>
<td>miR-130a (elevated in early disease recurrences) miR-26a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(78) 2014</td>
<td>Plasma miRNA</td>
<td>Patients 17 Controls 13</td>
<td>miR-122-5p, miR-152-5p, miR-25-3p miR-145</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(66) 2015</td>
<td>Whole blood → serum</td>
<td>Patients 25 Benign 25</td>
<td>Let-7i, miR-122-5p, miR-152-5p, miR-25-3p miR-145</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(67) 2015</td>
<td>Serum miRNA</td>
<td>Patients 84 Benign 51 Controls 135</td>
<td>miR-200c, miR-141</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(73) 2015</td>
<td>Serum miRNA</td>
<td>Patients 74 Benign 19 Controls 50</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

EAOC, endometriosis-associated ovarian cancer. Text written in bold comprises miRNAs reported deregulated in more than one study.
Therefore, miR-212 could be a promising candidate for the treatment of OC (79).

Only a few studies investigating miRNAs specific for various histologic subtypes have been published, therefore further investigation on their potential to classify OC is needed. An overview is given in Table 3. All together the studies support the idea that cell-free-derived miRNAs potentially may be valuable biomarkers for screening, early diagnosis, prediction, and prognosis of OC. However, so far the identified circulating miRNAs lack validation in additional studies. Identified plasma/serum miRNAs that could be potential biomarkers in OC are summarized in Table 2.

### THE THERAPEUTIC POTENTIAL OF MiRNAs IN OVARIAN CANCER

As evidence has indicated, miRNAs can function as either oncogenes or tumor suppressor genes, and correction of the altered miRNA expression is an attractive target for cancer therapeutics. Correction of the alteration can be done either by using miRNA mimics (miRNA replacement therapy) to restore loss-of-function or by inhibition of the upregulated oncomiRs using antisense miRs (miRNA inhibition therapy) (80). In hepatocellular carcinoma, miR-34 was recognized to be frequently downregulated and has been the template for the first target of an ‘anticancer miRNA drug’ named MRX34. This drug is a miR-34 mimic that inhibits tumor growth and increases OS in mouse models, and has now entered a phase I clinical trial in patients with hepatocellular carcinoma or metastatic cancer that has spread to the liver (80, 81). Interestingly, the miR-34 family has been shown to be significantly downregulated in OC as well (19, 20, 49).

As functional studies proceed to verify the role of the interesting OC miRNAs mentioned throughout this review, they will become prime candidates for future therapeutic intervention for treating OC.

As mentioned it has been shown that miRNA expression profiles are able to predict sensitivity to chemotherapy, and this has introduced the field termed ‘miRNA pharmacogenomics’, where miRNAs are termed ‘pharmaco-miRs’. A database that identifies potential pharmaco-miRs, genes they regulate, and the drugs of interest has been established and is available online (http://pharmaco-mir.org/) (82). Some of the most imperative studies in this regard are listed in Table 4. Platinum-based chemotherapy is generally first-line treatment for OC patients, after debulking surgery, and since approximately 80% eventually develop resistance to the treatment, there is an urgent need for methods to prevent emergence of chemoresistance.

Van Jaarsveld et al. showed that overexpression of miR-141 (a member of the miR-200 family) enhanced resistance to Cisplatin, possibly through directly targeting KEAP1 (Kelch-like ECH-associated protein 1) resulting in repression of KEAP1 that contributed to the cisplatin resistance. Additionally they showed that inhibition of this pathway partially reversed the miR-141-mediated cisplatin resistance, thereby suggesting miR-141 as a promising future target (57). Other data show that the let-7 family is also likely to be a potential target in modulation of chemosensitivity.

