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Review

Key nodes of a microRNA network associated with the integrated mesenchymal subtype of high-grade serous ovarian cancer

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Abstract

Metastasis is the main cause of cancer mortality. One of the initiating events of cancer metastasis of epithelial tumors is epithelial-to-mesenchymal transition (EMT), during which cells dedifferentiate from a relatively rigid cell structure/morphology to a flexible and changeable structure/morphology often associated with mesenchymal cells. The presence of EMT in human epithelial tumors is reflected by the increased expression of genes and levels of proteins that are preferentially present in mesenchymal cells. The combined presence of these genes forms the basis of mesenchymal gene signatures, which are the foundation for classifying a mesenchymal subtype of tumors. Indeed, tumor classification schemes that use clustering analysis of large genomic characterizations, like The Cancer Genome Atlas (TCGA), have defined mesenchymal subtype in a number of cancer types, such as high-grade serous ovarian cancer and glioblastoma. However, recent analyses have shown that gene expression-based classifications of mesenchymal subtypes often do not associate with poor survival. This "paradox" can be ameliorated using integrated analysis that combines multiple data types. We recently found that integrating mRNA and microRNA (miRNA) data revealed an integrated mesenchymal subtype that is consistently associated with poor survival in multiple cohorts of patients with serous ovarian cancer. This network consists of 8 major miRNAs and 214 mRNAs. Among the 8 miRNAs, 4 are known to be regulators of EMT. This review provides a summary of these 8 miRNAs, which were associated with the integrated mesenchymal subtype of serous ovarian cancer.

Key words MicroRNA (miRNA), epithelial-to-mesenchymal transition (EMT), cancer, ovary, miR-506, miR-101

Cancer is a complex and dynamic disease that defies the normal differentiation process in which pluripotent embryonic stem cells are differentiated into almost all cell types; this process is driven mostly by epigenetic nuclear programming events such as DNA methylation and selective expression of a series of non-coding RNAs[1]. However, the extensive nuclear reprogramming that accompanies genetic events such as mutation and gene copy number alterations confers remarkable plasticity on cancer cells[2], particularly regarding the phenotypic switches often found in cancer[3]. Epithelial cells can adopt mesenchymal features, mesenchymal cells can adopt epithelial features, and mesenchymal cells can become endothelial cells[3,4]. The most well-studied cell fate switch is epithelial-to-mesenchymal transition (EMT), a process whereby epithelial cells lose both polarity and cell-to-cell contacts, thus acquiring increased motility and invasiveness. This pathophysiological transition is necessary for the conversion from a benign tumor to an aggressive, highly invasive carcinoma; it is the mechanism that allows tumor cells to escape from the primary tumor, evade into neighboring normal parenchyma, and enter lymphatic and blood circulation to initiate lympho-hematogenous metastasis. The early escape of certain epithelial cells in tumors was noticed and documented by pathologists more than 100 years ago in drawings[5]. EMT properties are acquired as a

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result of complex changes in cancer cells and their microenvironment that lead to the dissolution of intracellular junctions and detachment from the basolateral membrane; changes in the interactions between cancer cells and the extracellular matrix also contribute to EMT.

The phenotypic switch from epithelial to mesenchymal is characterized by profound morphologic changes, such as the loss of apico-basal polarity and reorganization in the distribution of organelles and cytoskeleton components that are related to a mesenchymal switch in the expression of cell lineage–specific genes and levels of proteins. The levels of epithelial proteins (e.g., E-cadherin, claudin, occluding, cytokeratins) progressively decrease while the levels of mesenchymal proteins [e.g., N-cadherin, vimentin, alpha-smooth muscle actin (α-SMA), fibronectin] increase.

In addition to these well-known marker genes, more complex gene signature sets that take into account the intrinsic heterogeneity of tumor cells have been proposed to define mesenchymal and epithelial subtypes[6-12]. Practically, these gene sets are used to estimate whether a cell population is more likely to express mesenchymal or epithelial features. Examining genes that cluster a group of tumors together is a frequently used strategy in cancer classification[6,9-11]. If these genes are enriched in a mesenchymal gene set, these tumors are often classified as mesenchymal subtypes. Using data from The Cancer Genome Atlas (TCGA), mesenchymal subtypes have been identified in multiple cancer types, including serous ovarian cancer[13].

Because of the importance of EMT and mesenchymal-to-epithelial transition (MET), a concerted effort has been made to determine the regulators of these processes[14-17]. In this article, we focus on the post-transcriptional regulatory mechanism based on microRNAs (miRNAs), which are 22-nucleotide, non-coding RNAs that suppress gene expression through mRNA destabilization or translational inhibition. miRNAs are deregulated in a wide variety of human cancers and have been shown to contribute to the control of cell growth, differentiation, and apoptosis, which are all important for the development and progression of cancer[18-26]. miRNAs regulate multiple signaling pathways involved in EMT[27]. Because of the intimate relationship between miRNAs and EMT signature genes, we believe that an integrated analysis of these two groups of genes (protein coding and non-coding) is critical to define the core regulatory network that may underlie specific phenotypes associated with cancer subtypes. In a recent report, we performed such an analysis and identified a core regulatory network that better describes an integrated mesenchymal subtype of serous ovarian cancer patients in the TCGA cohort. This network includes 8 key node miRNAs and 214 protein-coding genes (**Figure 1**)[28].

