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REVIEW

MicroRNA-Mediated Post-Transcriptional Regulation of Epithelial to Mesenchymal Transition in Cancer

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Abstract Epithelial to mesenchymal transition (EMT) program participates in tissue repair, embryogenesis and numerous pathological conditions, particularly cancer progression and tumor metastasis. A highly complex and strongly controlled post-transcriptionally regulated network of microRNAs (miRNAs) regulates the EMT process. miRNAs are critical parts of the post-transcriptional regulation of gene expression. A set of miRNAs target multiple components of major signaling pathways and downstream effectors of EMT. miRNAs associated with this process are involved in controlling tumor progression and invasiveness either as oncogenes or as tumor suppressors. Since several miRNAs directly affect EMT-related master regulators, they have been discovered to have the potential to be used as biomarkers or targets in EMT-based pathological conditions such as cancer. Therefore, comprehensive understanding of miRNA-EMT correlation with tumor metastatic spread may provide improvements to diagnostic tools or therapeutics for cancer. This review summarizes our current knowledge about some of these important miRNAs and focuses on their specific roles in regulation of the EMT process in cancer.

Keywords Cancer · Epithelial to mesenchymal transition · Post-transcriptional regulation · microRNAs

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Abbreviations

EMT	Epithelial to mesenchymal transition
MET	Mesenchymal to epithelial transition
EMT-TFs	EMT transcription factors
TGF-β	Transforming growth factor beta
miRNAs	microRNAs
oncomiRs	Oncogenic miRNAs
TSmiRs	Tumor suppressor miRNAs
EGF	Epidermal Growth Factor
FGF	Fibroblast growth factor
HGF	Hepatocyte Growth Factor
PDGF	Platelet Derived Growth Factor
VEGF	Vascular endothelial growth factor
ZEB	Zinc finger E-box binding homeobox
LIFR	Leukemia inhibitory factor receptor
YAP	Yes-associated protein
ATC	Anaplastic thyroid carcinoma
hECSs	Human embryonic stem cells
HNSCC	Head and neck squamous cell carcinoma
HCC	Hepatocellular carcinoma
NSCLC	Non-small cell lung cancer
CRC	Colorectal cancer
PCa	Prostate cancer
CEA	Carcinoembryonic antigen
Let-7	Lethal-7
RKIP	Raf kinase inhibitory protein
3′-UTR	3′-untranslated region

Epithelial to Mesenchymal Transition in Cancer

Tumor invasion and metastasis are critical steps that display the aggressive phenotypes of human cancers. Epithelial to mesenchymal transition (EMT) is an important event in the metastatic cascade and functions during reversible

developmental processes. During the tumor metastasis process, malignant epithelial cells detach from the primary tumor and acquire mesenchymal-like properties and migrate to distant organs to form metastatic lesions. Once mesenchymal cells arrive at their destination, they undergo a reverse program of mesenchymal to epithelial transition (MET) [1–4]. EMT is often associated with primary cancer cell dissemination, which leads to metastasis, whereas MET is correlated with the establishment of secondary tumors following metastasis [5]. During the EMT process, a network of transcription factors and signaling pathways involved in the development and progression of tumor becomes activated, while the same complex signals are suppressed in the initiation and progression phase of MET [6, 7].

Phenotypic changes associated with EMT are characterized by the loss of the cell-cell contacts, cell polarity, and E-cadherin, which co-occur with an increase in mesenchymal markers such as vimentin and fibronectin [8]. EMT induces the activation of transcription factors, microRNAs (miRNAs) and different signaling pathways based on specific physiological or pathological contexts [7]. Several important signaling pathways contribute to the initiation of EMT, including transforming growth factor beta (TGF-β), Notch and Wnt, Hepatocyte Growth Factor (HGF), Platelet Derived Growth Factor (PDGF), Epidermal Growth Factor (EGF), and fibroblast growth factor (FGF) [9, 10]. Expression of EMT transcription factors (EMT-TFs) triggers EMT and can be activated by these signaling pathways at the transcriptional and post-transcriptional level. EMT-TFs include three families: the zinc finger SNAIL (Snail1 and Snail2/Slug), ZEB (Zeb1and Zeb2/SIP1) and basic helix-loop-helix transcription factors (Twist1, Twist2 and E12/E47) [1, 2]. These transcription factors repress CDH1 (encoding E-cadherin) expression and induce the expression of mesenchymal markers [3, 7]. Furthermore, several miRNAs have been identified to regulate cancer progression by affecting the EMT process. Therefore, a better understanding of the molecular intricacies of EMT-miRNA correlation in cancer will lead to the designation of novel effective therapeutics against cancer.

