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Hamideh Aboutalebi[1*], Afsane Bahrami[2*], Farzad Rahmani[3,4], Majid Khazaei[5], Hamid Fiuji[6], Mojtaba Shafiee[4,7], Gordon A. Ferns[8], Amir Avan[2,9#], Seyed Mahdi Hassanian[3,9#]

1) Department of Anatomy, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
2) Department of Modern Sciences and Technologies, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
3) Department of Medical Biochemistry, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
4) Student Research Committee, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
5) Department of Medical Physiology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
6) Department of Biochemistry, Payame-Noor University, Mashhad, Iran.
7) Department of Nutrition, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
8) Brighton & Sussex Medical School, Division of Medical Education, Falmer, Brighton, Sussex BN1 9PH, UK.
9) Metabolic Syndrome Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.

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*Made equal contribution to this study.

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Corresponding Authors:
Seyed Mahdi Hassanian, Ph.D.
Department of Medical Biochemistry
Faculty of Medicine, Mashhad University of Medical Sciences
Mashhad, Iran.
Phone: (+98) 5138002375, Fax: (+98) 5138002389
E-mail: hasanianmehrm@mums.ac.ir

Amir Avan, Ph.D.
Molecular Medicine Group, Department of Modern Sciences and Technologies
School of Medicine, Mashhad University of Medical Sciences
Mashhad, Iran.
Phone: (+98) 5138002298, Fax: (+98) 5138002389
E-mail: avana@mums.ac.ir

Abstract

Ovarian cancer (OC) is often diagnosed at an advanced stage because of the late onset of symptoms, and this together with the lack of effective treatments, has meant it is associated with a very high mortality. The aberrant expression of MicroRNA (miRNA) contributes to the initiation and development of human tumors including OC. Several miRNAs are secreted by tumor cells and can be identified in body fluids. Serum miRNAs levels are associated with several clinical conditions, and may be used to predict prognosis and response to treatments in some cancers including OC. This review summarizes the current progresses regarding the potential applications of circulating miRNA as innovative biomarkers in OC.

Keywords: Ovarian cancer, Extracellular microRNA, circulating microRNA, Biomarker

Introduction

Ovarian cancer (OC) is one of the most common gynecologic malignancies worldwide with an incidence of approximately 240,000 per annum (1). Epithelial ovarian cancer (EOC) is the most frequent pathological type of OC, and accounts for the majority of gynecologic cancer mortality. Early stage disease (Stage 1) may be successfully treated by surgery with an overall five-year survival over 92% (2). Unfortunately, more than 70% of OC patients are diagnosed at an advanced stages and the five-year survival for stage 3 and 4 is less than 30% (3, 4). About 20%–25% of patients with OC have an inherited predisposition to OC (5). The most common mutated genes are reported to be BRCA 1 and 2. Women with BRCA 1 or 2 mutations have a higher risk of 40% and 18% for developing OC, respectively (6).

Cancer antigen-125 (CA-125), and Human Epididymis Protein 4 (HE4), are well-established markers for OC for monitoring disease progression, which in terms of sensitivity and specificity are limited (7). Therefore, it is necessary to identify and develop novel, sensitive, and noninvasive biomarkers to support early detection and personalized therapeutic approaches.

MicroRNAs (MiRNAs or miRs) are evolutionarily conserved single stranded short endogenous non-coding RNAs of 17–22 nucleotides in length (8). They are initially transcribed as long primary miR transcripts (pri-miRs) through RNA II polymerase and cut by the Drosha (an RNAse III) complex into premature miRs (pre-miRs). Next, pre-miRs exported to cytoplasm, which are further cleaved by the Dicer (an RNAse III) into mature miRs (9). MiRNAs are involved in post-transcriptional regulation through their binding to 3'untranslated regions or open reading frame region of mRNA targets, leading to their degradation or translational repression (10). In man, almost 1,000 miRs have been identified in the genome that regulate approximately 30% of protein coding genes (11) MiRs are regulators of several biological functions including cell differentiation, development, proliferation, propagation, apoptosis and metabolism (figure 1). Hence, The aberrant

expression of miRNAs is associated with many human conditions including cancers (12). It has been demonstrated that microRNAs are important in cancer progression by targeting thousands of cancer-related genes (figure 1) (13). Dysregulation of miRNA in OC tissue has also been observed and they may also assist in the prediction of response to therapy and relapse (14). In addition, miRNAs which are derived from monocytes, plasma, and exosomes can overtake among cells or tissues and organs through blood stream (15). These miRNAs are very stable in the peripheral blood circulation and may reflect tumor status; they have been found to be present in patients with hepatocellular carcinoma, lung cancer, prostate cancer, breast cancer, gastric adenocarcinoma and OC (16).

