

# MicroRNAs driving invasion and metastasis in ovarian cancer: Opportunities for translational medicine (Review)

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**Abstract.** Epithelial ovarian cancer is the fifth most frequent cause of cancer death in women. In spite of the advantages in early detection and treatment options, overall survival rates have improved only slightly in the last decades. Therefore, alternative therapeutic approaches need to overcome resistance and improve the patient survival and outcome. MicroRNAs are evolutionary conserved small non-coding RNAs that function as negative regulators of gene expression by inhibiting translation or inducing degradation of messenger RNAs. In cancer, microRNAs are aberrantly expressed thus representing potential prognostic biomarkers and novel therapeutic targets. The knowledge of novel and unexpected functions of microRNAs is rapidly evolving and the advance in the elucidation of potential clinical applications deserves attention. Recently, a specific set of microRNAs dubbed as metastamiRs have been shown to initiate invasion and metastasis in diverse types of cancer. We reviewed the current status of microRNAs in development and progression of ovarian cancer with a special emphasis on tumor cells invasion and metastasis. Also, we show an update of microRNA functions in oncogenic pathways and discuss

the current scenario for potential applications in clinical and translational research in ovarian cancer.

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## 1. Introduction

Epithelial ovarian cancer (EOC) is a metastatic disease with the highest mortality rate of all gynecologic cancers (1). Serous, endometrioid, clear-cell, and mucinous ovarian cancers are the most frequent histotypes. These different ovarian cancer (OC) histotypes are characterized by altered genomic and epigenetic patterns which greatly impact oncogenic signaling-pathways, behavior and clinical outcome. Hence, alternative therapeutic approaches are needed to improve the patient survival and outcome. Although no molecular predictors of clinical outcome are currently in use, several cellular factors have been studied as potential prognostic and predictive biomarkers (2). MicroRNAs (miRNAs) are small non-coding RNAs that function as negative regulators of gene expression (3). In cancer cells, miRNAs may function either as oncogenes or tumor-suppressors and may contribute to the heterogeneous biological behavior of ovarian tumors. One of the most deadly hallmarks of cancer cells is the ability to metastasize to other tissues and organs (4). Notably, metastasis can be promoted

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**Abbreviations:** miRNAs, microRNAs; EMT, epithelial-mesenchymal transition; MET, mesenchymal-epithelial transition; OC, ovarian cancer; EOC, epithelial ovarian cancer; SOC, serous ovarian carcinoma

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by specific sets of miRNAs which have the ability to target multiple genes related to cell migration and invasion (5,6). In OC, metastasis is greatly promoted by the epithelial-to-mesenchymal transition (EMT). A number of studies have focused on the discovery of key players in the EMT to identify potential targets for therapeutic intervention in precision medicine. Here, we reviewed the current status on the role of miRNAs in invasion and metastasis with a special emphasis in the potential applications in clinical and translational research in OC.

## 2. MicroRNAs: novel regulators of gene expression

miRNAs are small non-coding RNAs of ~22 nucleotides in length which function as negative regulators of gene expression. These small RNAs function as guide molecules to base pairing with target genes resulting in translation repression or messenger RNA (mRNA) cleavage. miRNA biogenesis (Fig. 1) begins with their transcription by the RNA polymerase II which synthesizes a primary miRNA (pri-miRNA) which contains both 5'-cap structure (<sup>7</sup>MGpppG) as well as 3'-end poly(A) tails (7). Pri-miRNAs are processed by the RNase III-type protein Drosha which is associated to DGCR8 (DiGeorge syndrome critical region gene 8), KSRP and p68 proteins in a complex denoted as microprocessor. The cleavage of pri-miRNA by Drosha generates a 70-nucleotide precursor miRNA (pre-miRNA) that is exported into the cytoplasm by the Ran-GTP-dependent exportin 5 (8). The second step of pre-miRNA processing (dicing) is then performed by Dicer, an RNase III enzyme which is associated to TRBP (TAR RNA-binding protein) or PACT, and Argonaute (AGO) proteins. This protein complex cleaves the pre-miRNA hairpin generating a double-stranded RNA of ~22 nucleotides in length known as the miRNA:miRNA\* duplex. Then the miRNA:miRNA\* interacts with the associated RNA-induced silencing complex (RISC), which preferentially includes the miRNA guide strand and AGO. The mature miRNA bound to AGO hybridizes to complementary sequence in the 3' untranslated region (3'-UTR) of mRNA target. If miRNA binds to mRNA with non-perfect complementarity, it leads to translation repression. In contrast, if miRNA binds to target with a high complementarity, cleavage and degradation of mRNA is induced (9). The decay of mRNA is initiated by shortening of poly(A<sup>+</sup>) transcripts by the canonical deadenylation machinery in cytoplasmic foci denoted as P-bodies (10,11).

## 3. OncomiRs: microRNAs with key roles in cancer

Aberrant expression of miRNAs is a common event during carcinogenesis as they regulate key transcripts involved in the initiation and progression of tumors, thus they have been denominated as oncomiRs (12). miRNAs with increased expression in tumors are frequently considered as oncogenes as they promote tumor development by inhibiting tumor suppressor genes. In contrast, miRNAs may function as tumor suppressors as they prevent tumor development by inhibiting oncogene expression or genes that control cell differentiation and apoptosis (13). Aberrant expression of miRNAs is produced from genetic or epigenetic alterations represented by: i) deletions, ii) amplifications, iii) point mutations, iv)

DNA methylation, and v) chromatin modifications. Notably, several studies have identified miRNAs as useful biomarkers of diagnosis and prognosis in OC patients (14,15).

## 4. Epithelial to mesenchymal transition: a key mechanism for invasion and metastasis

Epithelial to mesenchymal transition (EMT) is a morphogenesis program activated during embryonic development, tissues repair, fibrosis and cancer (16,17). During tumor progression the EMT activation induces changes in epithelial cells that promote loss of basal apical polarity and cell-cell junctions. This allows that epithelial cells acquire plasticity, invasive capacity, stem cell-like characteristics and gain the ability to invade adjacent tissues and colonize distant organs (18) (Fig. 2). Cells that have undergone EMT also have the capacity to transform into epithelial cells in a reverse process known as mesenchymal to epithelial transition (MET). Both processes are important during embryo- and organogenesis, but in malignant transformation they represent a critical step to induce metastasis progression (19). Induction of EMT program is facilitated by growth factors produced from stromal cells that activate diverse signaling pathways including TGF- $\beta$ /BMP, WNT/ $\beta$ -catenin, Hedgehog, Notch, Sonic and PI3K/AKT signaling pathways represent crucial mechanisms promoting EMT (20). Crosstalk between diverse signaling pathways promotes the expression of repressors of E-cadherin transcription such as: i) the Snail family of zinc-finger transcription factors Snail, Slug and Smuc; ii) the basic helix-loop-helix factors, Twist and E12/E47; iii) the two-handed zinc-finger factors of  $\delta$ -crystallin/E2 box factor 1 (dEF1) family proteins, dEF1/zinc-finger E-box-binding homeobox 1 (ZEB1), and iv) the Smad-interacting protein/ZEB2 (21). In particular, E-cadherin plays an important role in the induction of EMT phenotype in cancer cells. E-cadherin is a membrane glycoprotein that mediates calcium-dependent cell-cell adhesion which is located at the adherens junctions predominantly associated with  $\beta$ -catenin. Under normal circumstances  $\beta$ -catenin binds E-cadherin providing a direct link between the E-cadherin cell-adhesion complex and the intracellular cytoskeleton (22). During EMT activation,  $\beta$ -catenin influences the transcriptional repressors Snail, Slug, and Twist to inhibit E-cadherin transcription (23).