Core EMT-Transcription Factors

EMT is regulated by various signaling pathways culminating in core transcription factors of the process, such as Snail, Slug, Zeb1/2, and Twist [11]. The SNAIL family of zinc-finger transcription factors consisting of three members, Snail 1, 2, and 3 (in vertebrates also termed Snail, Slug, and Smuc, respectively) [12]. The first member of the SNAIL family, Snail, was first described in *Drosophila melanogaster* as a transcription factor essential for the formation of mesoderm [13]. Regarding that loss of E-cadherin appears to be critical to the process of EMT and Snail strongly represses E-cadherin expression, it seems that Snail may serve as a prominent inducer of EMT during development and carcinogenesis [14]. Moreover, the expression of Snail confers tumor cells with cancer stem-like traits and positively correlates with tumor grade, nodal metastasis, drug resistance, tumor recurrence, and poor prognosis in different types of tumors [3, 15–18]. It has been also demonstrated that the transcriptional repressor proteins Snail and Slug (another member of the SNAIL family) promote EMT through activating transforming growth factor-β (TGF-β) signal transduction [19]. In a model of TGF-β-induced prostatic EMT, Slug functioned as a "gatekeeper" of EMT. In this model, depletion of *Slug* inhibited EMT through repressing expression of a variety of other EMT regulators such as Zeb2 and Snail1 [20].

The two members of the zinc finger E-box binding homeobox (ZEB) family, Zeb1 and Zeb2, are among the first described EMT-transcription factors and trigger EMT through a combination of repression of epithelial and activation of mesenchymal markers [21, 22]. Twist, a basic helix-loop helix transcription factor, is a master regulator of morphogenesis participating in EMT during mesoderm differentiation in Drosophila and during neural crest migration in vertebrates. Twist-1 is up-regulated in various epithelial tumors such as breast [23, 24], prostate [25] and gastric [26] carcinomas. It has been demonstrated that Twist-1 causes the transcriptional repression of E-cadherin through the E-box elements present in its promoter region [27]. Overall, as loss of E-cadherin-mediated cell to cell adhesion and acquisition of the mesenchymal phenotype are frequent events during EMT, it seems that core EMT-transcription factors might modulate several crucial processes during cancer development leading to both tumor cell survival and invasion capability.

MicroRNAs: an Overview in Cancer

microRNAs (miRNAs or miRs) are a class of non-coding small RNAs of approximately 20–22 nucleotides, which regulate gene expression at the post-transcriptional level. They bind to complementary sequences mainly located at the 3′-untranslated region (3′-UTR) of their target mRNAs for degradation or the induction of translational repression [28–32]. In recent years, increasing number of studies have indicated that deregulated miRNAs are involved in primary tumor formation and cancer metastasis [33]. Interestingly, about half of human miRNA genes are frequently located at fragile sites or cancer-related genomic regions involved in cancer-related chromosomal abnormalities [34].

miRNAs regulate different steps of carcinogenesis, including primary cell transformation, tumor cell proliferation, marginal invasion, EMT, and metastasis [31, 35]. The association between aberrant expression of miRNAs and development of various human tumors suggests that different miRNAs directly modulate oncogenesis [35–38]. miRNAs control gene

expression in key biologic pathways related to cancer [31, 35]. Depending on their target transcript, miRNAs function either as tumor suppressor or as oncogene, and can down- or up-regulate target gene expression during carcinogenesis. Oncogenic miRNAs (oncomiRs) frequently inhibit tumor suppressor genes and are usually up-regulated during cancer initiation. On the other hand, tumor suppressor miRNAs are down-regulated in cancer. Expression of tumor suppressor miRNAs (TSmiRs) is decreased in malignant cells, resulting in the over-expression of their target oncogenes that affect cell differentiation or apoptosis [28, 36, 39]. Moreover, oncomiRs repress epithelial characteristics and TSmiRs inhibit mesenchymal progression [7]. Genetic mutations, epigenetic alterations and defects in miRNA biogenesis machinery also play critical roles in deregulating the miRNA expression [34, 40, 41]. Furthermore, loss of expression of tumor suppressor miRNAs and over-expression of oncogenic miRNAs depends on the cellular context of their target genes [41]. Because of the importance of miRNAs in tumor biology, there is a wide range of possible applications for miRNA research including diagnosis, prognosis and therapeutic applications [42]. In the following sections, we describe important miRNAs which have been discovered through tremendous investigations to identify their relationship to EMT-induced metastasis (Table 1).

MicroRNAs in EMT-Induced Metastasis and their Targets

In recent years, emerging evidence has demonstrated the implication of different miRNA signatures in EMT through targeting EMT-TFs and EMT-related signaling networks in human cancer (Table 1). Our appreciation of EMT has been improved by identifying the effects of miRNAs on signaling pathways and downstream events controlling EMT at the molecular levels [43–45]. Schematic representation of core EMT-TFs, significant miRNAs targeting transcription factors and miRNAs enhanced or repressed by transcription factor activity is depicted in Fig. 1.