In the integrated miRNA-mRNA network, 3 of the 8 miRNAs (miR-101, miR-200c, and miR-141) are well-known regulators of EMT, and the work performed by our group and others has also shown that miR-506 is a potent regulator of EMT[28-30]. The role of other 4 miRNAs (miR-25, miR-29c, miR-182, and miR-128) in EMT is less clear, although some of these have been shown to affect cell migration, invasion, and metastasis. This article briefly summarizes these 8 miRNAs and their roles in cancer. We first review the newly defined EMT suppressor miR-506, followed by the other known EMT regulators (miR-101, miR-200c, and miR-141), and then the remaining 4 miRNAs.

miR-506

miR-506 is located in Xq27.3, a chromosomal region associated with fragile X syndrome. Female patients with fragile X syndrome suffer from primary ovarian insufficiency[31]. miR-506 belongs to a chrXq27.3 miRNA cluster that is associated with early relapse in advanced stage ovarian cancer[32]. In our previous study, we demonstrated that miR-506 is a potent inhibitor of the mesenchymal phenotype and transforming growth factor β (TGF-β)-induced EMT by directly targeting snail family zinc finger 2 (SNAI2), a transcriptional repressor of the epithelial protein E-cadherin[28]. Subsequently, we further illustrated a broader role of miR-506 in the suppression of EMT via its direct regulation of 2 well-known mesenchymal proteins, vimentin and N-cadherin, in all epithelial ovarian carcinoma subtypes[29]. Therefore, miR-506 represents a novel class of miRNA that regulates both E-cadherin and vimentin/N-cadherin to suppress EMT.

The expression of E-cadherin and vimentin/N-cadherin represents 2 spectrums of EMT and MET. Accumulating evidence suggests that these pathways crosstalk closely and regulate one another. In our study, we found that the knockdown of vimentin up-regulated E-cadherin expression[29]. Rodriguez *et al*.[33] reported that vimentin inhibited E-cadherin and induced EMT via glycogen synthase kinase 3β (GSK-3β), an upstream regulator of SNAI1. Our recent study showed that miR-506 inhibited the expression of forkhead box protein M1 (FoxM1) by directly down-regulating cyclin-dependent kinase 4 (CDK4)/CDK6[34]. FoxM1 is a transcriptional activator of SNAI1, another major E-cadherin repressor[35,36]. miR-506 also targets nuclear factor kappa B (NFκB) p65[37], which was implicated in the regulation of EMT. Kuphal *et al*.[38] identified the NFκB-binding site in the N-cadherin promoter and reported that the loss of E-cadherin activated NFκB and induced N-cadherin expression during the EMT of melanoma cells. Vimentin expression was also transactivated by NFκB[39]. Therefore, miR-506 has emerged as a key network gatekeeper for epithelial and mesenchymal lineage switches by simultaneously regulating multiple nodes in the sophisticated regulatory network (**Figure 2**).

Because miR-506 functions as a potent suppressor of EMT, it may be useful as a small-molecule therapeutic agent for cancer. We tested this possibility in a preclinical study in which nanoparticle delivery of miR-506 effectively suppressed tumor growth and spread in 2 orthotopic ovarian cancer models[28,29]. In addition to its effect on EMT, this tumor suppression function of miR-506 may also be partly caused by its recently recognized role in the inhibition of cell proliferation and the promotion of senescence by directly targeting its binding sites on the 3'- untranslated regions (3-UTRs) of CDK4 and CDK6[34]. CDK4/6 is a druggable target for cancer therapies[40]. The CDK4/6 inhibitor PD-0332991 is currently undergoing clinical testing in several cancer types[41-43]. The transcription factor network CDK4/6-FoxM1 is activated in more than 80% of high-grade serous ovarian cancer cases[13]. Collectively, these data suggest that miR-506 is a