Studies related to the characterization of EMT-induced signaling pathways have demonstrated that WNT signaling pathway is predominantly activated in EOC. Additional evidence reported that WNT signaling is negatively regulated by E-cadherin which in turn suppresses  $\beta$ -catenin expression and suppresses transcriptional activation of the EMT-related genes in OC (24). A number of studies have shown that Sonic Hedgehog signaling pathway also participate in the process of EMT. Upregulation of hedgehog glioma-associated oncogene 1 (shh-Gli1) promotes the activation of EMT event by crosstalk with PI3K-AKT pathways in OC (25). In this context, another mechanism has been described that sustains EMT by cooperative signaling. Rac-1, a specific guanine nucleotide exchange factor induce EMT in OC through simultaneous activation of mitogen-activated extracellular signal-regulated kinase 1/2 (MEK1/2) and Src signaling pathways (26). Another sophisticated regulatory network that regulates EMT

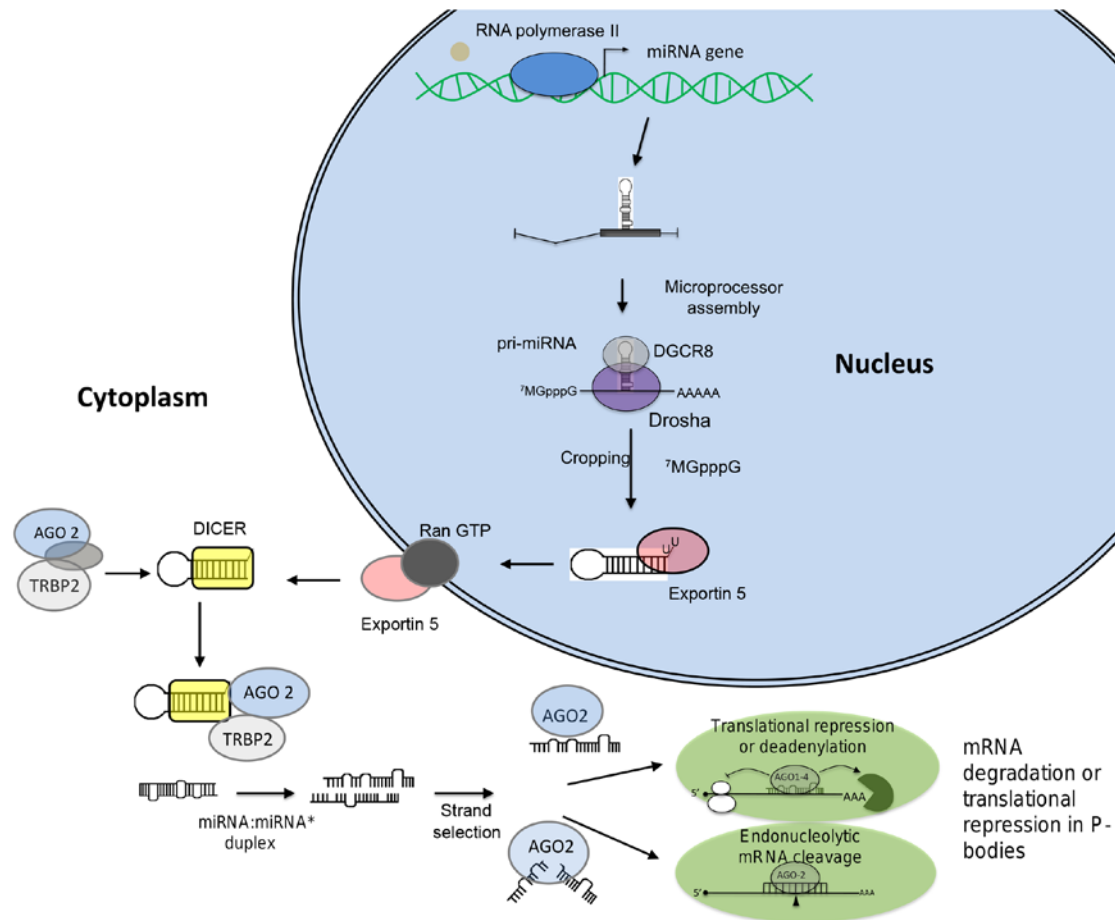


Figure 1. MicroRNA biogenesis. The main molecular steps required for miRNA process in the nuclei and cytoplasm.

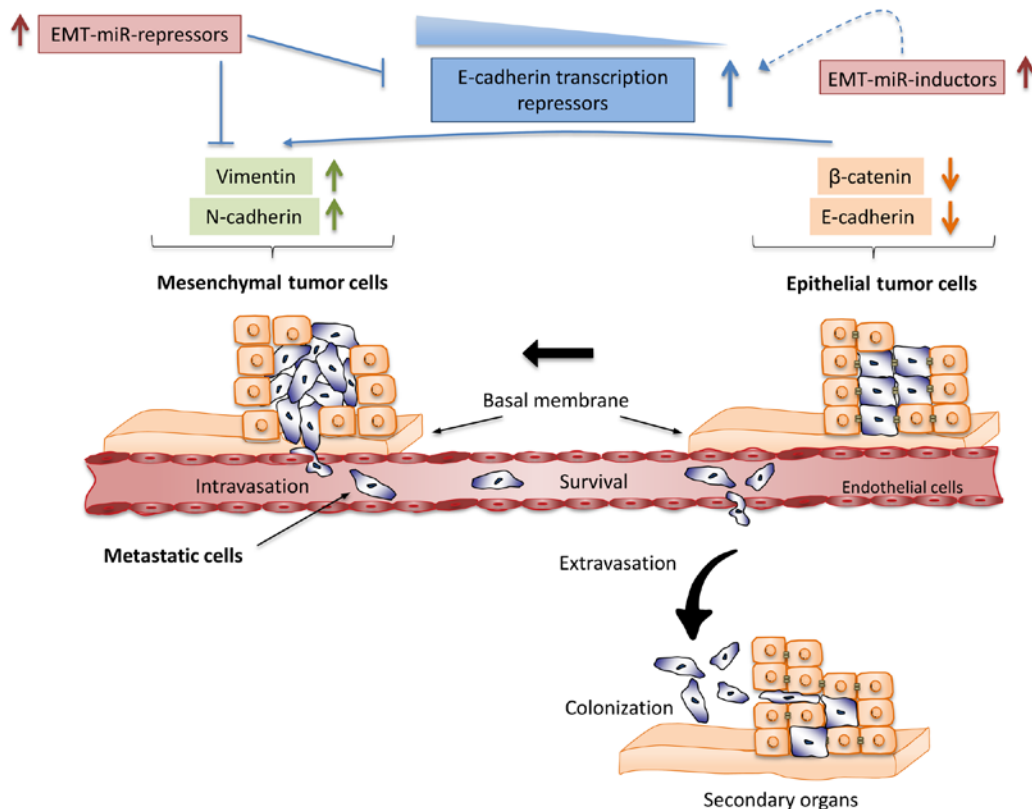


Figure 2. Schematic representation of epithelial-to-mesenchymal transition (EMT). The molecular mechanism by EMT converts epithelial cells into mesenchymal cell and shows the major molecular players that regulate the EMT process.

is exerted by TGF- $\beta$  family. Recently, it was found that Smad4 mutations increased homodimerization of Smad4 with its receptor to promote nuclear localization leading to reduction of E-cadherin expression and increases of N-cadherin (27). In addition, crosstalk between TGF- $\beta$  and stem-cell promoting pathways facilitates the formation of Smad complex during EMT. Interestingly, the molecular basis of this cooperative mechanism indicates that many EMT signaling molecules interact with Smads to form complexes engaged in both repressing epithelial genes and activating mesenchymal genes (28). A number of studies have showed that microRNAs are important in the regulation of signaling proteins involved in the control of EMT. miRNAs are frequently engaged in feedback loops with signaling molecules, thus alterations in miRNAs expression may result in an imbalance of signals that promotes the activation of EMT program in cancer (29).

### 5. MicroRNAs are negative regulators of epithelial to mesenchymal transition in ovarian cancer

*MicroRNAs regulating ZEB1/ZEB2 transcription factors.* Expression of genes responsible for EMT is mainly under control of ZEB1 (TCF8/ $\delta$ -EF1) and ZEB2 (SIP1/ZFXH1B) transcription factors which repress E-cadherin whereas enhance vimentin and N-cadherin. These changes in ZEB1 and ZEB2 expression constitute a signal for starting transition from epithelial to mesenchymal phenotype. Remarkably, it has been well demonstrated that miR-200 family members orchestrate the initiation of EMT by controlling the expression of ZEB1 and ZEB2 genes. The miR-200 family is expressed as two separate polycistronic pri-miRNAs: i) miR-200a, miR-200b, and miR-429 located on chromosome 1, and ii) miR-200c and miR-141 at chromosome 12 (30-34). Interestingly, miR-200 family members are differentially expressed in the bulk tumor. For instance, miR-200 members are expressed at low or negligible levels in normal ovarian surface cells and increased their expression in OC, whereas expression of ZEB1 and ZEB2 shows the opposite pattern. Ovarian surface cells acquire a more epithelial phenotype as they undergo transformation switching from a miR-200 family low and ZEB1/2 high state to a miR-200 family high and ZEB1/2 low phenotype (34). An early study which identified the relationship between miR-200 family and EMT process in EOC was carried out in the panel of 60 human cancer cell lines (NC160). One the most relevant findings of this study was the identification of miR-200 as a marker for E-cadherin-positive and vimentin-negative cancer cells in both the NC160 cells as well as in tumors from OC patients. This outstanding report showed that miR-200 targets the E-cadherin transcriptional repressors ZEB1 and ZEB2. Furthermore ectopic expression of miR-200 induced upregulation of E-cadherin in diverse cancer cell lines and reduction in their motility, while inhibition of miR-200 reduced E-cadherin expression and induced EMT associated to increased ZEB1 levels (33). On the other hand Chen *et al* performed a genome expression profile of two mesenchymal-like OC cell lines with different metastatic potential and demonstrated that expression of miR-429, a member of the miR-200 family, resulted in the reversal of the mesenchymal phenotype through activation of the MET which was associated to changes in E-cadherin, ZEB1 and ZEB2 expression