Some mechanisms target EMT-TFs that directly regulate the EMT process, while others affect genes or signaling pathways involved indirectly in EMT. Indirect mechanisms target EMT-TF modulators, proteins implicated in migration and invasion, and regulatory enzymes involved in miRNA biosynthesis. As discussed previously, a hallmark of EMT is the loss of E-cadherin expression, which is responsible for the epithelial integrity, and some miRNAs directly mediate *CDH1* gene deactivation [43]. For instance, miR-9 down-regulates E-cadherin gene expression by directly targeting the *CDH1* transcript in breast cancer [46]. Other miRNAs such as miR-200 and miR-34 directly regulate EMT-TFs and are implicated in double negative feedback loops that help maintaining the epithelial or mesenchymal state [43, 47, 48].

EMT-inducing signaling pathways such as TGF-β and p53 control EMT-regulatory miRNA networks [43]. TGF-β activates transcriptional repressors of E-cadherin such as Zeb, Snail and Twist. In addition, TGF-β can function downstream or upstream of the EMT master regulators [30]. TGF-β signaling modulates the activity of several miRNA networks, which are implicated in TGFβ-induced EMT [26, 31, 32]. miR-1 and miR-200b target Slug and form a double negative feedback loop, where TGFβ-induced EMT promotes Slug expression, which in turn represses the expression of both miR-1 and miR-200b [49]. Furthermore, there are other regulatory loops between miR-200 family and miR-205 with Zeb1/Zeb2 as well as between miR-203 and Snail in EMT progression [50, 51].

Table 1 MicroRNAs associated with metastatic progression and their EMT-related targets

microRNA(s)	Upstream regulator(s)	Downstream target(s)	Cancer type(s)	Reference(s)
miR-1	Snail2 (Slug)	Snail2	Prostate cancer	[49]
miR-9	MYC	CDH1	Colon cancer, Breast cancer	[46, 127]
miR-29b		Snail	Prostate cancer	[63]
miR-30	NA*	Snail1	NSCLC, Breast cancer	[68]
miR-34	Snail1, p53	Snail1	Breast cancer	[51, 78]
miR-103/107	NA	DICER	Breast cancer	[79]
miR-141	NA	Zeb2 (SIP1)	Colorectal, breast, lung	[128]
miR-200 s	ZEB1/2, SNAI1/2, p53	Zeb1/2, SNAI2	Pancreatic cancer, Breast cancer	[91, 92, 94, 97]
miR-10b	Twist	HOXD10, CADM1, RHOB, KLF4, Tiam1	Breast cancer, liver cancer, Glioma, Esophageal Squamous Cell Carcinoma (ESCC)	[80, 129–131]
miR-192	p53	Zeb2	Hepatocellular carcinomas	[90]
miR-203	Zeb1, Snail1/2	Snail1/2	Breast cancer, Prostate cancer	[106, 107]
miR-205	TGF-β	Zeb1/2	ESCC, Breast cancer, Prostate cancer	[92, 105]
Let-7	Lin28,MYC	RAS, HMGA2	Breast cancer, Colorectal cancer	[113, 115]