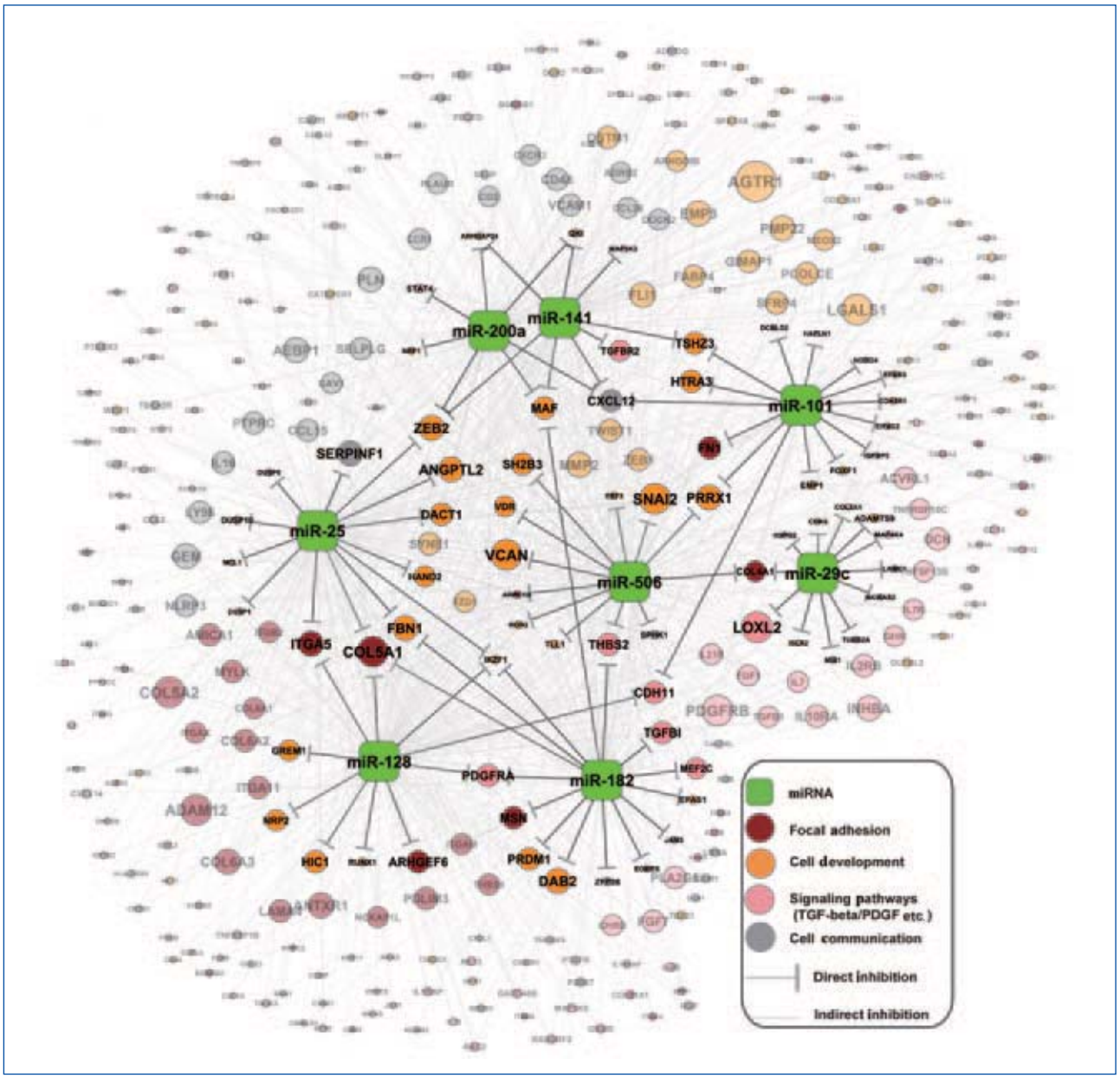

Figure 1. **The key microRNAs (miRNAs) in the integrated mesenchymal subtype of The Cancer Genome Atlas (TCGA) high-grade serous ovarian cancer cases. This figure is part of Figure 3 in Yang *et al.*[28] (used with approval from the publisher). The miRNA-gene network shows the relationships between 8 key miRNAs and epithelial-to-mesenchymal transition (EMT) signature genes they are predicted to regulate. The size of each gene node indicates the number of predicted key miRNAs regulators; the colors indicate the annotated function of the gene. Only genes with gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) annotations are shown in this network. TGF-β ,transforming growth factor β; PDGF, platelet-derived growth factor.**

potential therapeutic agent for ovarian cancer, and further studies are needed to validate the clinical value of miR-506 in the treatment of ovarian cancer.

The role of miR-506 in EMT inhibition, cell senescence, and differentiation has also been demonstrated in several other cancer types, including breast cancer, lung cancer, cervical cancer, and neuroblastoma[30,37,44,45], indicating that miR-506 functions as a tumor suppressor in a wide spectrum of cancers. However, the regulation of miR-506 expression remains understudied. We previously reported that miR-506 is partially regulated by methylation[28]. This is consistent with the result of a recent large-scale screening of epigenetically regulated miRNAs in ovarian cancer, which showed the Xq27.3 miRNA cluster (including miR-506) was regulated by epigenetic mechanisms[46]. Further studies using larger sample sizes are needed