and migration and invasiveness (35). In addition, restoration of individual miR-200 members (miR-200a, miR-141, miR-200b and miR-200c) in HEY ovarian cancer cells also resulted in the acquisition of morphological epithelial characteristics, elevated expression of KRT8, KRT18 and KRT7 epithelial markers and reduced levels of ZEB1 and ZEB2. Remarkably, ectopic expression of miR-200 family members influenced the sensitivity of EOC to platinum-based drugs, which was associated to the inhibition of EMT that is known to reduce the sensitivity of chemotherapy (36). Besides these findings Chen *et al* reported that overexpression of miR-429 in a primary cell line (OCI-984) isolated from the ascites fluid an advanced stage OC patients also induced morphological, functional and molecular changes consistent with MET (35). Overexpression of miR-429 in OCI-984 cells also increased the sensitivity to cisplatin. Another study indicates that upregulation of miR-200c in ovarian cancer tissues was inversely associated with advanced clinical stage. miR-200c inhibited E-2 cells migration and invasion capacity through repression of ZEB2 (37). Moreover, miR-200c showed a potential function in stemness as it was downregulated in CD44<sup>+</sup>/CD117<sup>+</sup> enriched stem-like cells derived from SKOV3 cells. Ectopic expression of miR-200c resulted in the inhibition of migration and invasion by downregulation of ZEB1 and vimentin, which in consequence upregulated E-cadherin in CD44<sup>+</sup>/CD117<sup>+</sup> subpopulations. In addition miR-200c overexpression inhibited the EMT process and subsequently metastasis in CD44<sup>+</sup>/CD117<sup>+</sup> xenograft in nude mice (38).

On the other hand, ZEB1 was upregulated in high-grade serous ovarian carcinoma (SOC) specimens compared to normal fallopian tube tissue. In addition, miR-1236-3p suppressed migration and invasion abilities through targeting of 3'-UTR of ZEB1 in A2780 and SKOV3 cell lines (39). On the other hand Wu *et al* identified that ZEB2 is overexpressed in CD133/1<sup>+</sup> ovarian cancer stem cells by downregulation of its repressor miR-200a. Conversely, ectopic expression of miR-200a in CD133/1<sup>+</sup> resulted in suppression of ZEB2 causing the increase of E-cadherin expression. Noteworthy these data show that loss of miR-200a expression may play a critical role in the repression of E-cadherin by ZEB2, thereby enhancing migration and invasion in CD133/1<sup>+</sup> cells possibly through the acquisition of EMT phenotype (40).

Another microRNA that targets ZEB1 and control the EMT process is miR-150 which was downregulated in primary EOC tissues (41). Lower expression of miR-150 was associated with advanced clinical stage (III-IV) as well as with poor prognosis. Ectopic expression of miR-150 inhibits cell proliferation, invasion and metastasis (42). Targeted silencing of ZEB1 using shRNAs in SKOV3 cells resulted in enhanced expression of miR-200c and reduced EMT and metastasis. Downregulation of ZEB1 also decreased the growth of tumors in xenograft mouse model (43). Likewise, overexpression of miR-101 suppressed cell migration, invasion and EMT phenotypes by targeting ZEB1 and ZEB2 in SKOV3 cells. These phenotypes were associated to a significant increase in E-cadherin levels and decreases in mesenchymal markers (fibronectin, N-cadherin and vimentin). In other related studies, Zhou *et al* reported that miR-153 also inhibits the EMT by targeting ZEB2 and Set7 (Set domain containing 7) genes (45). Set7 is a methyltransferase enzyme that trimethylates lysine 4 on

histone 3 and epigenetic mark that has been found enriched in promoters of activated genes (44). In agreement with these findings and using an RNA mimic approach, it was demonstrated that miR-153 also promotes degradation of SET7/ZEB2 transcripts which in turn leads to the inhibition of cell proliferation and EMT reducing the invasive potential of OC cells. In addition, miR-153 expression was downregulated in ovarian tumors and correlated with poor survival rates in EOC patients (45).

*MicroRNAs regulating SNAI family of transcription factors.* SNAI family of transcription factors consists of SNAI1 (often designated as SNAIL), SNAI2 (also known as SLUG) and SNAI3 (also known as SMUC), and they also suppress the expression of epithelial genes such as E-cadherin and activate the expression of mesenchymal genes including N-cadherin and fibronectin (46). Yang *et al* performed an integrated genomic analysis and found that miR-506 inhibited cell migration and invasion and prevented TGF- $\beta$ -induced EMT by targeting SNAI2 in SOC. In addition, miR-506 expression was associated with decreased SNAI2 and VIM, as well with increased E-cadherin levels. Interestingly, using a nanoparticle delivery system for miR-506 in orthotopic mouse models, a significant suppression of SNAI2/VIM and upregulation of E-cadherin expression was found (47). Functionally, miR-506 directly binds to the 3'-UTR of vimentin and N-cadherin genes in EOC (48). In addition using tissue microarrays including 204 EOC subtypes it was found that miR-506 expression was positively associated to E-cadherin and negatively correlated with vimentin and N-cadherin. Noteworthy, all these findings confirms that loss of miR-506 expression resulted in mesenchymal phenotype in EOC. Thus, miR-506 is a potent regulator of both E-cadherin and vimentin/N-cadherin to suppress and control the EMT process and represents an innovative therapy against most aggressive ovarian cancers (49).

Another miRNA that regulates SNAI genes is the tumor suppressor miR-203. In human SOC tissues, miR-203 was found repressed whereas its target SNAI2 was upregulated. In addition, overexpression of miR-203 inhibited cell proliferation, migration and invasion in SKOV3 and OVCAR3 cells by suppression of EMT which was associated to E-cadherin upregulation and vimentin and SNAI2 downregulation. Outstandingly, through subcutaneously injection of miR-203-expressing SKOV3 and OVCAR3 cells transduced with a lentiviral luciferase vector into immunodeficient NSG female mice, Zhao *et al* revealed that miR-203 inhibited EMT process and tumor growth in a xenograft mouse model (50).

Recently, Ye *et al* reported that during the induction of EMT by TGF- $\beta$ , the miR-30b, miR-30c and miR-30d were suppressed in SKOV3 and 3AO cancer cells. In addition, restoration of miR-30d in TGF- $\beta$ -induced EMT cancer cells induced the regression of EMT phenotypes including the morphological changes and the expression of E-cadherin and N-cadherin markers. Also evidence that miR-30d directly targets the SNAIL gene was provided (51). Likewise, overexpression of miR-34a also inhibited the TGF- $\beta$ -induced EMT process and leads to MET phenotype by downregulating SNAIL (52).

On the contrary, miR-520h regulates Twist an oncogenic transcription factor that plays a key role in EMT-mediated

intravasation of tumor cells. Forced expression of miR-520h in SKOV3ip1 cells enhanced invasion and metastasis. In addition, E-cadherin was downregulated by miR-520h and associated to increased expression of Twist (53).

*EMT-related genes are regulated by microRNAs.* Several reports have identified diverse EMT-related genes as targets of microRNAs. For instance, miR-187 is an independent prognostic factor in OC and was associated with recurrence-free survival. Overexpression of miR-187 induced cell proliferation and inhibited cell migration. On the other hand, inhibition of miR-187 resulted in the upregulation of Dab2, repression of E-cadherin and increased vimentin levels which in turn promote the EMT phenotype. This means that miR-187 has a dual function; during the initial steps of tumorigenesis miR-187 may induce proliferation, but in the late stages it inhibits the EMT and tumor invasiveness by the suppression of Dab2. Another miRNA involved in the posttranscriptional regulation of EMT-related genes is miR-29b. This small RNA modulates the EMT by targeting the high mobility group A2 gene (HMGA2), a non-histone DNA binding protein that plays a very important role in fetal development and carcinogenesis. As an oncofetal gene it is overexpressed in tumors of both epithelial and mesenchymal tissues (54). Overexpression of HMGA2 in OSE cells induced spindle-like cell morphology, whereas its repression restored their epithelioid phenotype. In addition, HMGA2 overexpressing xenograft tumors resulted in the loss of E-cadherin and the increase of vimentin. Global expression profiling indicated that HMGA2-mediated tumorigenesis was associated with changes in the expression of miRNAs and target genes involved in EMT process. Among these, lumican (LUM) a tumor suppressor that inhibits EMT was found transcriptionally repressed by HMGA2 in OSE cell lines (55).