In a recent study by Cai et al. (83) they demonstrated that treatment of OC with let-7e antagomirs combined with cisplatin enhanced the intratumoral concentration of cisplatin and significantly reduced the growth of tumors in mice models. Another recent study by Guo et al. (84) discovered that glycogen synthase kinase 3 (GSK-3)β repressed the production of let-7 in OC cells, and identified GSK-3β as an indirect therapeutic target of let-7 in OC. In resemblance Yan et al. demonstrated that prostatic secretory protein 94 (PSP94) expression is decreased in an acquired chemoresistant OC cell line, apparently by regulating downstream signaling of Lin28b, which is a direct upstream target and inhibitor of the let-7 family. They further showed that restoration on PSP94 expression resensitized

### Table 3. MiRNAs identified in ovarian cancer tissue according to histologic type

<table>
<thead>
<tr>
<th>Type of OC</th>
<th>Upregulated</th>
<th>Downregulated</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serous</td>
<td>miR-7, miR-21, miR-22, miR34c-5p, miR-141, miR-200a,-b,-c, miR-214, miR-302b, miR-490a, miR-519a, miR-3373</td>
<td>Let-7b, miR-22, miR-31, miR-34a,-b,-c, miR-99a, miR-125b, miR-148b, miR-211</td>
<td>(19, 38, 51, 89, 96, 114)</td>
</tr>
<tr>
<td>Endometrioid</td>
<td>miR-141, miR-200a/b/c</td>
<td>miR-20a</td>
<td>(19)</td>
</tr>
<tr>
<td>Clear Cell</td>
<td>miR-29b, miR-30a-3p, miR-30c, miR-30e-5p, miR-200a/c, miR-486-5p</td>
<td></td>
<td>(19, 47, 51, 89)</td>
</tr>
<tr>
<td>Mucinous</td>
<td>miR-141, miR-200b</td>
<td></td>
<td>(51)</td>
</tr>
<tr>
<td>miRNAs</td>
<td>Alteration</td>
<td>Cellular function</td>
<td>Target</td>
</tr>
<tr>
<td>--------</td>
<td>------------</td>
<td>-------------------</td>
<td>--------</td>
</tr>
<tr>
<td>miR-200 family</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-200a</td>
<td>Mixed</td>
<td>Decreased migration and invasion upon miR-200a mimic</td>
<td>ZEB2</td>
</tr>
<tr>
<td>miR-200b</td>
<td>Downregulated</td>
<td>Metastasis, angiogenesis</td>
<td>IL8, CXCL1</td>
</tr>
<tr>
<td>miR-141, 200a</td>
<td>Mixed</td>
<td>Oxidative stress response, paclitaxel sensitivity upon overexpression</td>
<td>MAPK14</td>
</tr>
<tr>
<td>Let-7 family</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Let-7e</td>
<td>Downregulated</td>
<td>Overexpression increases platinum sensitivity</td>
<td>EZH2 CCD1</td>
</tr>
<tr>
<td>Let-7i</td>
<td>Downregulated</td>
<td>Overexpression increases platinum sensitivity</td>
<td>H-RAS, HMGA2</td>
</tr>
<tr>
<td>Let-7 (indirect)</td>
<td>Downregulated</td>
<td>GSK-3β inhibition increased cell death</td>
<td>GSK-3β</td>
</tr>
<tr>
<td>Let-7 (indirect)</td>
<td>Downregulated</td>
<td>Overexpression of PSP94 increases paclitaxel sensitivity</td>
<td>PSP94 (Lin28/let-7)</td>
</tr>
<tr>
<td>miR-506</td>
<td>Low in chemoresistant cells</td>
<td>Overexpression increases platinum sensitivity, inhibits proliferation, and senescence Suppresses tumor growth and EMT</td>
<td>RAD51, CDK4, CDK6, SNAI2</td>
</tr>
<tr>
<td>miR-520d-3p</td>
<td>Low expression associated with shorter survival</td>
<td>Overexpression inhibits proliferation, migration, and invasion</td>
<td>EPHA2, EPHB2</td>
</tr>
<tr>
<td>miR-199/214 cluster</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>miR-199a/214</td>
<td>Elevated</td>
<td>Knockdown: reduces OC growth, increases chemosensitivity Stemness</td>
<td>IKKβ/NF-κB, PTWN/AKT</td>
</tr>
<tr>
<td>miR-199a/214</td>
<td>Elevated</td>
<td>Overexpression increases platinum sensitivity</td>
<td>PTEN/AKT</td>
</tr>
<tr>
<td>miR-31</td>
<td>Downregulated</td>
<td>Overexpression increases chemosensitivity. Overexpression inhibits proliferation and induces apoptosis</td>
<td>Stathmin1, MET, E2F2, STK40, CEBPA</td>
</tr>
<tr>
<td>miR-187</td>
<td>Overexpressed</td>
<td>Continued increased levels of miR-187 inhibits EMT</td>
<td>Dab2</td>
</tr>
<tr>
<td>miR-125a</td>
<td>Downregulated</td>
<td>Overexpression inhibits EMT</td>
<td>ARID3B</td>
</tr>
<tr>
<td>miR-138</td>
<td>Downregulated</td>
<td>Overexpression inhibits EMT</td>
<td>SOX4, HIF-1α</td>
</tr>
<tr>
<td>miR-7</td>
<td>Downregulated in metastatic EOC</td>
<td>Overexpression inhibits invasion, migration, and EMT</td>
<td>EGFR</td>
</tr>
<tr>
<td>miR-137</td>
<td>Downregulated</td>
<td>Overexpression reduces OC growth. Overexpression inhibits proliferation, migration, and invasion. Induces apoptosis and suppresses tumor growth</td>
<td>AEG1</td>
</tr>
<tr>
<td>miR-92a</td>
<td>Downregulated</td>
<td>Overexpression inhibits cell adhesion, invasion, and proliferation. Reduces peritoneal metastasis</td>
<td>ITGA5</td>
</tr>
</tbody>
</table>
resistant cells to drug treatment, and introduced PSP94 as a potential target for reversing drug resistance (85).