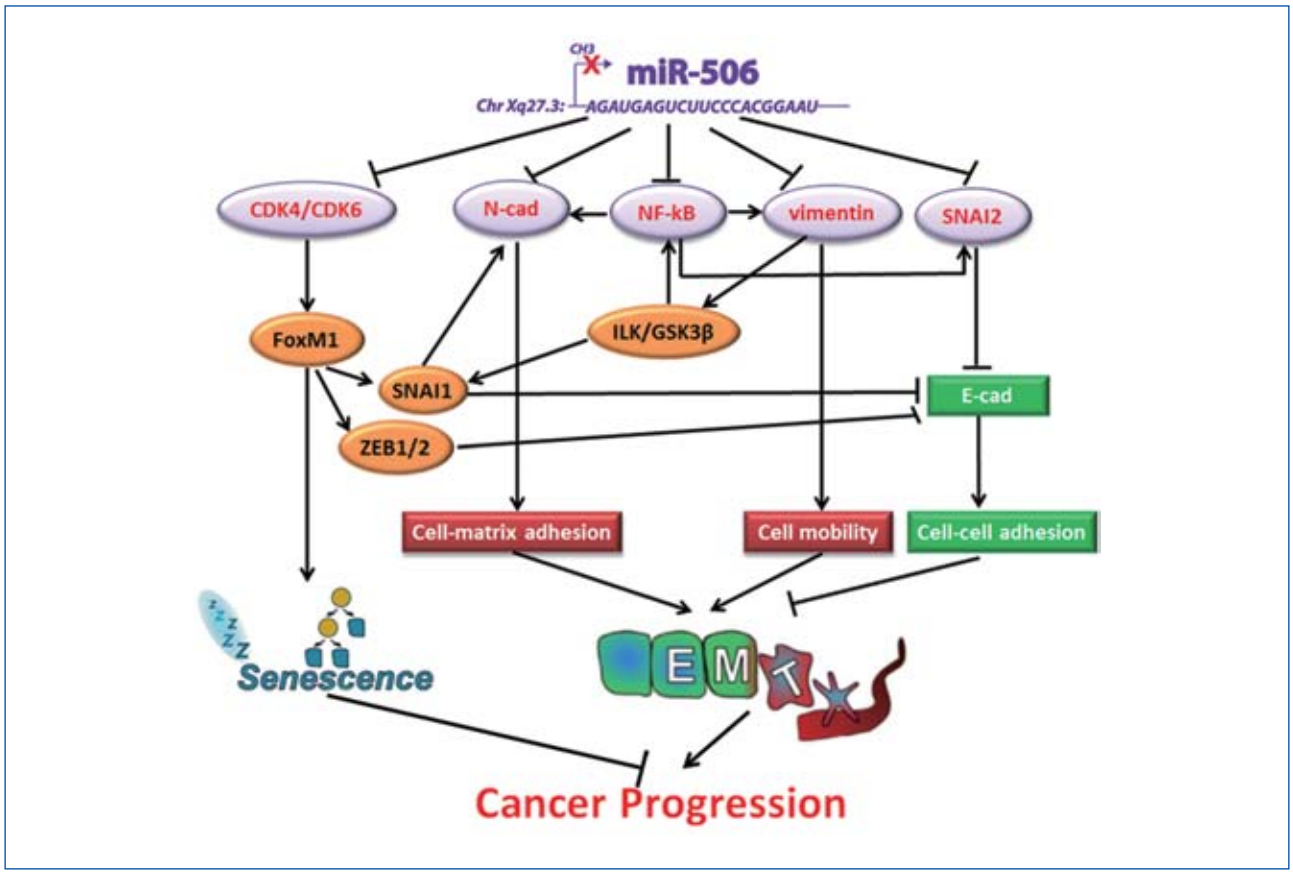

Figure 2. **The miR-506 network regulates EMT and cellular senescence.** miR-506 directly targets SNAI2[28], vimentin[29], N-cad[29], NFκB[37] and CDK4/CDK6[34]. miR-506 down-regulates SNAI2 which increases E-cad expression and subsequently promotes cell-cell adherence[28]. miR-506 directly down-regulates vimentin and N-cadherin, which reduces cell mobility and cell-matrix adherence[29]. miR-506 also targets NFκB p65[37] which transactivates N-cad and vimentin and is implicated in the regulation of EMT[38,39]. miR-506 inhibits the expression of FoxM1 by directly down-regulating CDK4/CDK6, which not only promotes cellular senescence[34] but also inhibits EMT via suppressing the expression of SNAI1 and ZEB1/2[36]. Therefore, miR-506 inhibits cancer progression through suppressing EMT and promoting cellular senescence. Decreased expression of miR-506 partially results from promoter methylation[28]. EMT, epithelial-to-mesenchymal transition; E-cad, E-cadherin; SNAI2, snail family zinc finger 2; N-cad, N-cadherin; NFκB, nuclear factor kappa B; CDK4, cyclin–dependent kinase 4; CDK6, cyclin-dependent kinase 6; FoxM1, Forkhead box protein M1; SNAI1, snail family zinc finger 1; ZEB1, zinc finger E-box binding homeobox 1; ZEB2, zinc finger E-box binding homeobox 2; ILK, integrin-linked kinase; GSK-3β, Glycogen synthase kinase 3β.

to reveal the relationship between miR-506 methylation and miR-506 expression.

miR-101

There are 2 separate copies of the miR-101 gene, located on 1p31.3 and 9p24. Both regions have been identified as fragile regions of the genome that are associated with abnormal deletion or amplification in cancer[47]. Down-regulation of miR-101 has been observed in bladder cancer[48], intraductal papillary mucinous neoplasms of the pancreas[49], and ovarian carcinoma[50-52], suggesting that miR-101 plays a role in tumor progression. Recent reports showed that miR-101 is methylated in several cancer types, explaining its decreased expression[53].

Abnormal expression of miR-101 may lead to a more malignant phenotype and promote cancer progression. A recent study found that low miR-101 expression in several subtypes of ovarian cancer tissues is significantly associated with poor cell differentiation, advanced International Federation of Gynecology and Obstetrics (FIGO) stages, and resistance to cisplatin[51]. By contrast, miR-101 overexpression reduced the proliferation and migration of ovarian cancer cells and re-sensitized drug-resistant cancer cells to cisplatin-induced cytotoxicity[51]. Thus, miR-101 may act as a suppressor of tumor progression. miR-101 may suppress tumor proliferation and migration and induce apoptosis by targeting enhancer of zeste homolog 2 (*EZH2*)[54,55] and Janus kinase 2 (*JAK2*)[56]. miR-101 may also induce senescence in breast cancer cells by targeting ubiquitin-conjugating enzyme E2N (*UBE2N*)- and SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily A, member 4 (*SMARCA4*)[57] and inhibit the G_1-to-S phase transition of cervical cancer cells by targeting FBJ murine osteosarcoma viral oncogene homolog (*Fos*)[58].

Like most miRNAs, miR-101 acts as a tumor suppressor in cancers by targeting the 3-UTR of multiple genes, including *EZH2*[54], *UBE2N* and *SMARCA4*[57], mitogen-activated protein kinase 1 (*MAPK1*) and *Fos*[59], Kruppel-like factor 6 (*KLF6*)[60], DNA (cytosine-5-)-methyltransferase 3 alpha (*DNMT3A*)[61], and cyclooxygenase-2 (*COX-2*)[62].