Furthermore, miR-373 was downregulated in cholangiocarcinoma and colon cancer and inversely correlated with clinical stage and histological grade in human EOC (43,56). Ectopic expression of miR-373 in EOC cells suppressed cell invasion, metastasis and EMT process. Functional assays identified small GTPase Rab22a as a target of miR-373. Interestingly knockdown of Rab22a inhibits invasion, migration, and suppress N-cadherin in EOC cells. Metastasis was suppressed after restoration of miR-373 in xenograft ovarian carcinoma metastatic nude mice (57).

Recently, Hsiao *et al* reported that interferon  $\alpha$ -inducible protein 27 (IFI27) was upregulated in different types of cancer including OC. The IFI27 gene encodes a hydrophobic membrane protein that is highly induced by interferon- $\alpha/\beta$  to generate long-lasting immune response. By sending extracellular signals to activate other pro-apoptotic genes IFI27 functions as an apoptotic inducer (58). Notably, overexpression of IFI27 in OC cells induced EMT, cell migration, invasion, tumorigenicity, stemness, and drug resistance. Interestingly, in response to IFI27 the miR-502 expression was inhibited whereas its restoration inhibits IFI27-induced tumorigenicity, migration and drug resistance (59).

In another study conducted by Baskar *et al* it was found that the receptor tyrosine kinase orphan receptor 1 (ROR1) was overexpressed in chronic lymphocytic leukemia (60) and involved in the regulation of EMT mediated by miR-382. Using

diverse experimental approaches it was shown that miR-382 expression was decreased in human OC tissues and cell lines. In addition, miR-382 inhibited cell proliferation, migration, invasion and EMT. These phenotypes were associated to a decrease of N-cadherin and vimentin levels, as well as an increase of E-cadherin. In addition, using a luciferase assay it was revealed that miR-382 binds to the 3'-UTR of ROR1 gene (61). Other genes implicated in EMT that are *bona fide* targets of miR-138 were both the hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) and the SRY-related high-mobility group box (SOX4). In agreement with these findings Yeh *et al* reported that the absence of HIF-1 $\alpha$  resulted in decreased SLUG levels. Likewise, knockdown of SOX4 resulted in the downregulation of epidermal growth factor receptor (EGFR) a gene involved in the activation of EMT. In highly invasive OC cell lines, miR-138 was found downregulated and its overexpression inhibited metastasis in ovarian cancer (62).

In another study miR-125, which is a tumor suppressor in different types of cancer also inhibited EMT process by targeting the AT-rich interactive domain 3B (ARID3B) gene (63). ARID3B belongs to a family of AT-rich interactive domain (ARID) proteins that are involved in chromatin remodeling and gene expression regulation. ARID genes participate in development, tissue-specific gene expression and its aberrant expression is involved in tumorigenesis (64). Interestingly, miR-125 targets ARID3B and induced the conversion of highly invasive ovarian cancer cells from a mesenchymal to an epithelial morphology. Using multiple biochemical approaches, authors revealed that EGFR signaling leads to transcriptional repression of miR-125a through the ETS family transcription factor PEA3, which in consequence releases ARID3B to promote EMT (63).

*MicroRNAs modulate EMT-associated signaling genes.* Growing evidence indicates that activation of AKT, ERK1/2 and TGF- $\beta$  signaling pathways are essential for the induction of EMT genetic program. Since EMT-signaling pathways largely control metastasis, the investigations have been focused in the study of the interplay between microRNAs and novel genes modulating the EMT phenotype. Zhou *et al* reported that miR-7 plays a major role in the reversion of EMT by the inactivation of AKT and ERK1/2 pathways in EOC. Ectopic expression of miR-7 induced morphological changes from an elongated, spindle-shaped, mesenchymal phenotype to a rounded epithelial-like phenotype in highly metastatic HO-8910 and ES-2 cell lines. In addition, miR-7 expression was significantly downregulated in EOC cell lines and tissues, whereas EGFR expression correlated positively with metastasis in both EOC patients and cell lines. Moreover, miR-7 overexpression leads to the upregulation of CK-18 and B-catenin, and downregulation of vimentin by inactivation of EGFR/AKT and AKT/ERK1/2 pathways. These changes were enough to inhibit cell migration, invasion and EMT events indicating that miR-7 is a tumor suppressor in EOC (65).

In SKOV3 and HO8920 spheroid cultures expressing EMT markers, Yuan *et al* found an increase of AKT1 levels. AKT1 belongs to the AKT/PKB subfamily of the serine/threonine kinases activated by growth factors through PI3K pathway and inhibited by phosphatase and tensin homolog deleted on chromosome 10 (PTEN) (66). Interestingly, transfection of

miR-20a downregulated the expression of PTEN resulting in the acquisition of cancer stem-like cells (CSCs) properties and induction of the EMT phenotype (67). Likewise, another report showed that miR-181a also induced the EMT process through the activation of TGF- $\beta$  pathway by repression of SMAD7 gene. Ectopic expression of miR-181a leads to increased cellular survival, migration, invasion, drug resistance and *in vivo* tumor burden and dissemination. In contrast, miR-181a inhibition resulted in a significant reversion of these phenotypes in high-grade SOC (68).

Another miRNA that controls EMT related pathways to suppress tumor cells invasion is miR-17-5p which was previously found increased in chemotherapy resistant colorectal tumors (69). In OC cells, miR-17-5p enhances cellular invasion, migration and EMT by targeting both PTEN and AKT (70). Introduction of miR-17-5p in OC cell lines repressed E-cadherin while increased N-cadherin, Snail and vimentin expression. In addition, forced expression of miR-17-5p decreased PTEN while increased p-AKT in OVCAR3 and SKOV3 cell lines (70).

Other studies have focused on the role of miRNAs and metastasis in cancer stem cells. For instance, downregulation of miR-155 in OC stem-like cells (CD44<sup>+</sup>/CD117<sup>+</sup>) resulted in downregulation of claudin-1 (CLD-1). *In vitro* and *in vivo* assays showed that restoration of miR-155 expression inhibits the migration and invasion in CD44<sup>+</sup>/CD117<sup>+</sup> subpopulations by suppression of CLD-1. In addition, transfection of miR-155 did not induce changes in MMP-2 and MMP-9 expression, but a significantly increase of E-cadherin and  $\beta$ -catenin was found in agreement with acquisition of epithelial phenotype (71). All these reports evidence the alterations in the regulation network of miRNAs and EMT-related genes which are summarized in Fig. 3.

## 6. MetastamiRs: microRNAs driving invasion and metastasis in ovarian cancer

During the last decade a number of aberrantly expressed miRNAs have been identified as key promoters of cell migration and invasion in OC (Fig. 3). In early studies, Iorio *et al* reported a miRNA expression profile in different histotypes and clinical stages of EOC. They reported the downregulation of miR-144 and miR-216 in lymphovascular invasion; and miR137, miR-101, miR-215 and miR-211 in OC with tubal involvement. In contrast, upregulation of miR-101, miR-182\*, miR-22 and miR-133a was found in OC with ovarian surface involvement, miR-302c in pelvic peritoneum involvement, and miR-133a-2, miR-143, miR-145, miR-1, miR-147 and miR-126 in OC with uterus involvement. Subsequent functional studies gave insights into the role of some deregulated miRNAs in invasion and metastasis in OC (72).