*NA: Not Applicable

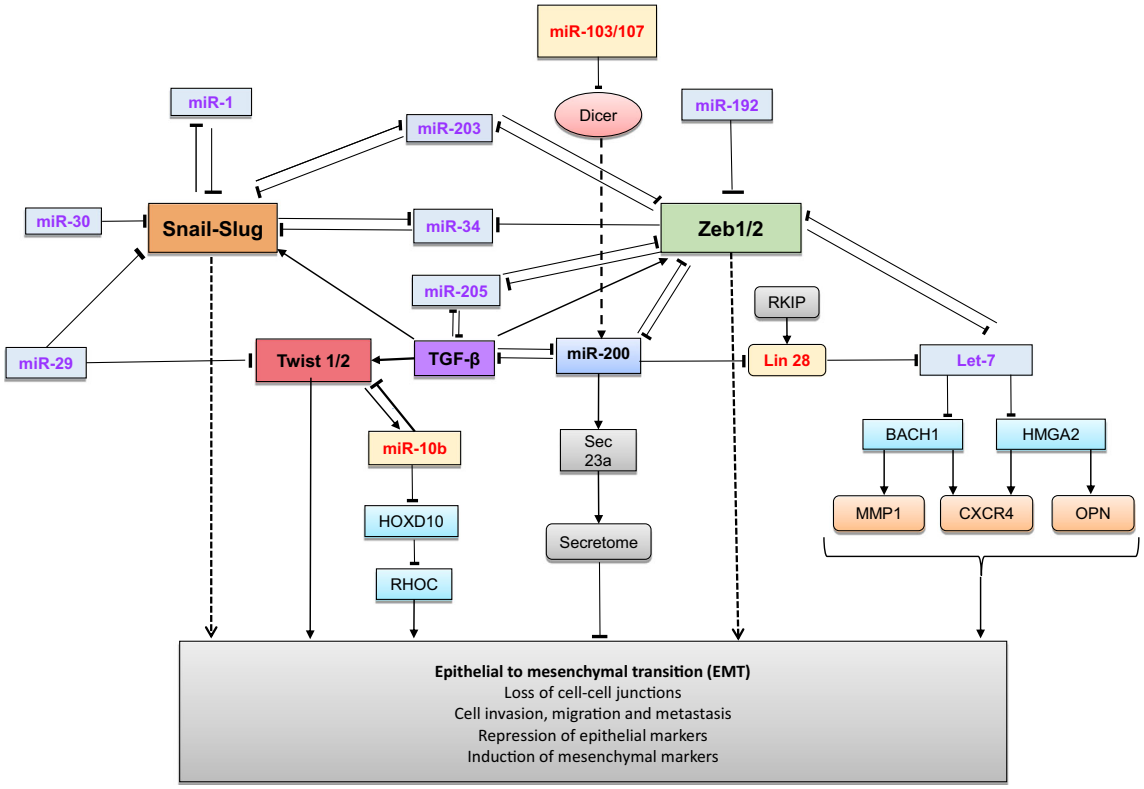

Fig. 1 MicroRNAs and EMT-transcription factor regulatory networks. Several miRNAs regulate the expression of EMT-TFs (including Snail, Zeb1/2, and Twist) and control epithelial-mesenchymal plasticity. Oncogenic miRNAs (such as miR-9, miR-103/107 and miR-10b) promote EMT through regulating core-EMT-TFs and consequently leads to the loss of cell to cell junction, repression of epithelial markers, and induction of mesenchymal markers which is associated with the migratory, invasive and metastatic behavior of the cells. In contrast, tumor suppressor miRNAs (such as miR-29b, miR-34, miR-192, miR-200, miR-203, miR-205, and let-7) suppress EMT by interacting with certain transcription factors. Some of miRNAs operate in negative feedback loops through regulating core-EMT-TFs and contribute to maintain the epithelial or mesenchymal states. Oncogenic miRNAs promoting EMT is shown by red and tumor suppressor miRNAs inhibiting EMT is shown by blue. miR-200 which represents a dual oncogenic or tumor suppressive role is depicted by black

Here, we review the influence of several important miRNAs and their involvement in EMT and metastasis.

miR-1

Down-regulation of miR-1 is widely detected in a number of cancers, including prostate [52], hepatocellular [53], head and neck squamous cell carcinoma (HNSCC) [54], and colorectal cancer [55]. miR-1 functions as a tumor suppressor and inhibits EMT by negatively regulating Slug as one of its targets; however, decreased expression of miR-1 is inversely associated with the progression of prostate adenocarcinoma [49]. miR-1 controls EMT through a Slug-dependent mechanism and its over-expression leads to deactivation of Slug and consequently the loss of mesenchymal markers. Additionally, miR-1 affects prostatic tumorigenesis via Slug-independent targets. In this mechanism, miR-1 and Slug act in a regulatory loop involving the direct targeting of Slug by miR-1 and direct transcriptional suppression of miR-1 by Slug. An imbalance in this negative feedback loop is implicated in prostatic tumorigenesis and tumor progression [49].

miR-9

Pro-metastatic miR-9 was the only known miRNA that was described to directly target E-cadherin gene [56]. miR-9 expression is significantly up-regulated in tumors of patients with metastatic breast cancer compared to its expression in those with no metastasis. Suppression of *CDH1* expression by miR-9 has a critical role in inducing malignant transformation and enhanced cell motility and invasion. Besides, miR-9 inhibits E-cadherin by up-regulating vascular endothelial growth factor (VEGF), which promotes tumor angiogenesis. miR-9 levels also correlate with v-myc avian myelocytomatosis viral oncogene neuroblastoma derived homolog (MYCN) amplification, tumor grade and metastasis status. Ma et al. [46] showed that at the earlier stages of tumor progression, MYC and MYCN oncoproteins activated the expression of miR-9 by binding to the miR-9-3 locus. Elevated expression of miR-9 induced EMT by down-regulating E-cadherin gene expression in some human tumors. Leukemia inhibitory factor receptor (LIFR) is a new metastasis suppressor and is down-regulated in invasive breast carcinoma with lymph node metastasis. LIFR plays a role in localization of