Likewise miR-506 has been presented as a potential target to enhance chemosensitivity. Liu et al. (86) first reported that miR-506 could inhibit CDK4/6-FOXM1 signaling, the transcription factor network, which is activated in most high-grade OCs, and later they showed that systemic delivery of miR-506 in vivo significantly amplified cisplatin and olaparib responses (87).

Using TCGA, Nishimura et al. identified miR-520d-3p as an independent prognostic marker for EOC, and showed that miR-520d-3p functioned as a tumor suppressor, upstream of the EPHA2 gene. They investigated the effect of dual targeting of EPHA2 with nano-liposomes loaded with miR-520d-3p and EphA2-siRNA and showed synergistic anti-tumor efficiency and greater therapeutic efficacy in vivo than either monotherapy alone (88). Combined these studies support the idea of miRNA-based combination therapies to improve the efficacy of chemotherapy.

Elevated expression of miR-214 has been reported in several types of cancers, and has been associated with chemoresistance and metastasis (89–91). It has been demonstrated that miR-214 represses p53 in OC cells and knockdown of miR-214 increases sensitivity to cisplatin and doxorubicin (92). Another study showed that TWIST1 (a highly conserved transcription factor) regulates the two pathways IKKβ/NF-κB and PTEN/AKT through the expression of the miR199a-2/214 cluster (93). Yang et al. (89) correspondingly showed miR-214 and miR-199 to be altered in OC, and that miR-214 significantly induced cell survival and cisplatin resistance through targeting the PTEN/AKT pathway. Latest, Zheng et al. found that miR-214 disturbs DNA damage responses through downregulation of RNRF8 (a protein that plays a key role in DNA damage response) and causes chromosomal instability in OC.

MiR-31 is another miRNA that has been associated with acquired resistance to chemotherapy. First Mitamura et al. showed that downregulation of miR-31 and high expression of MET (c-Met or hepatocyte growth factor receptor) was associated with taxane-resistant OC cells. They found that miR-31 directly targets the 3’UTR of MET, and that reintroduction of miR-31 resensitized the OC cells to paclitaxel both in vitro and in vivo (94). More recently the same group found that Statmhin 1, a microtubule-depolymerizing molecule, known to be involved in chemoresponses, was elevated in chemoresistant cells, and that reintroduction of miR-31 reduced Statmhin 1 and restored chemosensitivity (95). These studies introduce a miRNA-based therapeutic strategy, aiming to overcome resistance to paclitaxel. Creighton et al. likewise showed that miR-31 presented the largest downregulation in OC, and forced expression of miR-31 inhibited proliferation and induced apoptosis in cell lines with a dysfunctional p53 pathway (96).