*MicroRNAs functioning as tumor suppressors.* Degradation of the extracellular matrix is a complex process that stimulates cell migration, invasion and metastasis in many types of cancer. In endometrial carcinoma histotype, 28% of cases exhibit metastasis at diagnosis. Some miRNAs functioning as a tumor suppressor have been identified as deregulated in this particular OC. For instance, Habata *et al* found that BCL2-associated athanogene (BAG3) inhibited the expression of



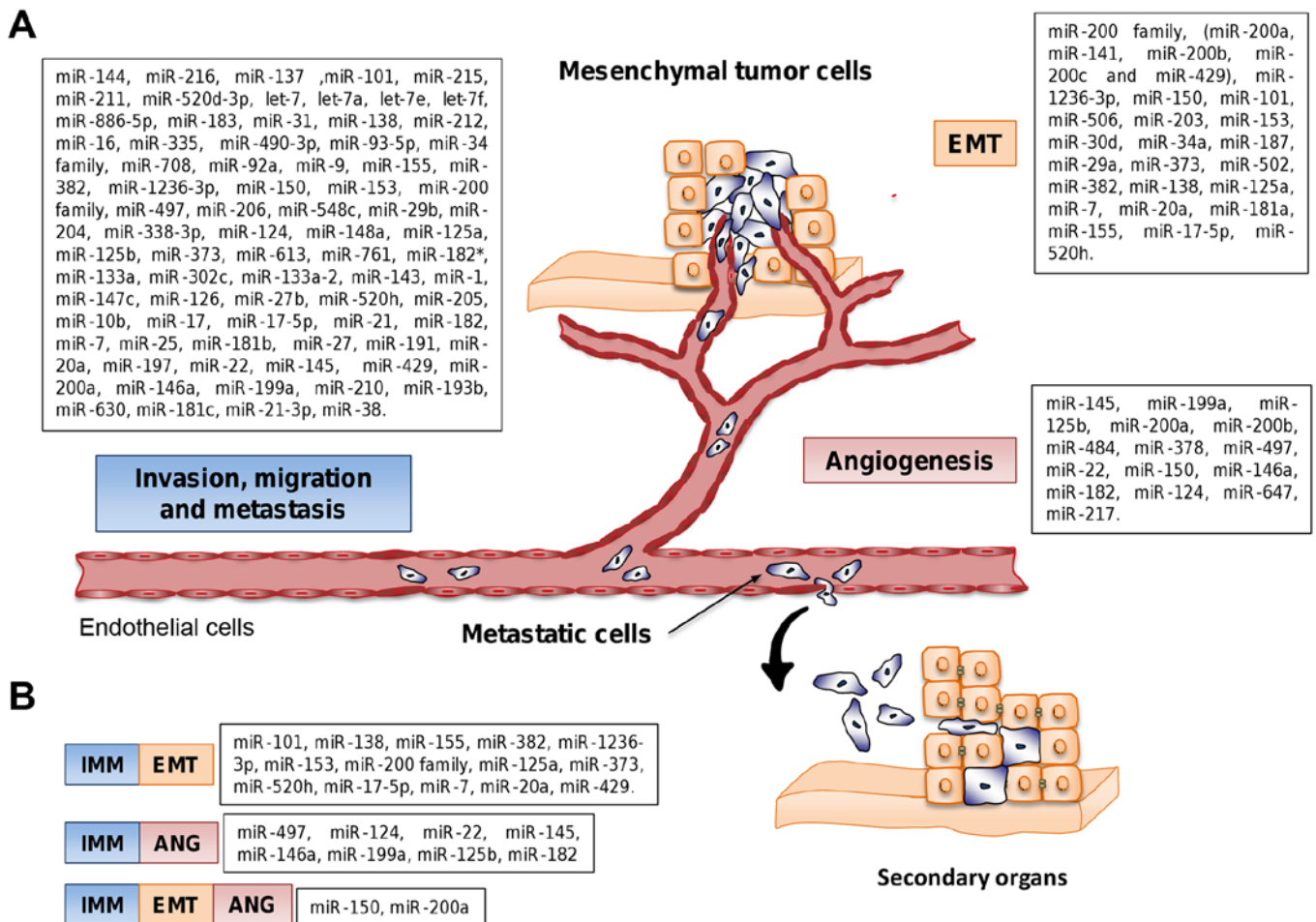


Figure 3. MicroRNAs involved in ovarian cancer. (A) The microRNAs involved in the regulation of epithelial-to-mesenchymal transition (EMT), angiogenesis (ANG), invasion, migration and metastasis process (IMM). (B) The miRNAs involved in common cellular processes.

miR-29b in endometroid adenocarcinoma cells. Congruently, knockdown of BAG3 led to increased levels of miR-29b in Ishikawa and HEC108 cells. Restoration of miR-29b using RNA-mimics decreased migration and invasion presumably by inhibition of metalloproteinase 2 (MMP2) gene (73). On the other hand, Sun *et al* demonstrated that Twist regulates miR-548c expression. Ectopic expression of miR-548c inhibited cell proliferation, migration and invasion, while Twist restoration abrogates the suppressive effect of miR-548c in cell migration and invasion in RL95-2 and HEC-1 ovarian cells (74).

**MicroRNAs functioning as oncogenes.** A number of miRNAs activates the cancer hallmarks thus functioning as oncogenes in OC. For instance, miR-205 is frequently overexpressed in endometrial carcinoma (EC) and it is related to metastasis. Experimental inhibition of miR-205 decreased cell proliferation, migration and invasion in Ishikawa cells by targeting the estrogen-related receptor- $\gamma$  (ESRRG) tumor suppressor gene (75). Another study showed that upregulation of miR-27 was associated with myometrial invasion in ECC. In addition, FOXO1 transcription factor was found downregulated in invasive ECC indicating that miR-27-FOXO1 axis inhibits invasiveness and apoptosis in tumors (76). Moreover,

Dong *et al* showed that miR-191 had a negative correlation with tissue inhibitor of metalloproteinase 3 (TIMP3) expression in tissues and serum of patients with endometriosis-associated ovarian cancer (EAOC). Notably, miR-191 was overexpressed in EAOC and its knockdown significantly inhibited cell invasion by upregulation of TIMP3 in CRL-11731 cells. These data indicate that miR-191-TIMP3 axis is involved in the malignant transformation of endometriosis to EAOC (77).

#### Hormonal receptors and microRNAs in cell invasion

**MicroRNAs acting as tumor suppressors.** Hormonal receptors interplaying with miRNAs regulate invasion and metastasis of tumor cells. Expression of estrogen receptor- $\alpha$  (ER- $\alpha$ ) and downregulation of miR-22 were associated with cell invasion in ER- $\alpha$  positive EC subtype. In addition, it was demonstrated that ER- $\alpha$  is a direct target of miR-22. Likewise, ectopic expression of miR-22 in RL95-2 and Ishikawa cells abrogates the invasion induced by 17 $\beta$ -E2 (E2) through downregulation of cyclin D1 and MMP2 and MMP9. Thus, E2-ER- $\alpha$  axis represents a potential candidate for endocrine therapy in OC (78). On the contrary, it was reported that miR-206 was significantly downregulated in ER- $\alpha$  positive EC and that its suppression was modulated by E2. Ectopic expression of miR-206 decreased ER- $\alpha$  positive-dependent proliferation and

invasion in EC cells. Thus, miR-206 also could be a potential tool for endocrine therapy in ER- $\alpha$  positive EC patient (79,80).

*MicroRNAs associated with highly aggressive phenotypes of ovarian cancer*

*MicroRNAs acting as tumor suppressors.* Recent findings from diverse research groups pointed out that altered miRNAs expression was associated with aggressive phenotypes of OC. Downregulation of tumor suppressors let-7a, let-7e, let-7f, miR-886-5p and miR-22 have been associated with aggressive behavior of tumors and represents potential markers of invasion and metastasis in EOC (81). Particularly, miR-22 has a critical role on malignancy and metastasis in all subtypes of OC. Li *et al* showed that overexpression of Tiam1, a guanine nucleotide exchange factor, correlated with metastatic phenotypes of OC. Authors showed that miR-22 is downregulated in ovarian cancer and negatively associated with metastasis in SOC cells. Intriguingly, the manipulation of miR-22 expression had inhibitory effects on cell migration and invasion, but not in cell viability and apoptosis (82). In agreement with these findings, miR-22, miR-183 and miR-31 suppressed cell migration and invasion of SOC cells, at least in part, by downregulation of Tiam1 (83).

In other studies, Wen *et al* reported that miR-338-3p expression was repressed in EOC tissues and correlated with malignancy. Ectopic expression of miR-338-3p triggers apoptosis and activation of caspases 3, 8 and 9. In addition, miR-338-3p inhibits MMP-2 and MMP-9 resulting in reduced cell migration and invasion abilities. Overexpression of miR-338-3p induced the downregulation of Runx2, which in turn abolish the phosphorylation of PI3K (Tyr458) and AKT (Ser473 and Thr308). Cell migration and invasion were inhibited by PI3K/AKT signaling pathways (84). In other studies, it was found that miR-708 was downregulated in SKOV-I6iv cells and associated with advanced-stage ovarian cancer (III/IV stages). *In vitro* assays showed that the restoration of miR-708 attenuated the cell invasion and metastasis in orthotopic xenograft mouse model through suppression of Rap1B. Loss of Rap1B resulted in increased focal adhesions (85).

