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Regulation of epithelial—mesenchymal and mesenchymal—epithelial transitions by microRNAs

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Abstract

Epithelial–mesenchymal transition (EMT) and the reverse process, mesenchymal–epithelial transition (MET), are essential during development and in the regulation of stem cell pluripotency, yet these processes are also activated in pathological contexts, such as in fibrosis and cancer progression. In EMT and MET, diverse signaling pathways cooperate in the initiation and progression of the EMT and MET programs, through regulation at transcriptional, post-transcriptional, translational, and post-translational levels. MicroRNAs recently emerged as potent regulators of EMT and MET, with their abilities to target multiple components involved in epithelial integrity or mesenchymal traits. By affecting EMT and MET processes, microRNAs are involved in the regulation of stem cell pluripotency and the control of tumor progression.

Introduction: Epithelial–mesenchymal and mesenchymal–epithelial transitions

Epithelial tissues reorganize themselves as cells proliferate, and display epithelial plasticity responses that enable cells to collectively migrate in response to cues in their environment. A further step in epithelial plasticity occurs when cells lose their epithelial characteristics to acquire the appearance and behavior of mesenchymal cells, promoting individual cell migration and invasion of surrounding tissues. This process, known as epithelial–mesenchymal transition (EMT), is temporally and spatially tightly controlled during development, and integral for organogenesis and tissue differentiation [1]. Following EMT, cells can revert back and re-acquire epithelial properties. Although less characterized, this reverse process of mesenchymal–epithelial transition (MET) also contributes to the formation of tissues and organs during development [1].

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In the adult, EMT can be re-activated, for example, to achieve wound healing following tissue injury. EMT also occurs in pathologies such as fibrosis or cancer progression [1]. In carcinomas, cancer cells can undergo EMT to escape the primary tumor, invade surrounding tissues, and eventually colonize remote sites via blood or lymphatic routes to generate metastases. Metastatic cells can then revert through MET to re-acquire epithelial characteristics similar to cells in the primary tumor [2,3].

During EMT, epithelial cells lose their cell–cell junctions, which encompass adherens junctions, tight junctions, and desmosomes, thus facilitating cell individualization. In addition, the epithelial apical–basal polarity is lost, and a complete reorganization of the actin cytoskeleton enhances cell locomotion along rear-to-front polarity. EMT also enables cells to acquire invasive properties, thus degrading extracellular matrix and re-synthesizing extracellular matrix proteins [1] (Figure 1).

The molecular and cellular mechanisms underlying EMT and MET are complex as they can be initiated by multiple extracellular cues, transcription factors, and signaling pathways, depending on the physiological or pathological contexts [2]. Among extracellular factors that activate the EMT program, TGF-β represents a potent and prominent EMT inducer [1,4]. The reverse process, MET is therefore enhanced by blocking the actions of factors and signaling pathways that activate EMT. Additionally, several BMPs, members of the TGF-β family, can promote MET in a cell-dependent and context-dependent manner [4].

EMT is characterized by an epithelial–mesenchymal switch in marker expression, primarily controlled by three families of transcription factors: the zinc finger Snail (Snail/Slug), ZEB (ZEB1/ZEB2), and basic helix-loop-helix (e.g. Twist1) families. These transcription factors act to repress E-cadherin expression, a major component of adherens junctions and hallmark of the epithelial integrity [5]. They also regulate the repression of other epithelial marker proteins, and induce mesenchymal gene expression.

While transcriptional regulation of EMT has been extensively studied, post-transcriptional, translational and post-translational regulators are recently highlighted in several studies. Integral to post-transcriptional regulation, micro-RNAs emerge as potent regulators of EMT and MET events through their ability to target the expression of key proteins that regulate these processes [6,7] (Figure 1).