Several recent publications have demonstrated that miR-101 can suppress EMT in cancers, including colon cancer[63]. Our investigations of the miRNA network that regulates the EMT of ovarian carcinoma have identified miR-101 as a key regulator[28]. Recent studies have shown that miR-101 regulates EMT through its effects on EZH2[64,65] and the Wnt signaling pathway[63]. Our group has provided evidence that miR-101 suppresses EMT in ovarian cancer by directly targeting the E-cadherin suppressor genes zinc finger E-box binding homeobox 1 (*ZEB1*) and *ZEB2* via specific binding sites on their 3-UTRs[52]. Therefore, the literature consistently supports that miR-101 is a tumor suppressing miRNA and that one of the key cellular processes miR-101 regulates is EMT (**Figure 3**).

miR-200a and miR-141

miR-200 is a family of tumor suppressor miRNAs that consists of 5 members (miR-200a, miR-200b, miR-200c, miR-141, and miR-429), which are significantly involved in the inhibition of EMT. The miR-200 family is often down-regulated in human cancer cells and tumors as a result of aberrant epigenetic gene silencing[66,67]. Recent studies reported that the miR-200 family plays a critical role in suppressing EMT as well as cancer invasion and metastasis by targeting transcriptional repressors of ZEB1 and ZEB2[68]. Furthermore, ZEB1 and ZEB2 repress the expression of miR-200a and miR-141[69] by binding to a conserved pair of ZEB-type E-box elements proximal to the transcription start site in the promoter region[70]. Therefore, ZEB1 and ZEB2 and miR-200 family members repress the expression of one another in a reciprocal feedback loop, which may lead to an amplification of EMT. Targeting this loop may be a novel therapeutic strategy for cancer.

Extensive research has been performed to characterize the regulation of the miR-200 family. Both P300 and PCAF act as cofactors for ZEB1, forming a P300/PCAF/ZEB1 complex on the miR200c/141 promoter. This results in lysine acetylation of ZEB1 and abrogates ZEB1's suppression of miR-200c/141 transcription[71]. p53 has been reported to transactivate miR-200 family members by directly binding to the promoters of miR-200c and repress the expression of ZEB1 and ZEB2, leading to an inhibition of EMT[72,73]. Moreover, NPV-LDE-225 (Erismodegib) suppressed EMT by increasing the expression of miR-200a, miR-200b, and miR-200c[74]. By contrast, the overexpression of signal transducer and activator of transcription 3 (Stat3)[75], platelet-derived growth factor D (PDGF-D)[76], Notch1[77], and doublecortin-like kinase 1 (DCLK1)[78] in cancer cells led to a significant down-regulation of miR-200 family members, resulting in the up-regulation of ZEB1, ZEB2, and SNAI2 expression and acquisition of the EMT phenotype. Several miRNAs,

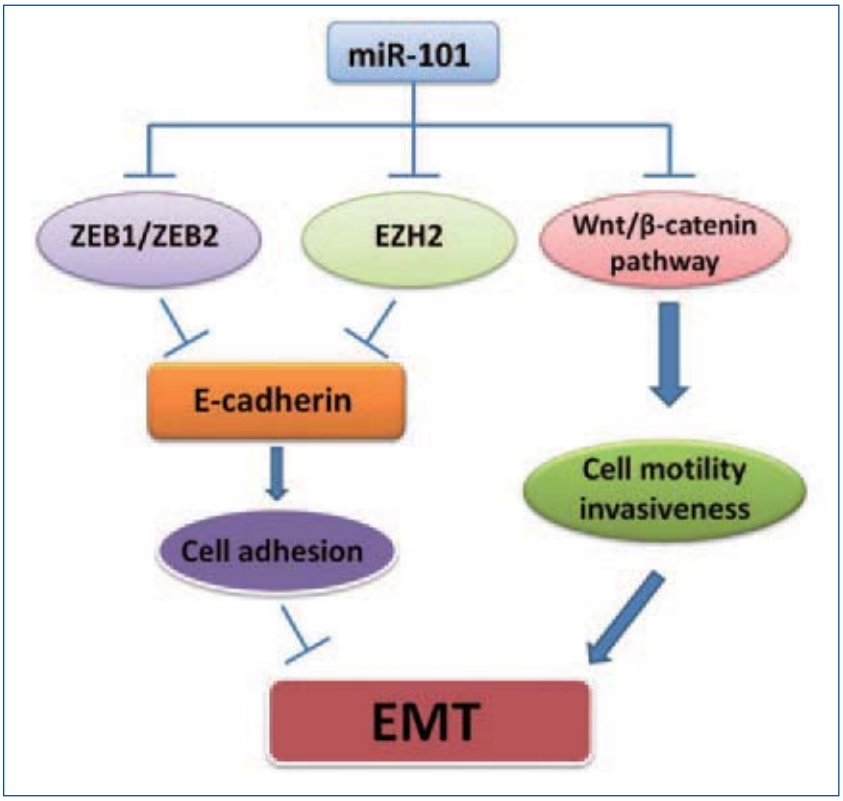

Figure 3. **The miR-101 network regulates EMT. miR-101 directly targets ZEB1/ZEB2[52], EZH2[64,65], and Wnt/β-catenin[63]. miR-101 down-regulates ZEB1/ZEB2 and EZH2, which increases E-cadherin expression and subsequently promotes EMT. miR-101 down-regulates the Wnt/β-catenin pathway, which promotes cell motility and invasiveness. Thus, miR-101 suppresses EMT through targeting these signal pathways. EMT, epithelial-to-mesenchymal transition; ZEB1, zinc finger E-box binding homeobox 1; ZEB2, zinc finger E-box binding homeobox 2; EZH2, enhancer of zeste homolog 2.**

such as miR-103 and miR-107, can induce EMT by down-regulating miR-200 via Dicer[79]. Moreover, miR-130b silencing can restore dicer 1 to a threshold level that allows miR-200 family members to repress EMT in endometrial cancer[80]. Together, these findings suggest that targeting these molecules may suppress EMT by increasing expression of the miR-200 family.

