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MiR-200, a new star miRNA in human cancer

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Abstract

MicroRNAs (miRNAs) are a set of non-coding small RNA molecules in control of gene expression at posttranscriptional/translational level. They not only play crucial roles in normal developmental progress, but also are commonly dysregulated in human diseases, including cancer. MiR-200 is a family of tumor suppressor miRNAs consisting of five members, which are significantly involved in inhibition of epithelial-to-mesenchymal transition (EMT), repression of cancer stem cells (CSCs) self-renewal and differentiation, modulation of cell division and apoptosis, and reversal of chemoresistance. In this article, we summarize the latest findings with regard to the tumor suppressor signatures of miR-200 and the regulatory mechanisms of miR-200 expression. The collected evidence supports that miR-200 is becoming a new star miRNA in study of human cancer.

Keywords

microRNA; miR-200; EMT; stem cells; ZEB; cell cycle; cancer cell

1. Introduction

MicroRNAs (miRNAs) are a class of short non-coding RNA molecules with a significant regulatory capacity on gene expression [1, 2]. Similar to protein-coding genes, the primary transcripts of miRNA (pri-miRNA) are derived from genomic DNA and synthesized by RNA polymerase II or III into a hairpin structure [3, 4]. After being further processed by the RNase III family enzymes, Drosha and DiGeorge syndrome critical region gene 8 (DGCR8), the excess nucleotides at both the 3' and 5' regions of pri-miRNA are cropped off to form precursor miRNA (pre-miRNA) that is ~70 nt in length [5-8]. Then, pre-miRNA will be exported out of the nucleus by the double-stranded RNA binding protein Exportin-5, and this intermediate product is subsequently cleaved by the RNase III family enzyme, Dicer, into imperfect mature miRNA:miRNA*duplexes in the cytoplasm [9-13]. The miRNA strand of approximately 18-22 nucleotides is incorporated into the RNA-induced silencing complex (RISC), which guides mature miRNA to trigger the target mRNA for subsequent silencing [14, 15]. Although the extent to which miRNAs regulate the human transcriptome is still under investigation, more and more evidence supports the fact that miRNA plays a crucial regulatory role in the control of gene expression.

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To date, a total of 2,578 mature miRNA sequences have been identified in humans in accordance to the latest release of the miRBase database (<http://www.mirbase.org/>). The miRNA-200 family consisting five members (miR-200a, miR-200b, miR-200c, miR-429, and miR-141), is of particular interest for human health and disease. Based on the chromosomal locations, the miR-200 family can be divided into two clusters: the miR-200ba/429 cluster containing miR-200a, miR-200b, and miR-429, which is located on chromosome 1p36, and the miR-200c/141 cluster, which contains miR-200c and miR-141 and is located on chromosome 12p13. As known, in miRNA biology, seed sequences, in terms of the complementary sequences to binding sites of miRNA within the 3' untranslated regions (UTRs) of the target genes, play a crucial role in regulatory effects of miRNA on gene expression [16]. Theoretically speaking, the members of a miRNA family contain highly conservative seed sequences; and miRNAs with the identical seed sequences may share the same putative target gene profiles. For the miR-200 family, two types of seed sequences are identified, which only have a nucleotide difference. MiR-200b, miR-200c, and miR-429 contain AAUACUG, whereas miR-200a and miR-141 possess AACACUG as their seed sequences. For the convenience of functional analysis, some studies named the miR-200bc/429 and miR-200a/141 clusters based on the seed sequences and potential similar target gene profiles [17], which are distinct from miR-200ba/429 and miR-200c/141 that are defined based on the chromosomal locations.

The first correlative report showing a relationship between the miR-200 family and human health demonstrated its high olfactory enrichment and neural expression patterns, consistent with a role in olfactory neurogenesis [18]. Soon afterwards, its involvement in human disease was confirmed when new studies demonstrated that miR-200 appeared to be downregulated during tumor progression and acted as a key inhibitor for epithelial-to-mesenchymal transition (EMT), tumor cell invasion, and metastasis. These benchmark results have been summarized in several high quality reviews [19-24]. To be complementary to these articles, we focus on presentation of the latest findings on studies of miR-200 tumor suppressive roles in human cancer, which include inhibition of EMT, repression of cancer stem cell (CSC) self-renewal and differentiation, modulation of cell division and apoptosis, and involvement in chemoresistance. Moreover, after sorting out many related publications, we summarize the mechanisms accounting for the regulation of miR-200 expression in various cancer cells. These results have not yet been collated to date but will be of importance to understand the tumor suppressor role of miR-200 in human cancer. We hope that this review article can provide useful insights into translation of the achievements gained from miRNA research into the future clinical applications.