Furthermore, miR-124 was downregulated in highly metastatic OC cells. Ectopic expression of miR-124 suppressed the expression of sphingosine kinase 1 (SphK1), a protein associated with tumor cell invasion and metastasis. Knockdown of SphK1 using miR-124 inhibited the cell motility in SKOV3 and HO8910 cells (86). Thereafter, Wen *et al* found that sphingosine-1-phosphate receptor 1 (S1PR1) was also suppressed by miR-148a. Overexpression of miR-148a resulted in the inhibition of cell migration and invasion in SKOV3 cells (87).

Moreover, two reports showed that low levels of miR-138 were associated with invasion and metastasis without affecting growth of tumors (62,88). In the first study, ectopic expression of miR-138 reduced the cell migration and metastasis ability by targeting SOX4 and hypoxia-inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ). Knockdown of SOX4 and HIF-1 $\alpha$  in SKOV-I6 cells abolished the invasion through the signaling downstream of EGFR and Slug. Thus low levels of miR-138 and high expression of SOX4 could represent a prognostic marker and a target for intervention of OC metastasis (62). In the second report, suppression of the expression of miR-138 was found in highly malignant phenotypes of OC tissues. Notably, miR-138 inhibits cell

invasion and metastasis by targeting LIM kinase 1 (Limk1) via Limk1/cofilin/p-cofilin axis and modulation of PCNA and Bcl-2 (88).

Another report indicates that EPH receptor A2/B2 (EphA2/B2) is implicated in malignancy of OC. Knockdown of EphA2 expression or ectopic expression of miR-520d-3p *in vitro* abolished the migration and invasion of cancer cells and decreased the tumor growth *in vivo* (14). It has been indicated that motility of the ovarian cancer cells can be promoted by ubiquitin ligases. Wang *et al* reported that higher levels of pro-metastatic factor SMAD specific E3 ubiquitin protein ligase 1 (SMURF1) were associated with shorter overall survival, whereas downregulation of miR-497 correlated with aggressive phenotypes of OC. Exogenous expression of miR-497 abolished the cell migration and invasion through direct inhibition of SMURF1 activity. In agreement with these data, restoration of miR-497 decreased invasiveness and was associated to better survival of patients (89). On the other hand, Lee *et al* elucidated a link between translation machinery and miRNAs. Ectopic expression of miR-125a and miR-125b decreased cell invasion and migration by targeting the eukaryotic translation initiation factor 4E binding protein 1 (EIF4EBP1) gene (90).

*MicroRNAs acting as oncogenes.* Some miRNAs could have a dual role as tumor suppressor or oncogene in cancer cells. For instance, miR-7 expression was identified downregulated in EOC tumors (65). However, Meng *et al* identified that miR-7 and miR-429 were overexpressed in serum of EOC patients. *In vitro* miR-429 inhibited the cell migration and invasion. Authors proposed that miR-429 could have a dual role acting as tumor suppressor and oncogene because miR-429 levels were high in patients with primary EOC while they diminished in patients with distant migration and metastasis (91).

*MicroRNAs regulating intercellular junctions and cytoskeletal dynamics in ovarian cancer*

*MicroRNAs functioning as tumor suppressors.* Actin cytoskeleton regulates the formation of F-actin-rich membrane protrusions leading to motility and cellular adhesion. Cao *et al* found that overexpression of miR-335 inhibited cell migration and invasion through the depolymerization of F-actin in OC cells. Moreover, miR-335 suppressed B-cell CLL/lymphoma w (BCL-w) and its effector MMP2. Lack of miR-335 expression resulted in the accumulation of BCL-w and acquisition of a more invasive phenotype (92).

Using high-resolution miRNAs comparative genomic hybridization (CGH), Imam *et al* identified a genomic loss of miR-204 in 44.63% of OC (93). Tumor suppressor miR-204 is frequently downregulated in diverse types of cancer (94,95). Authors demonstrated that miR-204 target the brain-derived neurotrophic factor (BDNF) gene which bind and activate the tropomyosin-related kinase B (TrkB). The BDNF/TrkB complex enhanced invasiveness and angiogenesis through subsequent activation of the small GTPase Rac1 and actin reorganization through the AKT/mTOR signaling pathway. Overexpression of miR-204 reduced colony-forming capacity and migratory and invasive capabilities of SKOV3 and HEK-293 cells lines. Interestingly, injection of the miR-204 in the breast cancer lung metastasis model led to suppression of



lung metastases (93). Likewise, expression of miR-9 inhibited the focal adhesion protein talin 1 (TLN1). Downregulation of TLN1 was associated with decreased cell migration and invasion in serous ovarian cancer through downregulation of FAK/AKT pathway (96). Besides, Ohyagi-Hara found that overexpression of the integrin subunit  $\alpha 5$  (ITGA5) correlated with peritoneal dissemination in OC. Restoration of miR-92a suppressed cell adhesion mediated by ITGA5, as well as cell invasion and proliferation in OC cells. ITGA5 was confirmed as a target of miR-92a and its inhibition resulted in the peritoneal dissemination of cancer cells (97). Finally, Doberstein *et al* found that overexpression of miR-21-3p modulated the expression of L1 cell adhesion molecule (L1CAM), which is a protein associated with metastasis in OVMz cells (98).

#### *MicroRNAs associated with p53 activity in ovarian cancer*

**MicroRNAs functioning as a tumor suppressor.** Loss or dysfunction of tumor suppressor p53 greatly contributes to tumorigenesis in more than half of all types of cancers. Chen *et al* demonstrated that restoration of miR-490-3p in EOC metastatic cells resulted in increased levels of p53. Overexpression of miR-490-3p *in vitro* suppressed cell migration and invasion as well as cell proliferation, and *in vivo* decreased the tumor development. Notably, miR-490-3p targets cyclin-dependent kinase 1 (CDK1) which participates together with Bcl-xL, CCND1, and MMP-2/9 enhancing tumorigenesis in OC. By decreasing CDK1 expression through miR-490-3p and stimulating the expression of p53, miR-490-3p potentially may suppress the tumorigenesis and metastasis progression in EOC (99). In addition, Chen *et al* suggested that the expression of tumor suppressor miR-93-5p activates p53 while represses poly (ADP-ribose) polymerase (PARP) expression through inhibition of RAS homolog gene family member C (RhoC), P70S6 kinase, Bcl-xL and MMP-9. Functional studies confirmed that RhoC is a target of miR-93-5p. In addition, overexpression of miR-93-5p *in vitro* inhibited invasion and proliferation of cancer cells, and the *in vivo* tumor development (100). Moreover, Liu *et al* showed that the expression of p53 and p21 was decreased because downregulation of serine/threonine kinase 11 (LKB1), which was inhibited by miR-17 in CD44<sup>+</sup>/CD117<sup>+</sup> cancer cells. Expression of miR-17 modulated the migration and invasion in CD44<sup>+</sup>/CD117<sup>+</sup> cells through suppression of the LKB1-p53-p21/WAF1 pathway. The suppression of LKB1-p53-p21/WAF1 by miR-17 resulted in increased invasiveness of CD44<sup>+</sup>/CD117<sup>+</sup> cells (101).

The miR-34 family plays a key role in cell proliferation and invasion of EOC cells. Corney *et al* showed that miR-34 family is downregulated in OC with or without p53-mutated. They found that miR-34b<sup>\*</sup>/c levels are lower in advanced OC and the expression of miR-34a was negatively associated with the expression of the proto-oncogene receptor tyrosine kinase (MET). Ectopic expression of miR-34 family members into SKOV-3 cells decreased MET significantly and resulted in the inhibition of cell motility and invasion (102). A further study highlighted that miR-34 family is activated in the presence of functional p53. MET can be suppressed through miR-34 in a p53-dependent way (103,104). In addition, Li *et al* reported that miR-34a inhibits cell invasion and proliferation by downregulating the AXL receptor tyrosine kinase (105).

Downregulation of miR-145 has been reported in tumor tissues, serum and OC cancer cell lines (106). Additional reports showed that p53 regulated the expression of miR-145 and p53 is dysfunctional in high-grade SOC (107,108). Functional studies using RNA mimics revealed a suppressive role of miR-145 in cell viability, invasion, cell growth, cell proliferation and colony formation in OC cells. Moreover, cell colony formation and invasion were mediated by P70S6K1 and MUC1, respectively. Both genes were confirmed as true targets of miR-145 (106). Another study found that miR-145 also targets the motif containing 2 (TRIM2) gene. Inhibition of TRIM2-BIM pathway through antagomiR-145 in EOC resulted in decreased tumorigenicity (109). Moreover, Dong *et al* found that metadherin (MTDH) oncogene, which was overexpressed in SOC, is a target of miR-145. Inhibition of MTDH by miR-145 abolished the cell migration and invasion, and reduced tumor growth and metastasis (108). In other studies, Kim *et al* found an inverse expression between miR-145 and HMGA2 during ovarian cancer metastasis. *In vitro*, ectopic expression of miR-145 inhibited cell growth and migration. Finally, low levels of miR-145 and high expression of HMGA2 represent biomarkers of poor prognosis in ovarian cancer (110).