MIRNAS IN INHIBITION OF METASTASIS

Epithelial-to-mesenchymal transition (EMT) is a well-known process in the initiation of metastasis for cancer progression (97). Extensive research has identified miRNAs as important regulators of EMT, mainly by targeting E-Cadherin transcription repressors.

The miR-200 family is shown to be a potential inhibitor of EMT and tumor cell migration, invasion and metastasis by downregulating ZEB1 (zinc finger E-box-binding homeobox) and ZEB2 (25, 98). In 2013, using the TCGA data, Pecot et al. demonstrated that miR-200 inhibits angiogenesis by targeting interleukin-8 and CXCL1 [chemokine (C-X-C motif) ligand 1] secreted by tumor endothelial cells, and further that transfer of miR-200 members into the tumor endothelium resulted in clear reduction in metastasis and angiogenesis, and vascular normalization in different cancers including OC, hereby again indicating that the miR-200 family is an interesting potential therapeutic agent in cancer therapy (99).
OC from the TCGA showed that miR-506 could prevent TGF-β-induced EMT by targeting the Ecadherin transcriptional repressor SNAI2. Further, they demonstrated that nanoparticle delivery of miR-506 in OC mouse models reduced tumor growth, indicating that miR-506 may represent a promising new target to suppress EMT and cancer progression (22). Likewise, Sun et al. found that miR-506 besides being a potential therapeutic target in enhancement of chemosensitivity, also plays a key role in EMT inhibition. MiR-506 simultaneously suppresses vimentin, N-cadherin and SNAI2 expression, while E-cadherin expression was increased, and nanoparticle delivery of miR-506 in EOC orthotopic mouse models resulted in dissemination of EOC cells (100).

Several other miRNAs have been identified as inhibitors of the EMT and thus as potential therapeutic agents in OC, including miR-187, miR-125a, miR-138, and miR-7 (101–104). Conversely, miR-181a has been identified as a promoter of EMT transition in EOC, and inhibition of miR-181a decreased cell survival, invasion, migration, drug resistance, and dissemination (59).

Studies identifying new potential therapeutic miRNAs for OC are published continuously with an accumulative rate. Some of the recent and notably published studies also describe miR-137, miR-92a, miR-106b, and the formerly mentioned miR-484 (58, 105–108).

DISCUSSION

MiRNA expression profiles in both tissue and blood have shown their profound potential as biomarkers for detection and surveillance of OC. However, the frequency and the pathologic significance of aberrant miRNA expression in OC has not yet been fully clarified.

The let-7 and miR-200 families are the most frequently reported deregulated miRNAs in OC, and both have been shown to be involved in EMT. EMT is considered the key regulator of the post-ovulatory repair process, and failure to undergo EMT may initiate OC (109). The let-7 family has also shown its potential as a marker for selection of chemotherapeutic agents in OC. Several studies have also identified the miR-200 family as a potential marker for chemosensitivity, however, the results have been conflicting.

Although the miR-200 family is one of the most often identified miRNAs altered in OC, it is also the group of miRNAs with the most diverging results. The family is believed to suppress metastasis, but most studies have reported an overexpression of miR-200 family in OC. The few studies that have reported a downregulation or even an unchanged expression of miR-200 in OC may be explained by sample size, study design, sample types (tumors, cell lines, etc.), clinical data (post-menopausal women, pre-treatment, residual disease etc.), differences in control groups and platforms (microarray, sequencing, etc.), or the inclusion of ovarian stromal cells that are known to lack miR-200 expression (110). Further it has been suggested that miR-200 could change expression as a result of the progression of the disease. It is possible that miR-200 is downregulated in early stage disease, when cancer cells exhibit invasive behavior, and subsequently becomes upregulated when the cells undergo mesenchymal-to-epithelial transition (111). Prisli et al. recently proposed another hypothesis for the difference in miR-200c. They suggested that the function of the miR-200c depended on the cellular location of the RNA-binding protein, HuR, to the 3’UTR of the mRNA of the class III β-tubulin. When HuR localization was only nuclear, miR-200c inhibits the class III β-tubulin resulting in a good prognosis, but when HuR localization was also cytoplasmic miR-200c enhanced class III β-tubulin resulting in a poor prognosis (46). These results are in concordance with the results of the study by Cochrane et al. (56) showing that miR-200c mediated class III β-tubulin downregulation resulting in enhanced chemosensitivity only is evident for endogenous class III β-tubulin, whereas miR-200c has no effect exogenous class III β-tubulin. It has also been suggested that the relationship between the miRNA expression and the response could be different for the distinct histologic subtypes (57).