2. Tumor suppressive signatures of miR-200

2.1. Inhibition of epithelial mesenchymal transition (EMT) and tumor metastasis

The involvement of miR-200 in cancer originates from several studies demonstrating a significant role for miR-200 in EMT, which is an important process in tumor progression as well as embryonic development. The expression of miR-200 family members was found to be highly associated with the epithelial phenotype of cancer cells, serving as a class of key markers for E-cadherin-positive and vimentin-negative cancer cell lines [25-27]. *ZEB1* and *ZEB2* are two members of the zinc-finger E-box binding homeobox family, and they have been defined as the master regulators in EMT [24]. The mechanism by which *ZEB1* and *ZEB2* facilitate EMT is that *ZEBs* can efficiently inhibit the cell-cell adhesion molecule E-cadherin, given that the aberrant expression of *E-cadherin* is a hallmark of EMT [28]. A number of studies have documented that, in epithelial cancer cells, highly expressed miR-200 represses the expression of *ZEBs*; whereas in mesenchymal cancer cells, impaired expression of miR-200 leads to induction of *ZEBs* and subsequent repression of *E-cadherin* [25-27]. Following the studies on the ability of miR-200 to regulate the expression of *ZEB1*

and *ZEB2*, dozens of others were carried out in various types of human tumors as summarized in Table 1[29-60]. While expression of the miR-200 family members was determined to be impaired in various human tumor cells leading to EMT and disease progression, increased miR-200 family member expression led to a reversal of EMT in bladder cancer, gastric cancer, nasopharyngeal carcinomas, ovarian cancer, pancreatic cancer, and prostate cancer [29, 39, 40, 44, 46, 52, 54]. In support of these results, studies by using clinical patient samples also indicated strong correlations between miR-200 expression and tumor progression in a variety of tumor types[31-33, 35, 37, 39, 41, 42, 45, 47, 48, 50, 53, 54, 56, 58-60]. Furthermore, it has been noted that low level expression of miR-200 could correlate with poor survival and serve as a prognostic marker for cancer patients [29, 38, 47, 58-60]. As such, the findings from the studies of miR-200 and EMT have become the benchmarks for further research on the tumor suppressive signatures of miR-200.

EMT is considered the initiating event for cancer metastasis. Two different groups simultaneously reported that miR-200 was significantly downregulated in metastases and metastatic-like primary tumors, thereby relieving the repression conferred by the mesenchymal transcription factor *ZEB1* *in vivo*. Forced expression of miR-200 abrogated the capacity of tumor cells to undergo invasion and metastasis, underscoring the role for miR-200 in the regulation of both EMT and subsequent metastases [61, 62]. A similar conclusion was drawn through studying different human ovarian cancer cell lines with distinct capabilities to metastasize [63], while miR-200 has also been implicated in the reversal of the metastatic phenotype of non-small cell lung cancer, as its re-expression has been shown to downregulate the expression of many prognostic markers for metastasis, such as alpha thalassemia/mental retardation syndrome X-linked gene (*ATR*), deleted in liver cancer 1 gene (*DLC1*), hereditary hemochromatosis gene (*HFE*), and heterogeneous nuclear ribonucleoprotein A3 gene (*HNRNPA3*)[43]. Given the mechanistic studies supporting that the Notch signaling pathway plays a crucial role in the regulation of EMT and thus metastasis during cancer progression [64], miR-200 was found to decrease expansion of human metastatic prostate cancer cells by targeting the Notch ligand Jagged1 and the mastermind-like coactivators Maml2 and Maml3, the key components in the Notch pathway [65, 66]. Complementary to these results, Yang et al. found that the Notch ligand Jagged2 was also able to inhibit miR-200 family expression at the transcriptional level by induction of GATA transcription factors, which eventually led to promotion of tumor metastasis *in vivo* [67]. These findings support a regulatory loop consisting of miR-200 and the Notch signaling pathway; the balance of their interaction can potentially decide the stages of tumor progression (Figure 1A). However, the molecular mechanisms accounting for the tumor suppressor roles of miR-200 are still largely unknown, although the Notch signaling pathway sheds light on understanding of its anti-metastatic activity. A recent study reported the controversial evidence that miR-200 was found to be upregulated in breast cancer 4T1 cells that formed macroscopic metastases *in vivo* when compared with related cells invading distant tissues but were unable to colonize. The authors proposed that miR-200 might be involved in promotion of the last step of the metastatic cascade when establishing macroscopic metastatic masses at distant sites [68]. Given most current findings supporting miR-200 as a tumor suppressor, additional evidence is needed to confirm such a hypothesis in which miR-200 plays an oncogenic role. Together with the myriad of aforementioned studies from a wide variety of cancers listed in Table 1, these data suggest that the miR-200 family plays a significant role in combating not only EMT, but also tumor cell invasion and metastases. Thereby, miR-200 has great potential to become a novel class of biomarkers for tumor prognosis and targets for new drug development against tumor progression.