miR-25

miR-25 is a member of the miR-106b-25 cluster, which is a part of the miR-92a family[81]. Recent studies found that miR-25 is located on the 13th intron of the minichromosome maintenance protein 7 (*MCM7*) gene of human chromosome 7q22.1[82]. The expression of miR-25 can be regulated at multiple levels. Liu *et al*.[83] reported that a single nucleotide polymorphism (SNP), rs999885, in the promoter region of the miR-106b-25 cluster influences the expression of miR-25. Kunej *et al*.[84] showed that the expression of miR-25 was regulated epigenetically in gastric cancer. Up-regulation of C-MYC induced the expression of a variety of miRNAs, including the miR-17-92 cluster, miR-106a-363 cluster, and miR-106b-25 cluster[85-87]. The transcription factor homeoprotein Sine oculis homeobox homolog 1 (Six1), a regulator of EMT, was shown to up-regulate the expression of the miR-106b-25 cluster[88]. miR-25 has been reported to regulate EMT. It is known that TGF-β has suppressive effects on normal epithelial cells and during the early stages of carcinogenesis. As cancer progresses, tumor cells become resistant to TGF-β–mediated growth inhibition, and TGF-β promotes tumor invasion and metastasis, partly via its promotion of EMT. It was reported that miR-25 targets the cell cycle inhibitor p21 and the pro-apoptotic factor Bim (also known as BCL2-like 11) in the TGF-β signaling pathway, thus inhibiting the TGF-β–mediated growth suppression of tumor cells[89,90]. Furthermore, it was shown that the miR-106b-25 cluster could also target the inhibitory Smad7 directly, resulting in increased levels of the TGF-β type I receptor and downstream activation of TGF-β signaling[88,91]. miR-25 was also reported to directly target the *CDH1* gene, which is closely associated with the lymphatic metastasis and invasion of esophageal squamous cell carcinoma (ESCC)[92,93]. Fang *et al*.[94] demonstrated that miR-25 could target desmocollin 2 (DSC2), a member of the desmocollin subfamily of the cadherin superfamily, which is involved in cell-cell adhesion and plays a critical role in maintaining normal tissue architecture in the epithelium. Down-regulated DSC2 promoted the aggressiveness of ESCC cells by redistributing the adherens junctions and inducing the transposition of β-catenin from the cytoplasm to the nucleus, thus further activating the β-catenin/T-cell factor (TCF) transactivation axis[94].

In our network analysis of integrated mesenchymal serous ovarian cancer, miR-25 had the largest number of connected protein-coding genes[28]. However, the current data on whether miR-25 acts as an oncogene or a tumor suppressor gene are inconsistent. miR-25 is more highly expressed in a variety of tumor tissues, including gastric cancer, prostate cancer, esophageal cancer, and colorectal cancer (CRC) tissues, than in normal tissue controls[91,94-96]. However, Li *et al*.[97] reported that miR-25 functions as a potential tumor suppressor by targeting SMAD family member 7 (Smad7) in colon cancer. They showed that the introduction of miR-25 inhibited the proliferation and migration of colon cancer cells. Furthermore, miR-25 suppressed the growth of colon cancer xenografts *in vivo*[97]. miR-25 was also suggested to act as a tumor suppressor in anaplastic thyroid carcinoma by targeting the polycomb protein EZH2[98].

Using an integrated analysis of TCGA cases, we found that miR-25 expression was decreased in our integrated mesenchymal subtype of high-grade serous ovarian cancer[28], suggesting that miR-25 is inversely associated with EMT. By contrast, miR-25 has been considered an oncogene in ovarian cancer. miR-25 was highly expressed in both clinical ovarian cancer samples and cell lines[99,100], and the miR-25 expression level was significantly positively associated with tumor stage, regional lymph node status, and poor survival in epithelial ovarian cancer[100]. Zhang *et al*.[99] demonstrated that miR-25 directly regulated apoptosis by targeting Bim in ovarian cancer. In ovarian cancer cells, the down-regulation of miR-25 induced apoptosis, whereas the overexpression of miR-25 enhanced cell proliferation. Feng *et al*.[101] reported that miR-25 promoted ovarian cancer proliferation and motility by targeting LATS2. However, the above results were from a single bioinformatics analysis[28], *in vitro* studies without *in vivo* validation[99,101], or a small-scale clinical analysis (86 cases)[100]. Therefore, further studies should be performed, such as measuring the expression of miR-25 in serous and other subtypes of ovarian cancer cases in a large-scale, multiple-center study and demonstrating the function of miR-25 in EMT, MET, and metastasis both *in vitro* and *in vivo*.

miR-29c

The miR-29 family consists of miR-29a, miR-29b, and miR-29c; miR-29b includes 2 members, miR-29b-1 and miR-29b-2[102]. Dysregulation of the miR-29 family is reported in many aspects of tumorigenesis and cancer progression, including cell proliferation, cell cycle, cell differentiation, apoptosis, and metastasis[102]. However, the mechanism responsible for the deregulation of miR-29 family members in tumors remains unclear. Zhang *et al*.[103] reported that miR-29 members were suppressed by c-Myc in B-cell lymphoma. Although not explicitly stated, the miR-29 family is involved in the regulation of EMT. miR-29 expression is induced by the TGF-β–Smad signaling pathway[104,105], which is a key signaling pathway for EMT. DNA damage–induced TP53 was shown to promote the expression of miR-29[106]. Furthermore, TP53-induced miR-200 expression provides critical evidence for the role of TP53 in EMT regulation. Further studies are needed to determine whether TP53-induced miR-29 also contributes to TP53-regulated EMT.