The presence of miRs in cell-free body fluids (serum, plasma, saliva, breast milk, amniotic fluid, colostrum, urine and etc) showed that circulating miRNAs (cirMiR) could be used as cost-effective and non-invasive biomarkers (17). Unprotected miRs are degraded via RNases in the circulation, but are stable when bound to RNA-binding proteins (18) or high-density lipoproteins (HDL) (19), or encapsulated within secrete extracellular vesicles (EVs) (20). There are several studies showing that cirMiRs have an essential function in carcinogenesis, and metastasis, and suggest that they may be an attractive class of molecules for diagnostic, prognostic purposes as a tumor biomarkers (21). We aim to give an overview on recent clinical studies conducted on cirMiRs and their potential role as diagnostic, prognostic and therapeutic targets in OC.

Role of circulating microRNAs in ovarian cancer

In the last decade, studies using miR/cDNA microarrays, or tissue arrays have revealed genome-wide transcriptional alterations in OC (22). Various miRs are markedly over-expressed or down-regulated in later stages or high-grade OC, suggesting that miRs may contribute to malignant cell transformation and proliferation.

Due to the stability and conservative properties of miRs, a variety of effective methods including cloning and sequencing, northern blot, western blot, microarray chip, RT-

quantitative PCR (RT-qPCR) have been developed for the assessment of cirMiR (23-25). Recently, the application of miRs as diagnostic, prognostic indicators and also therapeutic molecules in OC has emerged.

Circulating microRNAs for early diagnosis of OC

There is accumulating evidence that the profiling of cancer specific cirMiR could be used as a non-invasive biomarker for screening for early diagnosis of OC (Table 1). In 2009, Resnick et al. investigated the expression patterns of miRs among 28 serum samples from OC patients and 15 samples from normal controls using qRT-PCR, and reported that 8 miRs (miR-21, -29a, -92, -93, -99b, -126, -127, and -155) were dysregulated in OC patients (26). Häusler et al. observed high-expression of miR-574-3p in whole blood of OC patients (2). In a further study, four miRNAs (miR-19a-3p, -30a-5p, -150-5p, -645) were down-regulated and eight miRs (miR-34c-5p, -106b-5p, -191-5p, -320a, -206, -548a-3p, -574-3p, -590-5p) over-expressed in sera of cancer patients compared to controls (11). Similarly, up-regulation of miR-92, -200c, -221, and -141 were shown in OC patients compared to healthy controls (27-29).

Consistent with these findings, Shah et al. reported that CA-125 together with miR-34a-5p was the potential indicator of the complete surgical resection in women with high-grade serous OC (30). miR-34a-5p as a mature form of miRNA directly targets wild-type p53 (31). p53 is a protein that is often mutated in high-grade serous OC (32, 33). Further studies showed that miR-34a-5p is a circulating inflammatory microRNA (34) that is down-regulated in OC patients at higher disease stages (35). Moreover, Zhu et al. showed that compared to control group, miR-20a, miR-126, and miR-355 were down-regulated whereas miR-125b and let-7c were over-expressed in sera of patients with epithelial OC (36). They reported that serum miRNA-125b is a potentially more specific biomarker for diagnosis of EOC. Until now several functional target genes have been recognized for miRNA-125b including Sphk1, PI3K/Akt/mTOR, BAK1 and p53. Specifically, miRNA-125b inhibits the invasion and

metastasis of OC cells by targeting eIF4EBP1(37). To further support the diagnostic potency of miRNAs in OC, Yokoi and colleagues developed novel predictive methods by measuring a combination of eight cirMiRs in serum of OC patients (let-7d-5p, miR-26a-5p, -130b-3p, -142-3p, -200a-3p, -328-3p, -374a-5p, and -766-3p). This could potentially discriminate OC patients from healthy women with a sensitivity of 0.92 and specificity of 0.91; and early-stage OC in women with benign tumors (sensitivity=0.86 and specificity=0.83). This model also sub-classified four types of epithelial OC (38). Pendlebury et al. demonstrated increased expression of circulating miR200 family (miR-200a, -200b and -200c) in patients with high-grade serous OC (39). The miR-200 family is involved in cancer initiating and propagation by regulating epithelial to mesenchymal transformation. They also target E-box binding transcription factors including ZEB1 and ZEB2, which regulate E-cadherin expression and cell polarity (40).