2.2. Repression of cancer stem cell self-renewal and differentiation

The most essential feature of stem cells is the capability of self-renewal to produce dozens of differentiated cell types. The increased evidence shows that miRNAs play a crucial role in regulating stem cell self-renewal and differentiation. Tumor initiating cells, also called cancer stem cells (CSCs), have been identified in various types of cancer. The first report linking miR-200 and stem cell physiology came in 2009 from a study in which all five members of the miR-200 family were shown to be downregulated in human breast CSCs as well as in normal human and murine mammary stem/progenitor cells [69]. By targeting B lymphoma Mo-MLV insertion region 1 homolog (*BMI1*), a known regulator for stem cell self-renewal, miR-200c was shown to strongly suppress the ability of normal mammary stem cells from forming mammary ducts and tumor formation driven by human breast CSC *in vivo* [69]. Not only can miR-200c suppress tumor formation driven by breast CSCs, but miR-200b can downregulate CSC growth through targeting the Polycomb family embryonic stem (ES) cell pluripotency maintenance factor *Suz12* [70]. Moreover, Lim et al. reported that the immortalized human mammary epithelial cells convert from a non-stem to a stem cell-like phenotype accompanied with a loss of miR-200 expression. Restoration of miR-200 expression decreased stem-like properties while promoting a transition to an epithelial phenotype [71]. Thus, it is apparent that miR-200 family members play important roles in multiple events related to CSCs.

Many of the molecular factors needed to maintain pluripotency of stem cells have been found to regulate the expression of the miR-200 family of miRNAs, such as *c-Myc*, octamer-binding transcription factor 4 (*Oct4*), and sex determining region Y-box 2 (*Sox2*). Lin et al. reported that the expression of the miR-200 family was regulated by *c-Myc* in ES cells. The transcriptional induction of these miRNAs by *c-Myc* significantly attenuated the downregulation of pluripotency markers, which indicates that in ES cells, *c-Myc* acts, at least in part, through the miR-200 family to attenuate differentiation [72]. In addition, Wang et al. found that *Oct4* and *Sox2* induced the transcriptional activation of the miR-200 family, which promoted the mesenchymal-to-epithelial transition (MET) and the generation of induced pluripotent stem cells (iPSCs) by targeting *ZEB2* [73], thus leading to somatic cell reprogramming. However, in proliferating vMH (ventral midbrain/hindbrain) neural progenitors, miR-200 was required to promote cell-cycle exit and neuronal differentiation through targeting the expression of *Sox2* and the cell cycle regulator *E2F3* [74]. Samavarchi-Tehrani et al. also found that during the initiation phase of reprogramming, the bone morphogenetic protein (BMP) signaling could induce the expression of miR-200 and miR-205, forming a multistep mechanism that incorporates a BMP-miRNA-MET axis to promote somatic cell reprogramming [75]. Thus, as illustrated in Figure 1B, the miR-200 family has been demonstrated to bear integral importance in the process of self-renewal and differentiation of CSCs.

2.3. Modulatory role of miR-200 in cell division and apoptosis

Cell division is a vital process used by a single fertilized egg to develop into a mature organism, as well as to renew cells, tissues, and thus organs. The control of the cell cycle by miRNA is well established, and ectopic expression of certain miRNAs may contribute to tumor development by perturbing important cell cycle regulators. Uhlmann et al. were the first to describe a role for miR-200 in regulating cell cycle progression in breast cancer [17]. They found that overexpression of miR-200a/141 resulted in G1 arrest, which may be due to increased expression of cyclin dependent kinase inhibitor 1B (*p27/Kip1*) and decreased expression of cyclin dependent kinase 6 (*CDK6*). In contrast, elevating the expression miR-200bc/429 cluster caused a reduction of *p27/Kip1* expression and upregulation of inhibitory phosphorylation of cell division cycle 25C gene (*CDC25C*), thereby decreasing the G1 population and increasing the G2/M population [17]. It is noticed that, in this study,