#### *Long non-coding RNAs regulate tumor suppressor let-7*

**MicroRNAs acting as tumor suppressors.** Long non-coding RNA can act as molecular sponge to suppress miRNAs in cancer cells. Yan *et al* identified that H19 long non-coding RNA suppressed the activity of let-7 in A2780 cells. Silencing of H19 decreased the cell migration and invasion processes by restoration of let-7 activity and inhibition of its targets genes including HMGA2, c-Myc and Igf2bp3. A positive correlation between the expression of H19 and Hmga2, c-Myc and Igf2bp3 was also identified. Suppression of let-7 activity by H19 may explain why the overexpression of let-7 is associated with poor prognosis in ovarian cancer (111). On the other hand, Gao *et al* reported that HOST2 long non-coding RNA is overexpressed in EOC resulting in the inhibition of let-7b. Targeting of HOST2 leads to decreased cell migration and invasion in OVCAR3 cells (112).

#### *MicroRNAs regulating the microRNA biogenesis machinery*

**MicroRNAs acting as oncogenes.** Biogenesis of miRNAs is epigenetically and transcriptionally controlled resulting in tissue specific miRNA expression patterns. However, alterations in the basic components of the miRNAs biogenesis machinery can promote carcinogenesis. Guo *et al* identified that knockdown of DiGeorge critical region 8 (DGCR8), a component of the microprocessor complex, reduced cell migration and invasion through attenuation of ERK1/2, PI3-K and AKT pathways, as well as sensitizes cells to apoptosis induced by the chemotherapeutic drug cisplatin. In addition, DGCR8 knockdown resulted in dysregulated miRNA gene expression. miR-27b was identified as the most highly downregulated miRNA in DGCR8 knockdown cells and promoted cell proliferation in OC cancer cells (113). On the contrary, Rupaimoole *et al* showed that low levels of Dicer during hypoxia conditions were due to upregulation of miR-630 in ovarian cancer. In addition, using luciferase reporter assays Dicer was confirmed as target of miR-630.

Table I. MicroRNAs targeting EMT with potential therapeutic applications into translational medicine.

miRNA	Epithelial mesenchymal transition						Authors/ Refs.
	Expression in ovarian cancer	<i>In vitro</i> effect	Target genes	Downstream genes	<i>In vivo</i> effect	Potential therapeutic candidate	
miR-200c	Suppressed in CD44 <sup>+</sup> / CD177 <sup>+</sup> cells	Inhibition of cell migration/invasion and EMT	ZEB1	↑ E-cadherin ↓ Vimentin	Inhibits xenograft growth and lung metastasis by EMT	Yes	Chen <i>et al</i> (38)
miR-506	Suppressed in SOC specimens	Inhibition of cell proliferation migration/invasion and TGFB1- induced EMT	SNAI2 CDH2 VIM	↑ E-cadherin ↓ N-cadherin ↓ Vimentin	Inhibits tumor growth, EMT, invasion and metastasis in SKOV3/HeyA8 orthotopic model	Yes	Yang <i>et al</i> (47) Sun <i>et al</i> (48) Sun <i>et al</i> (49)
miR-203	Suppressed in SOC specimens	Inhibition of cell proliferation, migration/invasion and TGFB1- induced EMT	SNAI2	↑ E-cadherin ↓ Vimentin	Inhibits SKOV3/ OVCAR3 xenograft xenograft growth and EMT	Yes	Zhao <i>et al</i> (50)
miR-373	Suppressed in EOC specimens	Inhibition of cell migration/invasion and EMT	Rab22a	↑ E-cadherin ↓ N-cadherin	Inhibits metastasis in xenograft metastatic models	Yes	Zhang <i>et al</i> (57)
miR-138	Suppressed in OC specimens	Inhibition of cell migration/invasion and EMT	HIF-1	EGFR SLUG	Inhibits metastasis in GFP/SKOV-16iv orthotopic model	Yes	Yeh <i>et al</i> (62)
miR-181a	Overexpressed in EOC specimens	Increase of cell migration/invasion and EMT	SMAD7	↓ E-cadherin ↓ Keratin 8 ↑ N-cadherin ↑ Fibronectin ↑ Co13A1	Increases EMT and metastasis in intra- peritoneal and intra- bursal orthotopic xenograft mouse models of OC	Yes	Parik <i>et al</i> (68)
miR-155	Suppressed in OC	Inhibition of cel proliferation, migration and invasion	CLDN1	↑ E-cadherin ↑ B-catenin ↓ MMP-2 ↓ MMP-9	Inhibits tumor growth and reduces proliferation of OCICs xenograft mouse model	Yes	Qin <i>et al</i> (71)

OC, ovarian cancer; EOC, epithelial ovarian cancer.

The delivery of miR-630 in nano-liposomes in an orthotopic mouse model of OC resulted in increased tumor growth and metastasis (114).

#### MicroRNAs regulating cellular growth factors

**MicroRNAs as tumor suppressors.** Growth factors are key promoters of tumor cell motility, invasiveness and metastasis. For instance, Tang *et al* demonstrated *in vitro* that miR-16 suppressed invasion through downregulation of vascular

endothelial growth factor (VEGF), MMP-2 and BCL-2 genes (115). Another study showed that the heparin binding epidermal growth factor (HBEGF) was repressed by miR-212. This miRNA was found downregulated in tumor tissues and patient's serum with EOC. Overexpression of miR-212 in SKOV3 cells suppressed cell proliferation, migration and invasion (116). Recently, Li *et al* identified a correlation between downregulation of miR-217 with metastasis in EOC. Ectopic expression of miR-217 in EOC cells decreased cell prolifer-

Table II. MicroRNAs targeting invasion and metastasis with potential therapeutic applications in translational medicine.

miRNA	Migration, invasion and metastasis							Authors/ Refs.
	Expression in ovarian cancer	<i>In vitro</i> effect	Cancer cells	Target	Downstream genes	<i>In vivo</i> effect	Potential therapeutic candidate	
miR-204	Suppressed in OC tissue	Inhibition of colony-forming ability, migration/ invasion, tumor size	SKOV3	BDNF	↓ BDNF/TrkB complex ↓ Rac1 and actin reorganization ↓ AKT/mTOR	Inhibits lung metastasis in SCID mice	Yes	Imam <i>et al</i> (93)
miR-520d -3p	Suppressed in EOC	Inhibition of cell migration/invasion and tumor growth	SKOV3ip1/ HeyA8	EphA2	↓ EphB2 ↓ EphA8	Inhibits tumor growth, tumor weight, cell proliferation, angiogenesis and increase apoptosis in SKOV3ip1/ HeyA8 ortho- topic model	Yes	Chen <i>et al</i> (38)
miR-708	Suppressed in OC tissues	Inhibition of cell migration/invasion	SKOV3 SKOVI6iv TOV112D	Rap1B	↓ AKT2 ↓ p-FAK ↓ p-Paxillin	Inhibits lung metastasis, tumor size in orthotopic xenograft mouse model	Yes	Lin <i>et al</i> (85)
miR-182	Overexpressed HG-SOC	Inhibition of cell proliferation, colony-forming ability and migration/invasion	SKOV3 OVCAR	BRCA1 FOXO3 MTSS1	↑ CDKN1A ↑ CDKN1B ↑ CHEK2 ↑ CYLD ↑ FOXO1 ↑ PDCD4 ↑ RECK ↓ MYCN ↓ HMGA2	Inhibits tumor size, invasion and metastasis in orthotopic model	Yes	Xu <i>et al</i> (129)
miR-145	Suppressed in EOC and HG-SOC	Inhibition of cell proliferation, colony-forming ability and migration/invasion	SKOV3 HO8910 OVCAR3 A2780	TRIM2 MTDH	↑ BIM ↑ TP53	Inhibits tumor growth and metastasis in xenograft mouse model	Yes	Dong <i>et al</i> (108) Chen <i>et al</i> (109)
miR-490 -3p	Suppressed in EOC	Inhibition of cell proliferation, apoptosis and migration/invasion	OVCAR HO8910-PM	CDK1	↑ TP53 ↓ Bcl-xL ↓ MMP2/9 ↓ CCND1 ↓ SMARCD1	Inhibits tumor growth and its development in xenograft mouse models	Yes	Chen <i>et al</i> (99)
miR-93-5p	Suppressed in OC specimens	Inhibition of cell proliferation, apoptosis and migration/invasion	OVCAR HO8910-PM SKOV3/ DDP	RhoC	↑ TP53 ↓ P70S6K ↓ Bcl-xL ↓ MMP9	Inhibits tumor growth and its development in xenograft mouse models	Yes	Chen <i>et al</i> (100)