The reported functions of miR-29 family members consistently support their roles as tumor suppressing miRNAs in many systems, including melanoma, peripheral nerve sheath tumors, glioma, and nasopharyngeal, colorectal, gastric, hepatocellular, breast and lung cancers. Zhang *et al*.[107] reported that miR-29c dramatically suppressed CRC cell migration, invasion, and metastasis *in vivo*. These authors further demonstrated that miR-29c mediates EMT in CRC by directly targeting guanine nucleotide-binding protein alpha 13 (GNA13) and protein tyrosine phosphatase type IV A (PTP4A).

These 2 proteins are known to be involved in the ERK/GSK3β/β-catenin and Akt/GSK3β/β-catenin signaling pathways, respectively. Han *et al*.[108] showed that ectopic treatment with miR-29c mimics in gastric cancer cell lines resulted in reduced proliferation, adhesion, invasion, and migration and that high miR-29c expression suppressed xenograft tumor growth in nude mice by directly targeting integrin beta 1 (ITGB1). In hepatocellular carcinoma (HCC), miR-29c directly targeted and suppressed sirtuin 1(SIRT1) expression and blocked HCC cell growth and proliferation, thus suggesting a tumor suppressive role[109]. Consistently, miR-29c recapitulated SIRT1-knockdown effects in HCC cells. In addition, miR-29c expression was down-regulated in a large cohort of HCC patients, and low expression of miR-29c was significantly associated with poor prognosis of HCC. Currently, besides our report of the association between decreased miR-29c expression and the mesenchymal subtype of high-grade serous ovarian cancer[28], there are no other reports on miR-29c dysregulation in ovarian cancer. Further studies are needed to determine whether miR-29c is a strong tumor suppressor in ovarian cancer and the cause of its dysregulation.

miR-182

The miR-183 family is highly conserved and consists of miR-96, miR-182, and miR-183[110]. Several studies have demonstrated that the miR-183 family is abnormally expressed in various tumors and is directly involved in human cancer processes, such as cellular differentiation, proliferation, apoptosis, and metabolism[111-113]. Zhang *et al*.[114] performed a meta-analysis of the expression of the miR-183 family in human cancers and found that miR-182 expression was consistently up-regulated in 15 cancer types, including ovarian cancer, but inconsistently expressed in gastric cancer tissues and adjacent noncancerous tissues. Kong *et al*. [115] revealed that the miR-183 family was significantly up-regulated in gastric cancer tissues. However, Li *et al*.[116] observed that miR-182 was down-regulated in gastric adenocarcinoma tissues and may function as a tumor suppressor via down-regulation of cAMP responsive element-binding protein 1 (CREB1).

miR-182 has been consistently reported to be significantly up-regulated in ovarian cancer tissue[117-120]. Liu *et al*.[118] reported that miR-182 expression was significantly higher in serous tubal intraepithelial carcinoma, which is recognized as a precursor lesion of high-grade serous ovarian cancer, than in matched normal fallopian tube. Furthermore, miR-182 was significantly overexpressed in most high-grade serous ovarian cancer cases. Overexpressing miR-182 in immortalized ovarian surface cells, fallopian tube secretory cells and malignant ovarian cell lines resulted in increased tumor transformation *in vitro*, enhanced tumor invasiveness *in vitro*, and metastasis *in vivo*. miR-182 plays an ontogenic role in ovarian cancer partly via its effects on repairing DNA double-strand breaks, its negative regulation of breast cancer 1 (BRCA1) and metastasis suppressor 1 (MTSS1), and its positive regulation of the oncogene high-mobility group AT-hook 2 (*HMGA2*). Wang *et al*.[120] measured 1,722 miRNAs from 15 normal ovarian tissue samples and 48 ovarian cancer samples using a quantificational real-time polymerase chain reaction (qRT-PCR) assay and identified a 10-miRNA signature that distinguished ovarian cancer tissues from normal tissues. Wang *et al*.[119] demonstrated that miR-182 promotes cell growth, invasion, and chemoresistance by targeting programmed cell death 4 (PDCD4) in human ovarian cancer. Interestingly, inactivation of BRCA1, although less potent than that of BRCA2, has been shown to confer beneficial effects on ovarian cancer survival[121]. Among the 8 miRNAs in our network, the expression of miR-506 and miR-182 is associated with increased survival in the TCGA cohort[28].

In prostate cancer, miR-182 was reported to suppress EMT via its repression of SNAI2[122]. However, miR-182 was shown to increase the invasiveness of breast cancer by targeting reversion-inducing-cysteine-rich protein with kazal motifs (RECK), a matrix metalloproteinase inhibitor[123]. miR-182 also promoted gallbladder cancer metastasis partly by targeting cell adhesion molecule 1 (CADM1)[124]. Furthermore, miR-182 was shown to stimulate angiogenesis and promote non–small cell lung cancer (NSCLC) progression partly by directly targeting fibroblast growth factor receptor substrate 2 (FRS2)[125]. In addition, miR-182 drove metastasis of primary sarcomas by targeting MTSS1 and Ras suppressor protein-1 (Rsu1)[126]. Therefore, miR-182 may play different roles in the development and progression of various cancers depending on their target downstream genes.