the miR-200bc/429 and miR-200a/141 clusters are defined by using the different seed sequences and target gene pools, which account for, at least in part, their distinct functions in regulation of cell cycle. Xia et al. found that, in HeLa cells, miR-200b directly downregulated Rho family GTPase 3 (*RND3*), thereby promoting expression of the downstream cell cycle regulatory factor cyclin D1 (*CCND1*) that controls S-phase entry [76]. More recently, Yao et al. demonstrated that miR-200b was a critical regulator of the zinc finger transcription factor GATA-4, which regulates the expression of *CCND1* at the transcriptional level. Their results supported that miR-200b could regulate tumor cell growth and differentiation by targeting GATA-4 to downregulate the expression of *CCND1* [77]. Thus, these findings support that miR-200 can exert control over the cell cycle at many levels through the modulation of several different factors.

In addition to playing a role in the regulation of cell cycle, the miR-200 family has been shown to modulate apoptosis. Schickel et al. found that altering levels of miR-200c changed the sensitivity of cells to death receptor CD95-mediated apoptosis [78]. In addition, they identified the apoptosis inhibitor, Fas-associated phosphatase-1 (FAP-1), as a target of miR-200c, which was demonstrated to be responsible for the reduced sensitivity of CD95-mediated apoptosis in cells with inhibited miR-200. It was also reported that reactive oxygen species (ROS) could induce miR-200 family expression with subsequent downmodulation of *ZEB1*, which was likely to play a key role in ROS-induced apoptosis and senescence [79]. Figure 1C illustrates the involvement of miR-200 in regulation of cell division and apoptosis.

2.4 Reversal of chemoresistance

Although the mechanisms have not yet been completely understood, the chemoresistance to a few drugs, such as gemcitabine, paclitaxel, cisplatin, doxorubicin, docetaxel, EGFR inhibitors, and vincristine, has been reported to be associated with the downregulation of miR-200 [80-88]. Interestingly, some of these studies also reported that the restoration of miR-200 could effectively reverse the chemoresistance to certain drugs [82, 87, 88]. Meng et al. first reported that inhibition of miR-200b expression could increase the sensitivity of cholangiocarcinoma cells to the nucleoside analog gemcitabine [80]. Ali et al. reported that the curcumin analogue CDF (a novel turmeric spice analogue) was able to cause reactivation of miR-200b/c, which in turn resulted in the reversal of the EMT phenotype and thus sensitized pancreatic cancer cells to gemcitabine [81]. Cochrane et al. found that restoration of miR-200c could enhance the sensitivity of the antimicrotubule agent paclitaxel in resistant cancer cells through targeting *TUBB3*, which encodes class III β tubulin [82]. In breast cancer MCF7 cells, the miR-200 family was specifically down-regulated in those cells resistant to two therapeutic agents commonly used in the treatment of breast cancer, doxorubicin or cisplatin [83, 84]. By miRNA microarray, Wang et al. found that miR-200b was identified as the most downregulated miRNA in docetaxel-resistant human lung adenocarcinoma (SPC-A1/DTX) cells compared with the parental (SPC-A1) cells [85]. When examining the miRNA expression from lung adenocarcinoma patients treated with docetaxel-based chemotherapy, aberrant expression of miR-200b was correlated with decreased sensitivity to the antimitotic agent docetaxel [86]. Additionally, miR-200 expression correlated with sensitivity to epidermal growth factor receptor (EGFR) blocking agents in bladder cancer, and restoration of miR-200 increased the sensitivity in mesenchymal-like cell lines [87]. Recently, Zhu et al. reported that the miR-200bc/429 cluster was impaired in vincristine-resistant gastric cancer cells and cisplatin-resistant lung cancer cells. However, the restoration of the miR-200bc/429 cluster could sensitize the tumors cells to chemotherapy. The mechanistic studies suggested that this effect resulted from, at least partially, recurrent apoptosis led by the suppressive effect of miR-200 on two

anti-apoptotic factors, B-cell lymphoma 2 gene (*BCL-2*) and X-linked inhibitor of apoptosis protein gene (*XIAP*)[88].

In summary, aside from the well-documented inhibitory effect on EMT, many novel tumor suppressive signatures of miR-200 have been identified in various human cancer cells, such as inhibition of CSC self-renewal and differentiation, modulation of cell division and apoptosis, and reversal of chemoresistance. Figure 1 illustrates the tumor suppressor roles of miR-200 that have been discussed in this article. These findings will support that miR-200 not only is a valuable biomarker for tumorigenesis and progression, but can also become a potential target for new drug development.