Scribble to the cell membrane, which finally leads to the phosphorylation and inactivation of the transcriptional co-activator Yes-associated protein (YAP). miR-9 can directly target and suppress LIFR in non-metastatic breast cancer cells, and this suppression potentiates tumor cell migration, invasion and metastasis [57].

miR-29

Deregulation of the miR-29 family was reported in different cancers, including liver, lung, leukemia, hepatocellular carcinoma, and myeloma [58–61]. miR-29b is an important downstream target of GATA3 and is known as an anti-metastatic miRNA in different malignancies. GATA3 is a transcription factor that specifies and maintains luminal epithelial cell differentiation in the mammary gland by inducing miR-29b activation. Increased expression level of miR-29b by GATA3 is a significant event in metastasis. Furthermore, to form a positive feedback loop, miR-29b enhances the expression of GATA3 and loss of miR-29b leads to decreased GATA3 expression, suggesting that GATA3 and miR-29b cooperate to make cell fate decisions. The GATA3-miR-29b axis alters tumor microenvironment, suppresses metastasis and promotes differentiation. Furthermore, GATA3 expression is lost in breast cancer that causes miR-29b accumulation in luminal breast cancers, and loss of miR-29b increases metastasis and induces a mesenchymal phenotype [62].

Loss of miR-29b expression can induce metastasis in prostate cancer. In prostate cancer, miR-29b expression was lower in cancer cells compared to immortalized prostate epithelial cells. Functional miR-29b inhibited several steps in metastasis cascade including invasion, motility, cellular survival, and proliferation by suppressing the expression of multiple pro-metastatic proteins. In a study, which analyzed the effect of miR-29b on modulating the expression of epithelial and mesenchymal markers, E-cadherin expression was enriched in miR-29b transfected prostate cancer cells as compared with the cells expressing control miRNA. On the other hand, miR-29b over-expression reduced the expression level of mesenchymal markers, such as N-cadherin, Twist, and Snail in metastatic prostate cancer cells, which resulted in a less invasive phenotype. Therefore, miR-29 could be suggested as a new metastasis suppressor with therapeutic potentials in metastatic prostate cancer [63].

Increased level of miR-29a has been detected in the serum of colorectal and breast cancer patients, suggesting that miR-29a can serve as a non-invasive biomarker for early detection of advanced colorectal and breast cancer [64, 65]. Moreover, miR-29 over-expression induced EMT and metastasis by down-regulating tristetraprolin (TTP), a regulatory protein involved in degradation of mRNAs. An elevated level of miR-29a and reduced TTP was also observed in breast cancer patient samples. Accordingly, this miRNA can act either as an oncogene or a tumor suppressor, depending on the oncogenic context, which should be considered for future therapeutic applications [66].

miR-30

miR-30a, miR-30d and miR-30e are highly down-regulated in metastatic cancers, suggesting a possible role for miR-30 family in cancer and metastasis [67]. miR-30 has been considered as a tumor suppressor miRNA. This miRNA regulates EMT in aggressive tumor phenotypes and targets a number of EMT-associated genes. miR-30 directly targets the expression of EMT transcriptional regulator Snail, induces E-cadherin gene expression, which ultimately leads to the suppression of EMT in non-small cell lung cancer (NSCLC). miR-30a expression was significantly down-regulated in surgically resected tissues compared with normal tissue in about 80 % of NSCLC patients [68]. Although miR-30 was found to be down-regulated in tumors of lung, colon, liver, prostate and breast [69–74], one study showed that miR-30d promoted tumor invasion in hepatocellular carcinoma [75]. Furthermore, expression of miR-30 in mesenchymal anaplastic thyroid carcinoma (ATC)-derived cells promoted MET by modulating the expression of mesenchymal protein markers such as vimentin [76].

miR-34

miR-34a and Snail function in a mutual negative feedback loop to regulate EMT. Suppression of miR-34a leads to the up-regulation of Snail, resulting in cells displaying EMT phenotype, enhanced migration and invasion. On the other hand, the ectopic expression of miR-34a down-regulated Snail and induced MET. Furthermore, Snail could bind to E-boxes in the miR-34a/b/c promoters and repressed the expression of miR-34. Inhibition of miR-34 expression by transcription factors Zeb1/2 has also been observed which in turn leads to EMT. miR-34 as a tumor suppressor, also down-regulates the expression of Slug, Zeb1 and stem cell markers such as CD44 [51]. It was revealed that the expression level of miR-34a is correlated with the p53 status in prostate cancer (PCa) cells. p53, which directly activates miR-34a, also negatively regulates CD44. Down-regulation of miR-34a in $CD44^+$ PCa stem cells may contribute to PCa development and metastasis by regulating CD44 expression and the migratory, invasive and metastatic behavior of the cells [77]. A link between p53, miR-34 and Snail1 was confirmed in the regulation of EMT in cancer. miR-34 family members target Snail1, leading to the inhibition of cell migration and EMT associated invasion. Abnormal functioning of the p53–miR-34 axis causes tumor

progression. The Snail1-dependent EMT is regulated by p53/miR-34 axis and loss of p53 function induces Snail1-mediated EMT in colon, breast and lung cancers. In this process, Snail1 activity is suppressed as a result of decreased miR-34 levels. Moreover, loss of wild-type p53 function or silencing of the endogenous miR-34 leads to cancer cell invasion [78].