Numerous reports have suggested that miR profiles may be used as potential diagnostic biomarkers for distinguish OC type. Chung et al. found that serum let-7b, miR-26a, -132, and -145 could be useful for diagnosis of serous OC (41). miR-26a is situated on chromosome 3p22.2 and plays an essential role in the p53 tumor suppression, which regulate transformation-associated targets, such as PTEN, cyclinD2, SMAD1, and EZH2 (42). Also, miR-132 target cAMP response element and regulate function of neuronal system, immune system and the circadian timing proceeding (43). Similarly, Suryawanshi and colleagues reported three unique miR signatures (miR-21, -203, -205) that distinguished between normal controls, women with endometriosis, and women with endometriosis-related OC (44). MiR-21 has been found as one of the most high-amplified oncomirs in all cancer types (45), so it is plausible that the over-expression of cirMiR-21 may identify activation of a common oncogenic cascade that involves in endometriosis-related OC pathogenesis. These differentially expressed cirMiRs might be potential targets for the further inquiry of the molecular pathogenesis of OC and could be considered as beneficial biomarkers for early diagnosis of OC in future clinical setting.

Prognostic value of circulating microRNAs in ovarian cancer

There are several studies showing that alteration in the level of cirMiRs is associated with the prognosis of OC patients (Table 1). MicroRNA 200 family may not only be a diagnostic marker of epithelial OC, but could also provide useful information concerning the prognosis and therapeutic responses. Pendleburya et al. showed that circulating miR-200a, -200b and -200c concentrations are associated with the disease stage and tissue expression (39). Furthermore, it has been shown that elevated expression of miR-200a and miR-200c are related to altered responses to treatment and lower overall survival (OS) in epithelial OC patients (46-48). Moreover, Gao et al. investigated the prognostic value of the miR-200 family members (cirMiR-200c and -141) in OC patients and reported that higher miR-200c expression was significantly related to two-year OS rate, while low miR-141 expression was associated with higher OS rate (29). Zheng and colleagues also demonstrated that a low expression of let-7f in plasma was associated with worse progression-free survival (PFS) in OC patients at stage III/IV (49). miRs of the let-7 cluster, which are typically down-expressed in different tumors bind to human *RAS* oncogenes including *H-RAS*, *K*-RAS, and *N-RAS* (50). The Let-7 family has a regulatory activity as tumor suppressors through targeting the HMGA2 gene. Notably, let-7 can be predictors of prognosis of OC patients in which lack of let-7 expression associated with less differentiated tumor (51). Consistently, up-regulation of cirMiR-21 was linked with higher International Federation of Gynecology and Obstetrics (FIGO) stage, advanced tumor grade, lower OS, and poor prognosis in EOC patients (52, 53). Some studies have shown that increased expression of miR-193a, a tumor suppressor, induces apoptosis in human EOC cells (54). They also found that expression of miR-148b-5p was 3.2 fold higher post-chemotherapy in patients with prolonged PFS.

Consistent with the prognostic value of miRNAs in OC, It has been shown that there was a significant association between miR-92 expression with lymph node metastasis and stage of the disease (27). Similarly, serum levels of miR-221 was related with prognosis

(28). The molecular mechanisms of associations between miR-221 overexpression with carcinogenesis and development are not well known. Several potential targets have been suggested for miR-221 such as p27/kip1 and p57/kip2, c-Kit, Bmf, and PTEN(28). Furthermore, serum miRNA-125b level was significantly elevated in OC women in early stages I/II, and in women with no remaining tumor post-surgery. Moreover, the elevated miR-125b level was positively correlated with PFS and OS in OC patients (36).

Therapeutic potential of circulating microRNAs in ovarian cancer

Since each miR regulates the expression of hundreds of genes, miRs can control and coordinate many signaling pathways and processes. Several studies have shown the potential of miRNA profiles that may allow a better identification of the treatment approach for OC patients. CirMiR expression profiling can assist personalized treatment information for monitoring of therapy and prediction of drug resistance. It has been found miR-200 family members were associated with chemo-sensitivity in OC. In line with this, MiR-200c was related with better OS in patients treated with bevacizumab versus those received standard chemotherapy (48). Cochrane et al. have reported that miR-200c directly targets class III tubulin (*TUBB3*), which codifies a tubulin isotype typically exist in neuronal cells (55, 56). Restoration of miR-200c led to the inhibition the *TUBB3* expression and increment sensitivity to microtubule-binding chemotherapeutic agents such as epothilone B, paclitaxel, and vincristine and. In another study, Benson et al. analyzed miRNA profiles in plasma specimens of recurrent OC patients resistant to platinum that were treated with decitabine and carboplatin. This regimen led changes in 3 cirMiR (miR-193a, -339, and-375) concentrations (57). Comparison of miRNA expression profiles in cisplatin-sensitive and -resistant OC cells showed that elevated level of miR-141, -200c, -215, and -421 and down-regulation of miR-492-5p were associated with resistance to cisplatin in these cells (58).