3. Regulation of miR-200 expression

Given that miRNA is a class of non-coding small RNA molecules, the transcription factor involved regulation and epigenetic modulation are two major mechanisms that control miRNA expression. Due to the increased interest in miR-200 as well as its tumor suppressor role in a variety of human tumors, better understanding of miR-200 regulatory mechanisms can provide insights into the future translation of miR-200's tumor suppressive signatures into development of novel strategies for treating human cancer. Here, we will summarize the latest research results on studies of miR-200 expression regulation.

3.1 Transcription factor involved regulation

Regulatory mechanisms for miR-200's expression have been studied in dozens of cell types, during which several essential transcription factors have been determined to be involved. In addition to the master inducers of EMT, ZEB1 and ZEB2 are known as the transcription factors containing zinc-finger domains [24]. Within the putative promoter region of miR-200c/141 and in spacers between the miR-200c and miR-141 stem loops, there are two highly conserved Z-box and four E-box transcription factor binding motifs to which ZEB1 can bind for suppression of this family's polycistronic transcription [89]. Similarly, ZEB1 and ZEB2 can also repress miR-200ba/429 polycistronic transcription by binding to their regulatory E-boxes [90]. Interestingly, ZEB1 and ZEB2 were also demonstrated as targets of miR-200 so that they form a mutually inhibitory feedforward loop as shown in Figure 1A [24-27]. Additionally, two other related transcription factors known to be associated with EMT, Snail and Slug, were shown to be able to negatively regulate transcription of miR-200, providing additional evidence in support of the involvement of miR-200 in EMT [91, 92].

Members of various other transcription factor families have also been shown to be involved in the regulated expression of miR-200. Mizuguchi et al. reported that regulation of miR-200 transcription by ZEB1 could be modulated via the involvement of the transcription factors P300 and PCAF [93]. They found that the activation of miR-200c/141 transcription occurred when P300 and PCAF physically interacted to form a transcriptional complex involving ZEB1, while disruption of the P300-PCAF interaction significantly suppressed the transcriptional activity [93]. A recent study report that the proto-oncogene *c-Myb* was able to upregulate all miR-200 miRNA family members through the transcriptional regulation, whereas this inductive effect could be completely attenuated by ZEB1 at the onset of EMT [94]. A previous study also reported that miR-200b, miR-200c, and miR-429 target *c-Myb* and repress its expression [95]. Therefore, a reciprocal feedback loop may involve the mutual regulation between miR-200 and *c-Myb*. Another factor recently demonstrated to directly modulate the expression of miR-200a and miR-141 is the gene named proline, glutamic acid and leucine rich protein 1 (*PELP1*), a nuclear receptor that can be self-upregulated during the metastatic progression of breast cancer cells [96]. *PELP1* is found to form a complex with histone deacetylating enzymes (*HDAC2*) that binds to the miR-200's

promoters, thereby downregulating their expression [96]. Kim et al. reported that inhibitory effect of P53 on EMT involves the transactivation of miR-200; miR-200 repressing ZEBs is responsible for P53 regulated EMT [97]. In addition, P63 and P73, the members of the P53 transcription factor family, can also directly regulate transcription of miR-200 through binding with the p53/p63/p73 binding sites within the promoter regions of both miR-200 clusters [98]. Ahn et al. recently reported that Smad3 can transcriptionally induce miR-200 in a transforming growth factor β (TGF- β) independent manner, accounting for its suppressive effect on EMT in gastric cancer cells [99].

As discussed earlier, the transcription factors c-Myc, Oct4, and Sox2, which are largely essential for maintenance of pluripotency in stem cells, also play critical roles in regulating miR-200 family expression. In ES cells, c-Myc can upregulate miR-200b that leads to attenuation of ES differentiation [72], whereas in the endometrial cancer cells treated with tamoxifen, miR-200 repressed by elevated c-Myc and associated with the onset of EMT [100]. During iPSC reprogramming from fibroblasts, exogenous Oct4 and Sox2 can bind the promoter regions of the miR-200ba/429 and miR-200c/141 clusters, respectively, to activate their transcription [73], suggesting that miR-200 is involved in reprogramming and stem cell self-renewal, whereas Sox2 and E2F3 can activate the transcription of miR-200c/141 cluster *in vivo* [74]. In addition, the TGF- β pathway ligand, bone morphogenetic protein (BMP7), was shown to synergize with the Yamanaka factors (Oct4, Klf, c-Myc, and Sox2) to induce miR-200 expression during the initial reprogramming phase of iPSC generation involving MET [75]. As such, many different molecular factors have been documented to control the regulated expression of miR-200, which in turn shows variable downstream effects on human diseases.