miR-128

miR-128 is a brain-enriched miRNA. The expression of miR-128 exhibits tissue-specific and developmental stage-specific patterns. It is mainly expressed in neurons rather than astrocytes[127], and it is abundantly represented in the hippocampal region of brains of fetus, adults, and the patients with Alzheimer's disease[128]. miR-128 consists of 2 distinct genes, miR-128-1 and miR-128-2, which are embedded in the introns of the R3H domain containing 1 (*R3HDM1*) that is located on human chromosome 2q21.3 and in the introns of the cyclic AMP-regulated phosphoprotein, 21 kDa (*ARPP21*) that is located on 3p22.3, respectively[125]. miR-128-1 and miR-128-2 are processed to generate the same mature miRNA with an identical sequence, miR-128. It is also known that the majority of intronic miRNAs transcriptionally depend on the expression of their host gene[129]. However, researchers have found that approximately 26% of the mammalian intronic miRNAs may be transcribed using their own promoters[130]. Monteys *et al*.[131] demonstrated that miR-128-2 has a Pol III promoter in its 5'-flanking region, which may permit an independent expression from its host gene, *ARPP21*. Muinos-Gimeno *et al*.[132] also found that there were 3 SNPs located in the genomic region that corresponds to hsa-miR-128-1-R3HDM1 and that there was a strong geographical genetic variation among different populations from HapMap. Mi *et al*.[133] examined the DNA methylation status of CpG islands located in the miR-128b promoters of 10 acute lymphoblastic leukemia (ALL), 14 acute myeloid leukemia (AML), and 3 normal samples, and they found that the average methylation rate of the ALL group was 2.7%, lower than that of the AML group (17.1%). Their results suggested that the up-regulation of miR-128b in ALL patients may be due to a lower degree of CpG island

methylation in its promoter regions. miR-128 can also be regulated by transcription factors. SNAIL can down-regulate the expression of miR-128 by directly binding to its promoter regions of both E-box 1 and 2[18]. The mutant *TP53* can bind to the putative promoter of the miR128-2 host gene *ARPP21* and increase the expression of both miR-128 and *ARPP21* mRNA[134,135].

Aberrant expression of miR-128 has been observed in many malignant tumors. Under-expression of miR-128 was observed in glioma compared with tumor-adjacent brain tissue, particularly in the more aggressive subtypes, glioblastoma multiforme (GBM) and medulloblastoma, based on miRNA array, Northern blot, and qRT-PCR analyses[1,19,27]. However, the levels of miR-128 expression in other solid tumor tissues were highly variable. Using a large-scale miRnome analysis, Volinia *et al*.[136] measured 540 different malignant tumor samples and found that the expression of miR-128b was significantly up-regulated in tumor tissues of the colon, lung, and pancreas. By contrast, Katada *et al*.[137] measured the expression levels of miR-128 in 42 undifferentiated gastric cancer tissues and paired controls. Their findings showed that miR-128a was up-regulated, whereas miR-128b was down-regulated, in undifferentiated gastric cancer tissues. Khan *et al*.[138] measured 21 independent prostate specimens and found a significant reduction in the levels of miR-128 in a progressive fashion from benign prostatic hyperplasia to prostate cancer and then to metastatic prostate cancer. The level of miR-128 was also lower in more invasive ovarian cancer cells than in less invasive cancer cells[139].

Examining the role of miR-128 in EMT and tumor cell invasion and motility, Qian *et al*.[140] demonstrated that overexpression of miR-128 suppressed the morphologic transformation associated with EMT, retarded wound closing, and reduced cell migration and invasion in MDA-MB-231 cells. Evangelisti *et al*.[141] found that ectopic overexpression of miR-128 down-regulated glioblastoma cell invasion by directly targeting Reelin and doublecortin (*DCX*). Woo *et al*.[139] reported that overexpression of miR-128 in ovarian cancer cells resulted in reduced cell motility and adhesion by directly targeting colony-stimulating factor-1 (CSF-1). Because reducing cell motility and adhesion adversely affects cell migration, the function of miR-128 in ovarian cancer metastasis via its effects on CSF-1 needs to be studied *in vivo*. In addition, the regulation of miR-128 on multiple targets related to EMT should be further studied.

Conclusions and Future Directions

Most studies have shown that as post-transcriptional regulators, miRNAs play important roles in EMT and are important markers and tools in cancer diagnosis, prognosis, and therapeutics. Using an integrated analysis, we identified a core regulatory network, including 8 key node miRNAs and 214 protein-coding genes, related to an integrated mesenchymal subtype of serous ovarian cancer[28], suggesting that these 8 miRNAs can regulate EMT and MET in ovarian cancer. However, in various tumors, including ovarian cancer, the functions of some of the 8 miRNAs in EMT and MET are contradictory, possibly because miRNAs play different roles by targeting different targets in specific conditions. Therefore, further studies are needed on these miRNAs and their targets.

In addition, the function of a single miRNA in EMT and MET may be limited, thus the combination of several miRNAs may generate an entirely different cellular phenotype and therapeutic outcome. Shahab *et al*.[142] monitored the consequent changes in the global patterns of gene expression using microarray and qRT-PCR after transfecting 2 miRNAs, miR-7 and miR-128, and found that the changes in gene expression induced by the individual miRNAs was functionally coordinated but distinct. miR-7 transfection into ovarian cancer cells induces changes in cell adhesion and other developmental networks previously associated with EMT and other processes linked with metastasis. By contrast, miR-128 transfection induces changes in cell cycle control and other processes commonly linked with cellular replication. Therefore, the function of an individual miRNA in EMT and MET may be influenced by other miRNAs. The effects of combining several miRNAs should be investigated in the future. Preclinical mouse model studies have already provided evidence that miRNAs, such as miR-506, can exhibit strong tumor suppressive effects[28,29]. With the development and perfection of miRNA delivery techniques such as nanoparticles and mesenchymal stem cells, miRNAs are quickly becoming a promising therapeutic tool for cancer treatment.

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