Table II. Continued.

miRNA	Migration, invasion and metastasis							
	Expression in ovarian cancer	<i>In vitro</i> effect	Cancer cells	Target	Downstream genes	<i>In vivo</i> effect	Potential therapeutic candidate	Authors/ Refs.
miR-17	Overexpressed in OC CD44 <sup>+</sup> /CD117 <sup>+</sup> stem cells	Promotes cell proliferation, migration/invasion in CD44 <sup>+</sup> /CD117 <sup>+</sup> cells	CD44 <sup>+</sup> /CD117 <sup>+</sup> cells	LKB1	↑ p53-p21/WAF1	Promotes tumor growth and tumorigenesis in CD44 <sup>+</sup> /CD117 <sup>+</sup> cells	Yes	Liu <i>et al</i> (101)
miR-630	Overexpressed in EOC under hypoxia conditions	Inhibition of cell migration/invasion	A2780 OVCAR3	Dicer	↑ microRNAs biogenesis	Inhibits tumor growth and metastasis in orthotopic model	Yes	Rupaimoole <i>et al</i> (114)

OC, ovarian cancer; EOC, epithelial ovarian cancer.

eration, migration and invasion *in vitro*, as well tumor growth *in vivo*. miR-217 may exert its antitumor effects by targeting the insulin-like growth factor 1 receptor (IGF1R) (117).

**MicroRNAs as oncogenes.** VEGF is able to stimulate the expression of miRNAs leading to enhanced invasion and metastasis. For instance, Li *et al* showed that VEGF induced the expression of oncogene miR-205 in OC cells, and this in turn downregulated Ezrin and Lamin A/C. miR-205 promoted invasiveness, proliferation and inhibited apoptosis of OC cells (118). In addition, the expression levels of VEGF and miR-205 were found increased in the serum of EOC patients (119).

#### Other microRNAs involved in metastasis

**MicroRNAs functioning as tumor suppressors.** Fu *et al* identified that miR-613, a downregulated miRNA in OC targets the K-RAS oncogene. Congruently, *in vivo* ectopic expression of miR-613 suppressed cell migration and invasion (120). Likewise, Zhang *et al* reported that miR-137 is downregulated in EOC and its ectopic expression *in vitro* decreased cell migration and invasion (121). On the other hand, Shi and Zhang identified that miR-761 expression was severely downregulated in ovarian cancer. Ectopic expression of miR-761 *in vitro* inhibited invasion by increasing the expression of its target musashi RNA binding protein 1 (MSI1) (122). Yao *et al* showed that miR-181c was downregulated in OC patients with lymph node metastasis. Restoration of miR-181c expression led to decreased proliferation and metastasis in A2780 cells. Interestingly, protein kinase C  $\delta$  (PRKC- $\delta$ ) was identified as a target of miR-181c and its knockdown *in vitro* resulted in progression of metastasis (123).

**MicroRNAs as oncogenes.** Some studies showed that overexpression of miR-25 and miR-181b promotes cell migration and invasion of OC cells by targeting the large tumor suppressor 2

(LATS2). The restoration of LATS2 expression attenuated the oncogenic effects of miR-25 and miR-181b (124,125). Other studies showed that miR-21 repressed PTEN in EOC. Moreover, knockdown of miR-21 significantly inhibited cell proliferation and migration presumably through inhibition of programmed cell death 4 (PDCD4), because PDCD4 was correlated negatively with miR-21 expression (126,127). Wang *et al* identified that PDCD4 is the target of miR-182. In addition, miR-182 expression induced migration and invasion whereas its silencing inhibited cell viability and colony formation in OC cells due to the recovery of PDCD4 (128).

Likewise, Xu *et al* investigated the therapeutic potential of miR-182 in orthotopic model using SKOV3 (intrabursal xenografts) and OVCAR3 (intraperitoneal injection) cancer cells. Overexpression of miR-182 was associated with metastatic HG-SOC. The treatment with miR-182 inhibitors restored the expression of breast cancer 1 (BRCA1), FOXO3, metastasis suppressor 1 (MTSS1) and other genes involved in cell cycle control. All these tumor suppressor genes were miR-182 targets and their inhibition resulted in increased tumor growth, invasion and metastasis. Anti-miR-182 treatment restored the gene expression of their targets, which in turn reduced tumor invasion and metastasis in a model of SOC (129). In addition, Liu *et al* found that overexpression of miR-182 in immortalized ovarian surface cells; fallopian tube secretory cells and malignant ovarian cells lead to promotion of invasiveness and metastasis in female BALB/c nude mice. Oncogenic effects of miR-182 were driven by downregulation of BRCA1 and MTSS1, as well as overexpression of HMGA2 (130).

Nakayama *et al* showed that loss of homeobox D10 (HOXD10) gene due to overexpression of miR-10b enhanced the migration and invasion through increased activity of the pro-metastatic genes MMP14 and RhoC (131). Fan *et al* established that the inhibition of miR-20a suppresses the invasion by targeting amyloid precursor protein (APP) in OC (132). Similarly, Zou *et al* found that overexpression of miR-197

promotes the invasion of cancer cells through downregulation of its target nemo-like kinase (NLK) (133).

## 7. Possible exploitation of microRNAs in precision medicine

There is increased evidence that miRNAs play crucial roles as innovative therapeutic targets in OC. Due to their extreme stability and its potent activities that allow modulating relevant gene networks impacting tumor biology, miRNAs has also been studied as a prospective antitumor approach. The miRNAs can limit tumor growth and dissemination of tumor cells. These effects can be induced by inactivating oncogenic miRNAs or restoring the expression of miRNAs with tumor suppressor functions (134). The potential usefulness of a miRNA-based therapy has been exploited by different approaches such as: i) antisense oligonucleotides and synthetic analogues of miRNAs (antagomirs) to silence oncogenic miRNAs (135,136); and ii) synthetic miRNAs (mimics), chemically modified oligonucleotides or adeno-associated virus-based vector system to restore the expression of downregulated tumor suppressive miRNAs (137,138). Garzon *et al* showed that the effects of upregulated oncomiRs could be suppressed using antagomirs (139). For instance, Lu *et al* using a modified anti-miRNA antisense olideoxyribonucleotide (AMOs) revealed that the use of this innovative strategy silenced multiple-target miRNAs. This technology was used to targets oncogenic miR-21, miR-155, and miR-17-5 (140). In other report, Dai *et al* showed that using a chimera composed by a mucin 1 (MUC1) aptamer targeting the tumor cell surface MUC1 protein and miR-29b mimics inhibiting DNA methyltransferases, resulted in the expression of PTEN tumor suppressor. Interestingly, these findings provide evidence that delivery of miRNAs in a tissue-specific manner is possible and that it may exert potent antitumor effects (141). Another example of application of miRNAs as therapeutic tools is the miR-200 family. Restoration of miR-200c in combination with paclitaxel treatment decreased tumor development. miR-200c therapy before conventional chemotherapy reduces the effective dose of drugs resulting in increased response (142). Other miRNAs involved in cellular mechanisms that promote the development and progression of metastasis in OC have tumor suppressive effects when delivered *in vivo* into experimental models. For instance, miR-200c, miR-506, miR-203, miR-373, miR-138, miR-181a and miR-155 gained attention by suppressing EMT process and metastasis. In Tables I and II the miRNAs that have showed efficacy in decreasing the metastasis of tumor cells and that target EMT with potential clinical applications are listed. Such miRNAs are potential candidates for translational medicine in OC therapies (143).

## 8. Conclusion

The recent discovery of the role of miRNAs as tumor-suppressor genes and oncogenes has added an additional level of complexity to the mechanisms leading to tumorigenesis. The identification of miRNAs involved in invasion and metastasis will provide insights into molecular mechanisms underlying tumorigenesis. These miRNAs could be useful as clinical strategies to customize therapeutic targets that act in spatial and temporal manner during malignancy progression and

metastasis in ovarian cancer. The updated review presented here, showed that metastamiRs have emerged as new molecular players to regulate invasion and metastasis events in ovarian cancer. Some of these miRNAs are common regulators of cell motility and invasion in distinct types of cancers while others appear to be cancer specific. Understanding how metastamiRs are involved in regulating tumor invasion and metastasis processes will provide data to establish potential strategies for the development of new metastamiR-based treatments. However, further investigations and clinical trials challenging the potential applications of metastamiRs are required in order to use them as targets for personalized therapies, prognosis and diagnosis in the near future.

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