3.2 Epigenetic modification

Aside from the regulated expression conferred by the aforementioned transcription factors, epigenetic modification in the form of DNA methylation has been determined to control miR-200 family expression in both normal and cancer cells [101]. Substantial cytosine methylation, usually in CpG islands located within the gene's promoter region, has been documented as an epigenetic marker of gene repression. Studies have reported that the regulatory regions of both miR-200 clusters contain CpG-rich sequences. Li et al. reported the epigenetic regulation of miR-200 expression when they showed that miR-200a and miR-200b were hypomethylated and overexpressed in pancreatic cancer cells [52]. Vrba et al. found that in miR-200c/141-negative normal and tumor cells, its CpG island was heavily methylated, whereas in miR-200c/141-expressing cells, the CpG island was unmethylated [101], indicating a role for DNA methylation in miR-200c/141 regulation in both normal and tumor cells. In addition, mouse cells showed a similar correlation between DNA methylation and miR-200c expression. Wiklund et al. found that in muscle invasive bladder tumors and undifferentiated bladder cell lines, both miR-200 clusters were silenced concomitant with DNA hypermethylation, while in oral squamous cell carcinoma, miR-200 was epigenetically activated [29, 45]. More recently, the focal adhesion protein Kindlin 2 was found to form a complex with DNA (cytosine-5-)-methyltransferase 3 alpha (DNMT3A) in the breast cancer cell nucleus to induce CpG island hypermethylation of the miR-200 promoter in order to downregulate the expression of the miR-200 family [102]. It is of note that the aforementioned cases in which DNA methylation was used to downregulate miR-200 expression seemed to correlate with increased tumor formation and/or cell invasion.

In addition to DNA methylation, histone modification is another major class of epigenetic modulation used to regulate expression of the miR-200 family. Tellez et al. reported that a 4-week exposure of immortalized human bronchial epithelial cells to tobacco carcinogens

could induce EMT, most likely due to the silencing of miR-200b, miR-200c and miR-205, which occurred due to methylation initiated at histone H3 (H3K27me3) and later by increasing DNA methylation [103]. Recently, Lim et al. found that in the stem-like phenotype of immortalized human mammary epithelial cells, the miR-200ba/429 cluster was silenced primarily through histone modifications, whereas the miR-200c/141 cluster was repressed via DNA methylation [71]. More recently and interestingly, Zhang et al. found that the H19 long non-coding RNA (lncRNA) is associated with the hnRNPU/PCAF/RNA Pol II complex, which is able to activate the miR-200 family via histone acetylation [104]. Treatment of an endocrine-resistant breast cancer cell line with both a demethylating agent as well as a deacetylating agent resulted in an increase in expression of both miR-200b and miR-200c, leading to a repression of *ZEB1* and showing more epithelial-like characteristics [105]. Thus, it is clear that epigenetic modulation, including DNA methylation as well as histone methylation and acetylation, plays an essential role in the regulated expression of the miR-200 family.

The molecular mechanisms and related factors are mapped on Figure 2, illustrating the transcriptional regulation of miR-200 expression. In addition to the transcription factor involved regulation and epigenetic modification, other mechanisms/factors have also been studied. For example, Iliopoulos et al. reported that miR-200 was differentially regulated by two of the serine/threonine kinase Akt isoforms in breast cancer cells [106]. As they found that miR-200 family expression was decreased in either cells bearing Akt2 or cells of Akt 1 knockdown, but unaltered in cells of Akt2 knockdown or both Akt1 and Akt2 knockdown, it was inferred that miR-200 expression may be dependent on the balance between Akt1 and Akt2 [106]. However, in prostate cancer, silencing of Akt2 is able to inversely induce the expression of miR-200 [107]. These findings suggest that Akt is involved in the regulation of miR-200 expression, although the mechanism of action has not been uncovered.