MicroRNAs control EMT transcription factors

MicroRNAs are 22-nucleotide non-coding RNAs that suppress their targets through mRNA destabilization and translational inhibition. MicroRNAs are processed from longer transcribed pri-miRNAs. A single pri-miRNA can produce multiple mature miRNAs forming what is commonly called a miRNA cluster. MicroRNAs bind mRNA sequences through a complementary 7-nucleotide sequence, called the seed sequence, present at the 5′ end of the microRNA [8]. The seed sequence and to a smaller degree other nucleotides in the microRNA complement sequences in the 3′ untranslated region (3′UTR) and occasionally open reading frame of their mRNA targets. This minimal complementary requirement enables a single microRNA to regulate hundreds of mRNAs simultaneously. MicroRNAs are grouped into families based on shared seed sequences. Members of a family

are expected to have many overlapping targets. Individual microRNAs often target multiple mRNAs within a common pathway, increasing the robustness of their effects on cellular processes such as EMT, while single mRNAs can be regulated by multiple microRNAs [9].

Several microRNAs have been shown to directly target families of EMT transcription factors (Figure 1). The miR-200 family, which includes miR-200a, miR-200b, miR-200c, miR-141 and miR-429, targets ZEB1, and ZEB2 [10]. Their expression decreases during EMT resulting in enhanced ZEB1 and ZEB2 levels, and EMT progression. Conversely, their ectopic expression prevents EMT or enhances MET. Snail is targeted by several microRNAs, including miR-29b and miR-30a. Accordingly, enhanced expression of miR-29b in metastatic prostate cancer cells reverses EMT and inhibits the invasive phenotype [11], and miR-30a expression is reduced during TGF-β-induced EMT in murine hepatocytes [12]. The transcription factor HMGA2, a chromatin-binding protein that mediates TGF-β-induced EMT responses and activates Snail and Twist, is downregulated by the microRNA let-7 in a model of human pancreatic cancer [13], and by miR-365 in human lung adenocarcinoma cells [14], although the exact role of these microRNAs in EMT or MET remains to be characterized.

Multiple microRNA families can target a single EMT transcription factor. For example the miR-200 and miR-205 families target distinct sequences in the 3′UTR of ZEB1 and ZEB2, thereby, cooperating to enhance the repression of ZEB levels [10] (Figure 2). In a model of hepatocellular carcinoma, p53 expression prevents EMT progression by increasing the levels of miR-200 and miR-192/215 families of microRNAs, which targets ZEB1 and ZEB2 [15]. Furthermore, miR-1 and miR-200b both directly target Slug in prostate adenocarcinoma cells [16•]. In this system, TGF-β-induced EMT results in expression of Slug, which in turn transcriptionally represses the expression of both miR-1 and miR-200b, forming a double negative feedback loop.

Another double-negative feedback loop involves the mutual control of ZEB and miR-200 expression and activity, in which ZEB proteins repress the expression of miR-200 family members, which in turn suppress ZEB protein production, as mentioned above [17]. A double feedback loop also operates in the control of miR-34 and Snail expression. While Snail is targeted by miR-34, Snail binds E-boxes that control miR-34 expression and represses its transcription [18]. Consequently, in TGF-β-induced EMT of human colon adenocarcinoma cells, increased Snail expression is accompanied by repression of miR-34 levels. Finally, a feedback loop has been observed between Snail and miR-203 in human breast cancer cells, in which Snail expression reduces miR-203 expression, while ectopic miR-203 expression directly represses Snail [19]. These reciprocal feedback loops may amplify the activation of EMT and stabilize mesenchymal specification once EMT is completed. In addition, these double-feedback loops may explain the reversibility of EMT and MET programs through an imbalance in expression of microRNAs and EMT transcription factors (Figure 2).

A single microRNA species can also regulate several EMT transcription factors providing additional mechanisms to enhance EMT responses. In addition to Snail, miR-34a also represses Slug and ZEB1 [18]. Since Snail regulates the expression of other transcription

factors including ZEB1 and ZEB2, microRNAs that directly target Snail, may indirectly control the expression of other transcription factors. Interestingly, the 3′UTR region of ZEB2 contains a miR-34a seed matching sequence, although ZEB2 is not suppressed by miR-34a, suggesting additional mechanisms and regulation of microRNA functions [18].