miR-103/107

Association between the pathway of miR-103/107-Dicer-miR-200 in EMT with metastasis and poor outcome in breast cancer was studied by Martello et al. miR-103/107 is a known family of pro-metastatic miRNAs and high levels of miR-103/107 in metastatic cells reduces Dicer expression and loss of miRNA regulation in cancer cells. Inhibition of miR-200 by the miR-103/107-Dicer axis induces acquisition of mesenchymal properties and cell migration in breast cancer. At the molecular level, miR-103/107 promotes EMT by down-regulating miR-200 levels and repressing Dicer, which is a molecular target of miR-103/107. The decrease in Dicer level causes a general down-regulation in the miRNA biosynthesis. miR-107 also has the ability to down-regulate the miR-200 family levels by altering Zeb1/Zeb2 [79].

miR-10b

miR-10b is highly expressed in metastatic breast cancer cells compared with non-metastatic or normal epithelial cells and its level is correlated with the potential of these cells in invasiveness. Over-expression of miR-10b in non-metastatic breast tumor cell lines induces invasion and metastasis. As a key EMT inducing transcription factor, Twist promotes transcription of miR-10b primary miRNA by binding directly to the putative promoter of the *MIRN10B* gene, located in the HOXD gene cluster. miR-10b suppresses the expression of HOXD10, leading to increased expression of the pro-metastatic *RHOC* gene, followed by enhanced cell migration and invasion in breast cancer cells. Thus, loss of miR-10b could inhibit Twist-mediated cell migration and invasion [80].

miR-192

miR-192 could be regulated by p53 in various cell types [81–84] and studies have revealed that aberrant p53 expression plays an important role in inducing EMT [85–89]. The miR-192 family members were inactivated by p53 inhibition and re-induced by p53 activation in primary hepatocellular carcinoma (HCC) tumor samples and cell lines. p53 represses the expression of Zeb1/Zeb2 via miR-200 and miR-192 family members. Besides, miR-192 family members suppress EMT by repressing the expression of Zeb2, which consequently leads to decreased tumor migration, invasion and tumorigenicity providing a vital role for miR-192 in p53-regulated-EMT [90].

miR-200 Family

The miR-200 family consists of five members, miR-200a, miR-200b, miR-200c, miR-429, and miR-141. Based on their chromosomal location, the miR-200 family could be categorized into two different gene clusters: the miR-200b/miR-200a/miR-429 gene cluster, which is located on chromosome 1 and the miR-200c/miR-141 gene cluster on chromosome 12 [91]. In a study led by Geregory and colleagues, all members of the miR-200 family were down-regulated in canine kidney cells, which displayed EMT in response to TGF-β or ectopic expression of the protein tyrosine phosphatase, Pez. They also analyzed the expression of E-cadherin, Zeb1 and miR-200 family in human breast cancer cell lines. High expression levels of the miR-200 family and miR-205 were found in cell lines that expressed E-cadherin and showed features of well-differentiated epithelial phenotype. However, in invasive breast cancer cell lines with mesenchymal phenotype, expression of the miR-200 family was undetectable. Zeb1 and Zeb2 are critical promoters of cancer progression and their expression is regulated by miR-200 s. Expression of this miRNA family members is necessary for the maintenance of epithelial phenotype and their loss of expression in mesenchymal cancer cell lines leads to progression of cancer [92]. DNA hypermethylation of two miR-200 family loci was observed in human embryonic stem cells (hECSs) and it was confirmed that the miR-200c-141 locus was sensitive to DNA methylation mark, which resulted in down-regulation of miRNA-200 family during EMT. Furthermore, decreased miR-200 expression was found to be associated with the hyper-methylation of the miR-200c-141 locus in metaplastic breast cancer, a tumor displaying EMT-like features [93].