4. Conclusion and Perspective

The inhibitory effect of miR-200 on EMT by targeting ZEBs is the benchmark achievement to understand the tumor suppressor roles of miR-200 in human cancer. As discussed earlier, the promoter regions of both miR-200 clusters have binding sites for ZEB1 and ZEB2 transcription factors. Interestingly, both ZEBs can be targeted by miR-200, reciprocally, hinting at a possible unknown mechanism by which a feedback inhibition loop can be broken and cells can be switched between an epithelial and mesenchymal phenotype. Given the miR-200 family functioning as a potential suppressor of EMT to prevent malignant tumor progression, future work may improve our understanding of miR-200's role in cancer progression by identification and functional characterization of additional downstream targets of miR-200 that might be involved in multiple cellular events. Moreover, the newly identified tumor suppressor roles of miR-200, such as inhibition of CSC self-renewal and differentiation, modulation of cell division and apoptosis, and reversal of chemoresistance, are of significance to support that miR-200 is a new star miRNA in cancer research. In addition to the updates of new knowledge with regard to the tumor suppressive signatures of miR-200, we sort out the latest findings and summarize the mechanisms involved in the regulation of miR-200 expression. Given the non-coding nature of miRNA, transcription regulation involving multiple transcription factors and epigenetic modulation including DNA methylation and histone modification are major forms accounting for the regulatory basis of miR-200 expression. The understanding of these regulatory mechanisms can not only deepen our understanding of miR-200 anticancer activity, but also aid us in development of novel drugs to target these small molecules.

To date, there are 236 publications collected in PUBMED with regard to miR-200 and cancer; however, more than 75% of these publications are published over the past three

years (from September 2010 to August 2013), which indicates the growing interest in the study of miR-200. Due to the tumor suppressive signature of miR-200, a proposal for restoring miR-200 expression may be taken into consideration as a novel therapeutic to treat human cancer, although the development of such a strategy still depends on a better understanding of the mechanistic basis of miR-200 anticancer activity. In the near future, studying the relationship between miR-200 and CSCs, coupled with improvements of drug delivery systems, may also become a promising area to discover novel therapeutics for treating human cancer efficaciously.

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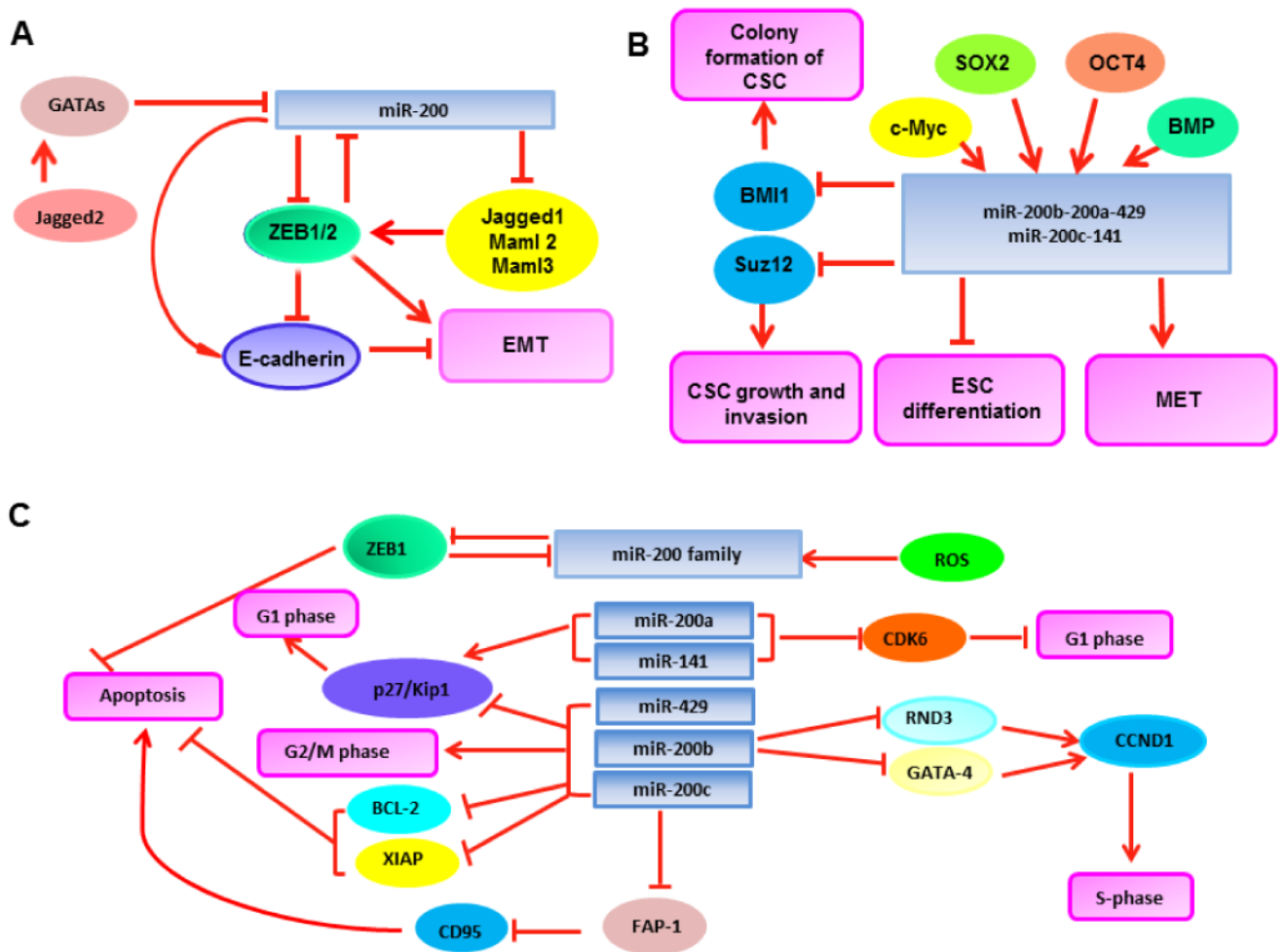