Multiple aspects of EMT are controlled by microRNAs

MicroRNAs also affect the integrity of the epithelial architecture during EMT progression (Figure 1). In human mammary epithelial cells, miR-9 directly targets E-cadherin, thus promoting the mesenchymal phenotype with increased cell migration and invasion [20•]. Loss of E-cadherin represents a hallmark of cancer progression and metastasis and accordingly, miR-9 expression is upregulated in a mammary tumorigenesis mouse model. During EMT, decreased E-cadherin expression is often accompanied by increased expression of N-cadherin, a mesenchymal marker involved in cell adhesion and migration. The expression of miR-194, which targets N-cadherin, is attenuated in advanced stage gastric cancer cells, while increased miR-194 expression in mesenchymal hepatic cancer cells reduces N-cadherin, cell migration, invasion, and the formation of metastasis [21,22].

During TGF-β-induced EMT in rat kidney epithelial cells, miR-491-5p targets Par3, an epithelial polarity complex protein. Consequently, Par3 downregulation contributes to destabilization of tight junctions, a major step in the initiation of EMT [23]. In addition, miR-661 is involved in the disassembly of epithelial cell junctions in Snail-expressing human breast cancer cells, by targeting Nectin-1, a protein involved in cell-cell adhesion, and StarD10, a phospholipid transfer protein that co-localizes with epithelial junctions [24]. The small GTPase protein RhoA, which plays a dual role in EMT by stabilizing tight junctions [25] and reorganizing the actin cytoskeleton into stress fibers [26], is controlled by the action of several microRNAs. TGF-β signaling activates the expression of miR-155, which targets RhoA expression, resulting in dissolution of tight junctions during EMT [27]. In keratinocytes, TGF-β treatment increases the level of miR-24 which targets Net1A, a Rho-GEF protein that mediates RhoA activation, promoting EMT through disruption of adherens and tight junctions, as apparent from decreased levels of E-cadherin and ZO-1, respectively [28]. In cancer progression, induction of miR-31 expression reduces the metastatic potential of breast cancer cells by targeting RhoA, and ectopic miR-124 expression in hepatocellular carcinoma cells decreases EMT, cell invasion and metastasis by targeting ROCK2, an effector of RhoA signaling [29,30].

Some microRNAs control EMT programs by targeting either receptors that initiate signals from EMT inducers, or multiple components of EMT signaling pathways (Figure 1). For example, by targeting TGFBR2, the gene encoding the TGF-β receptor TβRII, miR-204 maintains epithelial integrity of retinal pigment epithelium [31]. Inhibition of miR-204 enhances TGFBR2 and results in dissolution of tight junctions via decreased expression of Claudin 10, 16 and 19. MiR-204 also targets Slug, which is rapidly induced in response to TGF-β signaling during EMT, thus demonstrating dual roles and efficiency of this microRNA in maintaining epithelial integrity [31]. Introducing miR-302 and miR-372 expression in human keratinocytes targets TGFBR2 and inhibits TGF-β-induced EMT and associated Slug expression [32•]. Finally, in a squamous cell carcinoma model, miR-138

powerfully regulates EMT by targeting multiple components of EMT pathways such as ZEB2 and the epigenetic regulator EZH2, both repressors of E-cadherin expression [33], RhoC and ROCK2, leading to inhibition of cell migration and invasion [34], and vimentin, a mesenchymal component of intermediate filaments [33] (Figures 1 and 2).

By regulating EMT and MET programs in a controlled and efficient manner, through multiple mechanisms, modes of regulation, and feedback loops, and in a cell-dependent type and context, microRNAs represent tools to study and understand physiological and pathological processes.