An observation of E-cadherin level in 60 different human cancer cell lines representing 9 different tumor types showed that altered *CDH1* expression is caused by direct targeting of the Zeb1 and Zeb2 mRNAs by miR-200. miR-200 is a determining factor and powerful master regulator of EMT in cancer cells. Increased expression of miR-200 induces MET in established human cancer cells lines. In contrast, in mesenchymal cancer cells or in tissues undergoing EMT, the expression of miR-200 is decreased, which causes E-cadherin down-regulation, increased vimentin expression and EMT promotion [91]. miR-200 family serves as one of the master regulators of EMT by directly targeting the transcripts encoding Zeb1 and Zeb2, and increasing the E-cadherin expression. miR-141 and miR-200c form a loop with ZEB1 to promote EMT. In this loop, invading cancer cells with abnormal expression of ZEB1

initially suppress miR-200c and miR-141, which are inhibitors of Zeb1. This regulatory loop might be stabilized further by down-regulating miR-141 as a negative regulator of TGF-β2. In pancreatic, colorectal and breast cancer cells, the EMT activators TGF-β2 and Zeb1 are down-regulated by these two miRNAs. Furthermore, miR-200c and miR-141 are strong inducers of the epithelial phenotype and their over-expression strongly reduces cancer cell migration and invasion [94].

In contrast to previous reports, a new pro-metastatic role of miR-200 s was found in clinical and experimental models of breast cancer metastasis. Activation of the Zeb–E-cadherin axis and modulation of epithelial markers by miR-200 family induced metastatic colonization of breast cancer, through targeting Sec23a, which is responsible for the secretion of Igfbp4 and Tinagl1 tumor suppressor proteins. The miR-200 s and Sec23a pathway may provide opportunities for novel therapeutic applications in metastatic tumors; however, the biphasic and conflicting function of miR-200 s should be carefully considered in treating tumors with different stages of progression [95].

miR-141 is an epithelial-associated miRNA, which is expressed in different epithelial cancers including breast, lung, colon, and prostate [96]. It promotes MET by preventing the expression of its two targets, including Zeb1 and Zeb2, which finally results in repression of E-cadherin activity [94, 97]. Mitchell et al. [96] have found that miR-141 was over-expressed in prostate epithelial cells compared with prostate stromal cells and could be proposed as a molecular biomarker for prostate cancer. Studies have shown that the mir-200c-141 locus was repressed by DNA methylation at promoter and this epigenetic regulation was strongly related to the invasiveness phenotype observed in a panel of breast cancer cell lines [98]. In a similar study, DNA methylation of mir-141-200c locus was detected during EMT in both, in vitro (MDCK-EMT transfectants) and in vivo endometrial carcinosarcoma (ECS), which suggests this locus as a useful marker to differentiate ECSs [93]. Furthermore, miR-141 expression was decreased in metastatic breast cancer, which has a tumor entity similar to ECS and displays EMT-like features [99]. High level of plasma miR-141 in samples of colorectal cancer (CRC) patients was correlated with distant metastasis cases. Therefore, combination of miR-141 and the Carcinoembryonic antigen (CEA) could be used as a novel biomarker to detect metastatic CRC [100].

miR-205

miR-205 is significantly down-regulated in breast and colon cancer cells with EMT. This miRNA protects cells from EMT by targeting Zeb1/Zeb2; thus it can act as one of key regulators of EMT [92]. However, miR-205 was up-regulated in Esophageal squamous cell carcinoma (ESCC) tumors and human head and neck squamous cancer cell lines, which represents a dual oncogenic or tumor suppressive role for this miRNA [101–104]. miR-205 expression level in ESCC cell lines was the highest compared to other types of malignancies. In ESCC, miR-205 regulates cell invasion and migration through repression of Zeb2, which leads to EMT inhibition [105]. Previous studies have also identified two binding sites for miR-205 in Zeb2 locus that confirmed its role in EMT [50, 92]. These findings imply that miR-205 with tumor-suppressive function might function as a unique therapeutic target for ESCC [105].

miR-203

Expression of miR-203 is specifically decreased in advanced metastatic PCa and a reverse MET process was induced after miR-203 re-expression. miR-203 is an anti-metastatic miRNA in PCa that suppresses cancer progression and metastasis through repressing a group of pro-metastatic target genes including Zeb2, Bmi and survivin. Evidence also indicates that miR-203 in prostate cancer with bone metastasis inhibits several key steps of the metastatic cascade. miR-203 repressed bone-specific effectors such as Smad4, which is a central mediator of TGF-β intracellular signaling required for TGF-β–induced EMT [106]. Over-expression of miR-203 in primary tumors and non-metastatic cell lines was observed in other experiments; while it was down-regulated in metastatic breast cancer cell lines. In breast cancer cells, miR-203 down-regulated the endogenous expression of Snail and induced epithelial-like characteristics. Besides, re-expression of miR-203 could suppress cellular invasion and motility in metastatic breast cancer cell lines [107]. Hyper-methylation of mir-203 promoter could influence its expression in hepatocellular carcinoma and hematopoietic malignancies. In addition, some studies showed that miR-203 expression promotes apoptosis and inhibits cell proliferation in bladder and esophageal cancers [106, 107]. Accordingly, miR-203 silencing can induce tumor cell growth and invasion via Slug up-regulation, which leads to EMT [107]. In addition to Slug, Zeb1 was identified as a target of miR-203, which proposed miR-203 as a useful target for diagnosis and targeted therapy in prostate and breast cancers [106, 107].