In this review we also summarized the potential role miRNAs may play as biomarkers in prediction of chemotherapy response, and let-7 and miR-200 are the two important families in this regard, but should be further validated before any consensus can be made. Most studies focus on single miRNAs as possible markers, however, in other cancers miRNA predictors have been developed from the characterization of the miRNA profile and measured growth inhibition of different human cell lines in the presence of distinctive chemotherapeutics, and blinded validation of the predicted sensitivity showed to match the clinical response (112). It would be an interesting approach to study miRNA as a network in OC as well.

As miRNAs are present in plasma/serum, they allow a new possible method to achieve early diagnosis in OC, that could improve the patient outcome greatly, as chemotherapy interventions at early stage of OC is more successful, and have
lower recurrence rates. Even though the first study on circulating miRNAs in OC was published in 2008, the analyses in this field still need to be investigated and eventually validated in independent studies to clarify a possible role in clinical use.

The first anticancer drug, that entered a phase I clinical trial in patients in 2013 (MRX34, for hepatocellular carcinoma), could potentially be a future anticancer drug in OC as well, since miR-34 is frequently downregulated in OC. If the ongoing study of MRX34 shows positive and reliable results, this could be of great importance in the treatment of OC as well. However, the first two developed miRNA therapeutic drugs, MRX34 and miR-122 (for hepatitis C virus infection), are targeted hepatic diseases that are assumed to be easy to target as the hepatic circulation is the first major barrier for any systemic administered drug, and most of the systemic administered miRNAs are known to accumulate in the liver, spleen, and kidneys (80). For the MRX34, a liposome-based method of delivery is used, but several different modifications of methods for miRNA delivery, such as conjugation-, antibody-, nanoparticle-, and liposome-based methods are being tested in pre-clinical models (80). The same administration of MRX34 might not have same effect in OC. Instead, an intraperitoneal administration of miRNA therapeutics in OC could be a possible method, as intraperitoneal administration of platinum-based compounds sometimes are used in treatment of OC (113). Although, as miRNAs may affect the expression of a multitude of genes that occasionally could have opposing consequences, implies that the use of miRNAs in therapeutics should be introduced with great caution. And also the delivery to the appropriate cell type or tissue is an important aspect of effective miRNA mimicry to prevent unwanted side effects. However, targeting miRNAs rather than specific genes or proteins may be more effective, since they often target entire pathways.

A central problem that comprises the possibility to compare results of present studies on miRNAs in OC are the major variation in study methods and design. Further, the wide range of miRNAs that has shown to be deregulated in OC may also be a result of the considerable heterogeneity of the tumor. Together these differences in studies render it difficult to clarify the central miRNA profiles of OC, and most studies on miRNAs have been limited to a single histotype. Therefore, a general standardized analytical method should be presented, which can limit differences between studies and allow comparisons across different studies. In addition, validation of studies in independent series that ideally should be histotype-specific is essential to determine the clinical role of miRNAs in OC.

CONCLUSION

The research within miRNAs, their involvement and possible use as markers in diagnosis, prognosis and therapeutics in cancer has been enormous within the last decade. However, the path from identifying potential useful miRNAs to application in daily clinical diagnostics, prognostics, and treatments is still challenging. Studies performed in large well-characterized cohorts are needed and the findings independently validated before any clinical value of miRNAs can be evaluated. A greater understanding of miRNAs function and their interaction with therapeutics are still required. Nonetheless, miRNA may have a great potential in revolutionizing cancer diagnostics and personalized medicine in the coming years.

CONFLICT OF INTERESTS

The authors of this manuscript declare no conflicts of interests.

REFERENCES

MICRONAS AND OVARIAN CANCER