MicroRNAs: a tool for reprogramming

Introduction of four transcription factors, Oct3/4, Sox2, Klf4, and c-Myc can convert somatic cells into pluripotent cells, called induced pluripotent stem (iPS) cells [35]. The changes required for a somatic cell to attain pluripotency remain poorly characterized. However some insights have been made in recent years [36]. One of these insights is a role for MET early in reprogramming of mouse and human fibroblasts [32•,37•,38•]. Specifically, reprogramming can be separated into three phases by gene expression patterns [38•]. The earliest phase is highly labile and unstable, and is referred to as initiation. It is within this phase that MET occurs and it precedes the endogenous expression of transcription factors that drive pluripotency. Key molecular events in this phase include repression of mesenchymal genes encoding Snail, Slug, ZEB1, and ZEB2, and activation of epithelial genes encoding E-cadherin, Occludins, Ep-Cam, Claudins, and Crb3, all of which are components of epithelial junctions. Furthermore, suppression of MET blocks reprogramming when starting with a fibroblast population, while suppression of EMT promotes reprogramming when starting with an epithelial population [37•,38•]. This later finding suggests that even when starting with an epithelial population there can be activation of mesenchymal programs that suppress the efficiency of reprogramming.

The early phase of the reprogramming process in mouse fibroblasts is regulated by microRNAs belonging to the miR-200 and miR-205 families, which directly regulate the expression of key mesenchymal genes that drive EMT, such as ZEB1 and ZEB2 [38•]. This early stage of reprogramming can also be accelerated and enhanced by expression of the miR-302/miR-372 family of miRNAs [32•,39•] or the related miR-106 family [40]. During mouse and human reprogramming, these microRNAs target genes involved in the regulation of MET such as TGFBR2 and RhoC, which play key roles in maintaining the mesenchymal state. Thus, microRNA suppression of TGFBR2, RhoC, and ZEB protein levels results in enhanced reprogramming efficiency showing that miRNAs feed into the EMT pathways to regulate the de-differentiation of somatic cells to iPS cells (Figure 3a). However, miRNAs regulate hundreds of targets often in multiple pathways [9]. Indeed, the miR-302 and miR-372 family also targets cell cycle and epigenetic regulators that influence the efficiency of reprogramming [32•]. Therefore, the coordinated effects on MET and other pathways underlie the remarkable capacity of these miRNAs to promote de-differentiation of somatic cells to pluripotent stem cells.

MicroRNAs in cancer progression

EMT often correlates with cancer cell dissemination leading to metastasis, whereas MET can correlate with the establishment of secondary tumors following metastasis [41]. By regulating EMT and MET processes, microRNAs are therefore often involved in tumor progression with oncogenic microRNAs repressing epithelial characteristics, for example by targeting components of epithelial junctions, and tumor-suppressive microRNAs repressing mesenchymal progression, for example by targeting the expression of transcription factors that drive EMT [7]. In breast cancer progression, miR-221/222 expression is increased in aggressive basal-like subtype breast cancers. In these tumors, miR-221/222 directly represses the expression of the GATA family transcription factor TRPS1, a repressor of ZEB2, promoting downregulation of E-cadherin expression and EMT-associated increased cell migration and invasion [42•]. Conversely, miR-520/373 members act as tumor-suppressive microRNAs, and increased miR-520c or miR-373 expression inhibits the invasive behavior of breast cancer cells *in vitro* and *in vivo*, in part by targeting TGFBR2 [43] (Figure 3b). Preventing the expression of oncogenic microRNAs, or introducing tumor-suppressive microRNAs may therefore provide therapeutic strategies in attenuating cancer cell dissemination. For example, delivery of an antisense-based inhibitor of miR-10b, a microRNA expressed under the control of Twist and highly expressed in metastatic cancers, reduces the metastatic potential of cancer cells in a mouse mammary tumor model [44•]. Alternatively, increased expression of the tumor-suppressive miR-7 microRNA in fibroblast-like gastric cancer cells promotes MET and inhibits formation of metastatic tumors in mouse liver [45] (Figure 3b).