Let-7

Unlike most miRNAs which are named with “miR” prefix, the idiosyncratic prefixes “let” and “lin” have been preserved from the earliest days of miRNA taxonomy for the first identified miRNAs. The Let-7 (lethal-7) miRNA is one of the first miRNAs to be identified in the nematode, *Caenorhabditis*

elegans, and its biological functions is evolutionarily conserved in invertebrates and vertebrates. During *C. elegans* development (at the larval stage 4 to adult transition), the hypodermal skin cells (known as seam cells) stop proliferation and undergo differentiation to form alae. The seam cells harboring the let-7 mutation fail to differentiate leading to additional cycle of cell division and delayed and incomplete formation of adult alae. The majority let-7 mutants die by bursting through their vulvas, so giving the let-7 gene its name: lethal-7. As the timing of seam cell differentiation and alae formation is controlled by the let-7 miRNA, it could be considered as a stage-specific regulatory RNA critical for controlling developmental timing in *C. elegans* [108–110].

Unlike in *C. elegans*, higher animals have multiple let-7 isoforms [110]. The human let-7 family of miRNAs contains 12 related members of miRNAs organized in eight distinct clusters whose encoding genes map to various chromosomal regions that are frequently deleted in human cancers [34]. This is further supported by the validation of a tumor suppressor role for the let-7 family members in various types of human tissues, particularly in the lung, by negatively regulating the expression of various oncogenes such as RAS, MYC, and HMGA2 as well as other cell cycle progression genes [111, 112].

The let-7 family members were observed to be down-regulated in breast self-renewing tumor-initiating cells, but not in non-self-renewing cancer cells. Therefore, over-expression of let-7 decreases self-renewal and metastasis, which mediates differentiation of tumor-initiating cells to non-tumorigenic cancer cells [113]. There is a significant correlation between the expression of HMGA2, let-7b and Lin28B in ECSs. Expression analysis of members of the Lin28B–let-7–HMGA2 regulatory axis in a large cohort of endometrial and non-endometrioid carcinomas demonstrated that HMGA2 is an important factor in aggressive endometrial tumors. HMGA2 gene is consistently over-expressed in ECSs and mediates EMT by regulating transcription factors such as Snail, Slug, Zeb1, and Zeb2 [114]. Furthermore, inhibition of MAPK leads to Myc suppression, decreased expression of Lin28, and enhanced let-7 microRNA processing [115]. Besides, in the let-7/Lin28B pathway over-expressed Lin28B suppresses the biogenesis of miRNAs and is linked to tumor progression and metastasis [114, 116, 117]. Over-expression of HMGA2 and down-regulation of let-7 are also observed in other human tumors such as oral squamous cell carcinomas [118], gastric [119], colorectal [117], high-grade lung [120], hepatocellular [121] and ovarian carcinomas [122]. Raf kinase inhibitory protein (RKIP) is a metastasis suppressor that inhibits metastasis in primary human breast cancer in part through a let-7-dependent mechanism [115]. Several other studies also reported a decrease in RKIP expression in solid tumors such as those in prostate and breast [123–126]. RKIP/let-7 up-regulates bone metastasis-inducing genes including MMP1, OPN, and CXCR4 through let-7 targets (HMGA2, BACH1) and promotes invasion and metastasis [115].

Conclusions

Our understanding of the role of EMT/MET-related carcinogenesis has dramatically increased and the direct and indirect inducers of these processes are critical for the development of metastatic cancers. miRNAs have emerged as critical regulators of EMT and MET by targeting different signaling pathways and EMT-TFs. In recent years, a growing number of publications have reported the link between miRNAs and EMT processes during cancer dissemination (Fig.1 and Table 1). The redundancy observed in targeted EMT-related genes may be associated to differential expression of miRNAs during physiological or pathological events [43]. A better understanding of the roles of miRNAs in controlling EMT process suggests that these miRNAs might serve as novel biomarkers and therapeutic targets. Studies of EMT-miRNA pathways will ultimately yield the potential of miRNA-based therapeutics and lead to the early diagnosis, prognosis and treatment of human cancers. Therefore, targeting miRNA might be a useful method to regulate changes in the cell phenotype and a novel therapeutic approach for cancer treatment.

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Compliance with Ethical Standards

Conflicts of Interests None declared.

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