Exosomal miRs

Exosomes are membrane-bound lipoprotein nanoparticles of 30–100 nm in diameter that isolated from multivesicular bodies (MVBs) (59). They fuse with the plasma membrane and release to the extracellular space (60). Exosomes can exist in all extracellular fluids including blood, milk, urine, seminal, cerebrospinal and follicular fluids (61). Approximately 10% of cirMiRs are packaged in exosomes. They carry proteins, mRNAs, and microRNAs which involve in intercellular communication by moving proteomic and genomic substances between cells. Exosomes can transport miRs between neighboring cells and distant cells (62, 63). The RNA content of exosomes relies on the exosomes origin. The process of sorting and packaging of miRs into exosomes can be selective, preferring particular miRs for exosomal cargo over others (64). Extracellular miRs in exosomes are suggested to contribute in cell-to-cell communication and to provide as indicators of human diseases (65).

Exosome-derived miRs parallel miRs derived from tumor cells. Moreover, the quantity and composition of exosomal miRs are different between patients and healthy subjects. Thus, exosomal miRNAs emerge as potential noninvasive markers for identification of disease status.

Results obtained from analyzing the exosomes derived from the whole blood of OC patients demonstrated that exosomes which expressed epithelial cell adhesion molecule are significantly different from profiles of benign tumor or healthy controls, and elevate as the stage advances. Eight miRs, including: miR-21, -141, -200a, -200b, -200c, -203, -205, and -214 were also found to be increased in the blood-derived exosomes of patients with OC (14). Moreover, the expression of exosomal miR-200 family may be contributed in tumor progression. It has been shown that the miR-200b and miR-200c increased in women with FIGO stage III/IV compared to women with FIGO stages I/II (66). Exosomal miR-21-3p can also contribute to cisplatin resistance through targeting the *NAV3* gene. Exosomal miR-21 inhibits OC apoptosis and causes chemo-resistance through binding to the target, APAF1(67). (68) Furthermore, the exosomal miRNAs, miR-21, -103, -141, -200a-c, -203, -205, -214, and -373, are related with an unfavorable outcome (69).

Vaksman et al. investigated miRNA expression pattern in effusion-derived exosomes acquired from malignant peritoneal and pleural effusions samples among OC patients at later stages. In a univariate analysis, higher levels of miR-21, -23b, -29a were correlated with poor PFS, whereas elevated level of miR-21 was associated with poor OS (70). Urinary miRs may originate in the circulation and be excreted through renal elimination. Zhou et al. reported that urinary miR-30a-5p is a tumor suppressor was significantly increased in OC patients compared to the control group (71). Also, miR-30a-5p presented a similar expression pattern in exosomes and cells.

Discussion

The data presented in this review supports the view that cirMirs may hold great potential as a biomarker of OC, that may parallel biopsy findings, and may be used for screening, early diagnosis, prognosis monitoring, and sensitivity to chemotherapy in OC patients. Although, it have not yet been executed in the clinical setting due to the absence of concordance across researches. Until now, most of the studies seem to be initially, because they reported altered levels of cirMiRs in OC patient with relatively small sample sizes. Indeed, high reproducibility of quantified evidence about expression profiling of miRNAs in OC is necessary to allow their clinical use. A direct relationship between miRs expression concentrations in the blood and in the other body fluids such as milk or urine has not yet been obviously disclosed. Further efforts on the more rare histotypes of EOC including endometrioid, clear-cell, and mucinous, require to be performed because differentiation may lead to promoted therapy for specific subtypes maybe according to microRNA expression. To enhance the diagnostic sensitivity and specificity, more large studies with standardized procedures are warranted to carry out around the world. But, these pre-clinical researches hold promise for the near future.

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Figure legend

Figure 1. Schematic representation of regulatory mechanisms of microRNAs in ovarian cancer.