Tumors arising from carcinomas are heterogeneous in nature, primarily composed of differentiated cancer cells, leukocytes, endothelial cells and cancer-associated fibroblasts [46]. They also contain self-renewing cells named cancer stem cells (CSCs) or tumor-initiating cells (TICs), which are able to generate heterogeneous cancer cell populations and initiate tumor formation. CSCs are also believed to promote drug resistance and tumor recurrence in cancer therapies [46]. Several studies demonstrate that carcinoma cells can dedifferentiate and give rise to CSCs through EMT [47]. As a result, microRNAs that promote EMT have been linked to the generation of CSCs. For example, miR-21 acts as oncogenic microRNA, and its expression promotes EMT and CSC characteristics in breast cancer cells, notably through inhibition of PTEN expression [48,49]. In addition, the tumor-suppressive miR-200 family and miR-15b, target directly the expression of BMI1, a component of the Polycomb repressor complex 1 that is frequently elevated in CSCs and promotes acquisition of tumor-initiating capacity [50]. Interestingly, BMI1 cooperates with Twist1 in repressing E-cadherin expression and promoting EMT [51•]. Finally, expression of the miR-106b-25 cluster, which acts downstream of the transcription factor Six1, induces EMT and tumor-initiating characteristics in human breast cancer cells, through direct repression of Smad7, thus increasing TGF-β signaling [52] (Figure 3b). Intriguingly, members of the miR-106b-25 cluster can also promote a MET-like process and enhance the induction of iPS cell reprogramming through targeting TβRII [39•,40]. These studies position the miR-106b-25 family as inducers of stem cell properties through regulation of EMT or MET programs, and reflect the context-dependent activity of these micro-RNAs in

regulating such processes. These studies also highlight the roles of microRNAs as prognostic markers and therapeutic tools in EMT-associated cancer progression.

Concluding remarks and future perspective

MicroRNAs can potently regulate EMT and MET by targeting multiple components involved in transcription programs, signaling pathways or the integrity of the cell architecture. Distinct mechanisms, including double-negative feedback loops, define flexible and efficient control of EMT and MET processes by microRNAs. The redundancy observed in targeted EMT genes may be attributed to cell- and context-dependent expression of microRNAs at different stages during physiological or pathological events.

Several studies have defined microRNA signatures in EMT and MET processes involved in induction of iPS cell reprogramming, or during cancer progression. The identification of such microRNA profiles can translate into using microRNAs as tools, for example, to enhance iPS cell reprogramming in regenerative medicine. These microRNA profiles can also help define appropriate cancer treatments, by targeting signaling pathways affected by microRNAs. In addition, inhibiting the expression of oncogenic microRNAs or re-introducing tumor-suppressive microRNAs represent therapeutic opportunities to attenuate cancer progression. The main challenges in using microRNAs as therapeutic tools *in vivo* include developing efficient systems to deliver microRNAs or antisense oligonucleotides that target microRNAs, and improving the stability of these antisense oligonucleotides.

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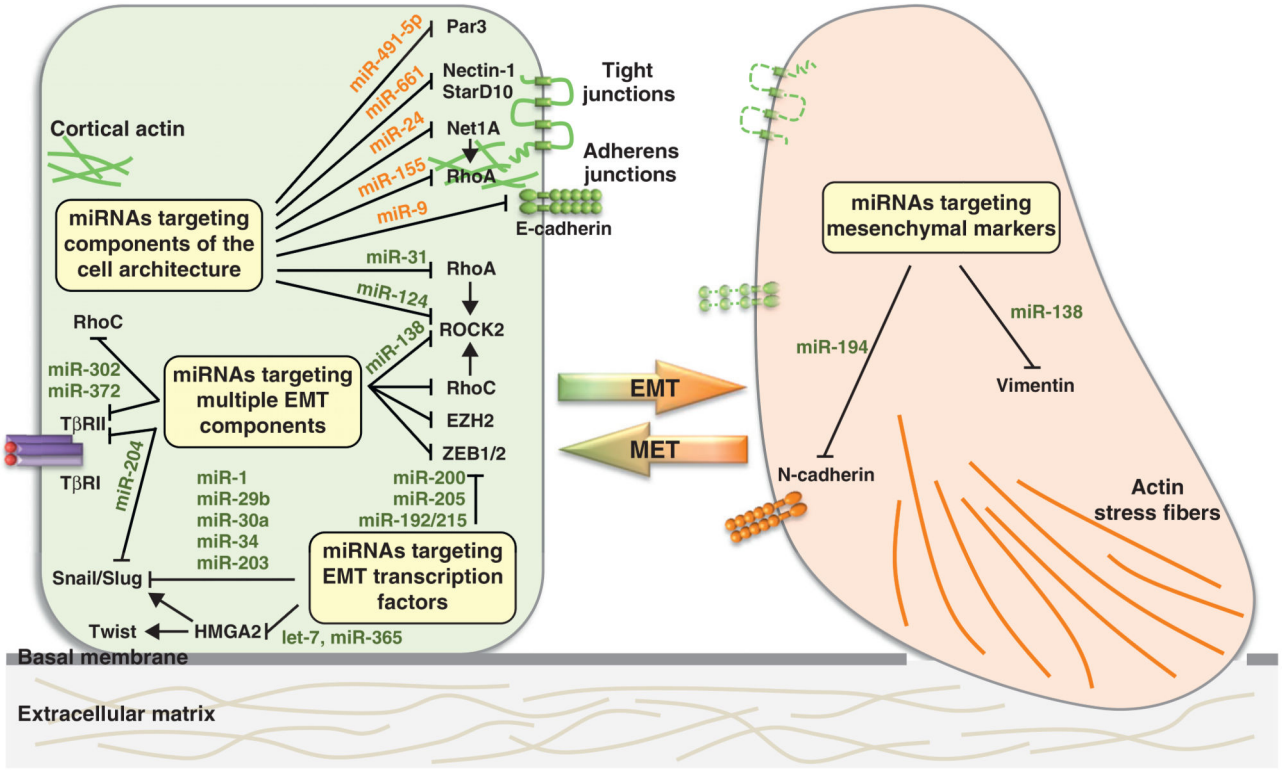

Figure 1.

MicroRNAs in EMT and MET. EMT is characterized by a disassembly of cell–cell junctions, loss of epithelial polarity, and reorganization of actin cytoskeleton. In addition to a decrease in epithelial marker expression, increases in expression of mesenchymal markers and invasive behavior are observed in cells undergoing EMT. The changes in gene expression programs are activated by families of transcription factors. MicroRNAs suppress production of these transcription factors as well as multiple markers and components that define the epithelial or mesenchymal characteristics. These microRNAs can therefore promote EMT (orange) or repress EMT and enhance MET programs (green).

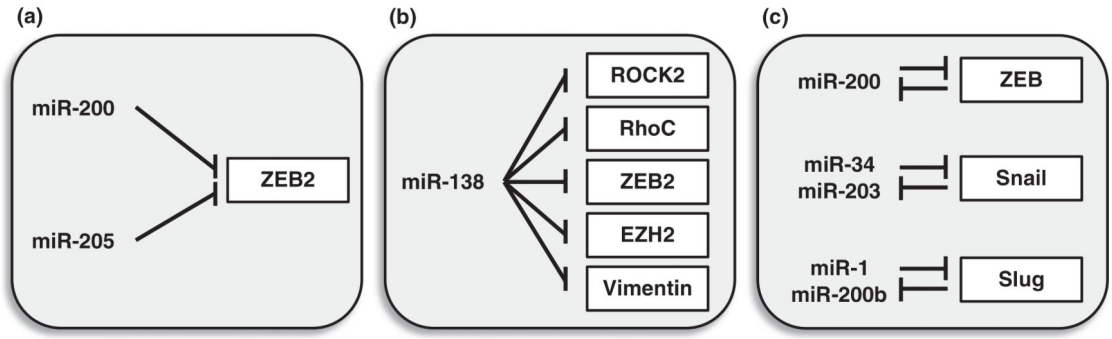

Figure 2.

Different mechanisms of action of microRNAs in regulating EMT. Three distinct mechanisms of regulation of EMT components by microRNAs can be discerned. (**a**) Two distinct microRNAs can cooperate to regulate the expression of a single transcript by targeting different sites in the transcript's 3′UTR. (**b**) One single microRNA can regulate multiple EMT components. (**c**) Double feedback loops are observed when the expression of microRNA is targeted and downregulated by its own EMT transcription factor target.

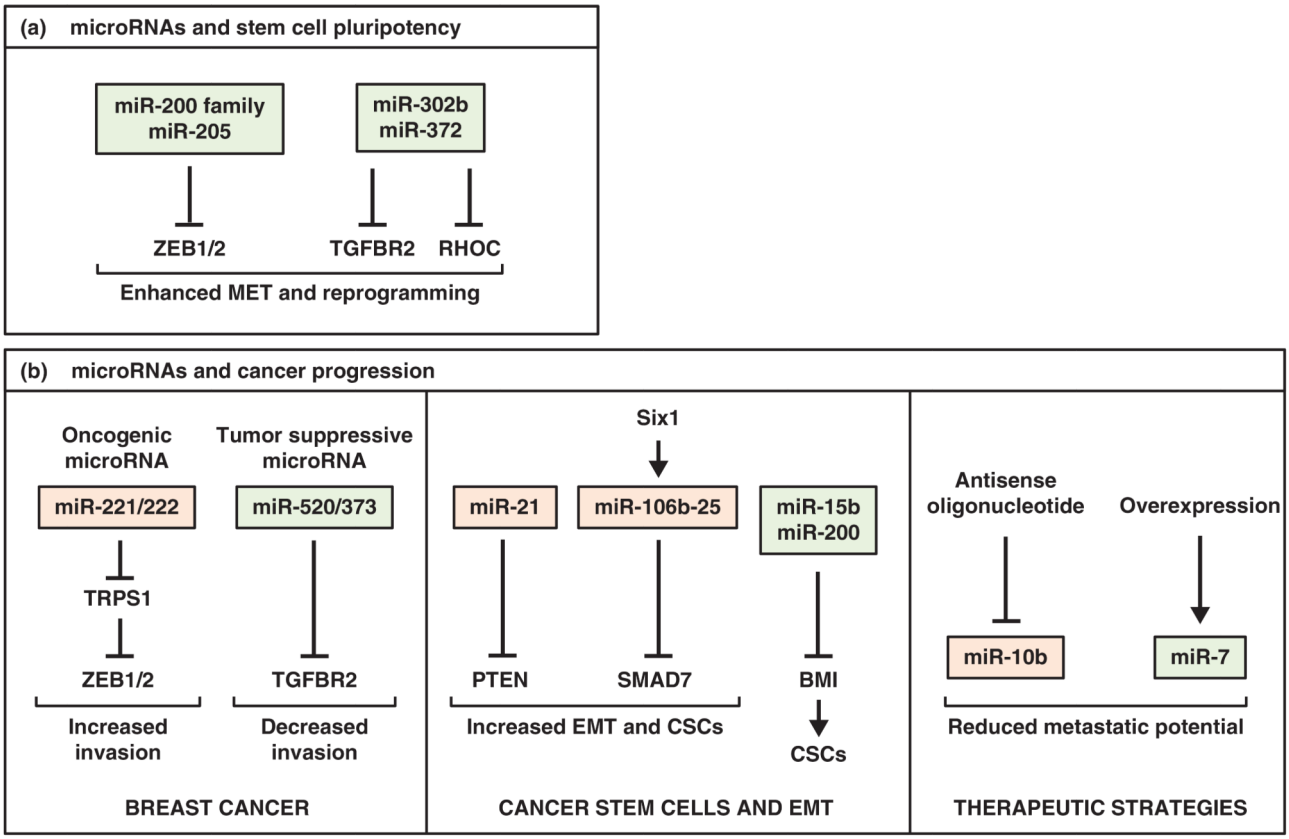

Figure 3.

MicroRNAs regulate EMT-associated and MET-associated stem cell pluripotency and cancer progression. (**a**) The induction of pluripotent stem cells requires the acquisition of epithelial characteristics and is initiated by an MET event. By inhibiting EMT and enhancing MET programs (green), microRNAs can enhance reprogramming and stem cell pluripotency. (**b**) EMT and MET programs have been associated with cancer progression. Therefore, microRNAs that regulate EMT or MET programs can present oncogenic (orange) or tumor-suppressive roles (green). The presence of cancer stem cells that arise from EMT events within a population of tumor cells can also be regulated by the action of microRNAs. Therefore, blocking oncogenic microRNAs or expressing tumor-suppressive microRNAs represent potent strategies against tumor progression.