Figure 1. Tumor suppressive signatures of miR-200. (A) MiR-200 inhibits EMT by interacting with ZEB1/2 and the Notch pathway. (B) MiR-200 represses self-renewal and differentiation in CSCs. (C) MiR-200 is involved in the regulation of cell division and apoptosis.

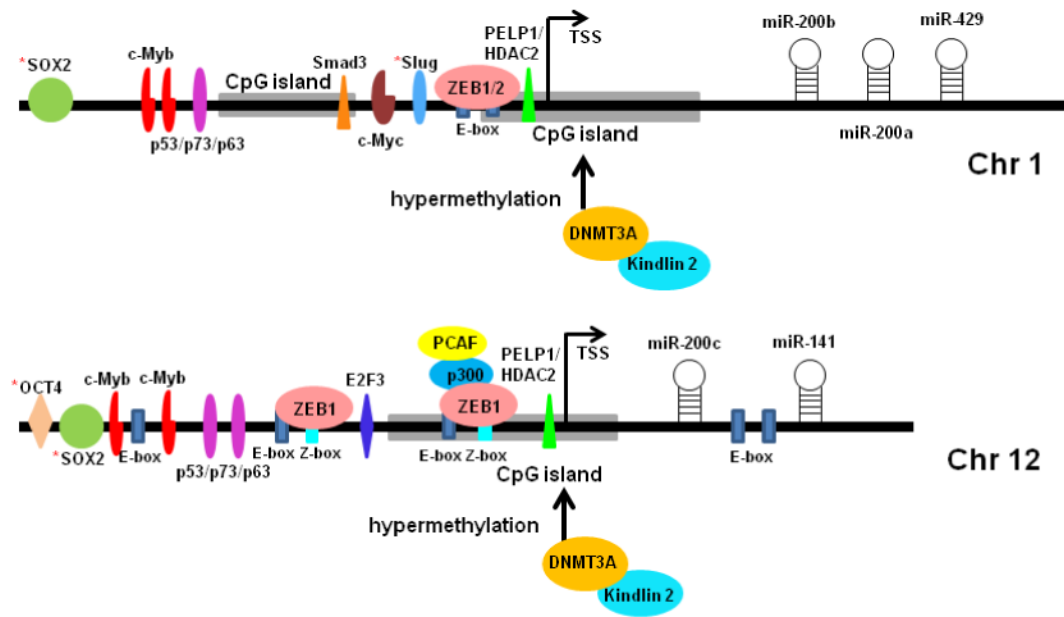


Figure 2. Regulation of miR-200 expression involving multiple transcription factors (TFs) and epigenetic modification. TSS represents transcription starting site. * TFs determined in mouse models.

Table 1
Dysregulation of miR-200 in various human cancers

Members of miR-200 family	Cancer types	References
miR-200a, 200b, 200c, 141	Bladder cancer	[29]
miR-200a, 200b, 200c, 141, 429	Breast cancer	[30-32]
miR-200a, 200b, 200c	Colorectal cancer	[33; 34; 58]
miR-200a, 200c	Cutaneous melanoma	[35]
miR-200a, 200b, 200c, 141, 429	Endometrial cancer	[36; 37]
miR-200a, 200b, 200c, 141	Gastric cancer	[38-41]
miR- 200c	Hepatocellular tumor	[42]
miR-200a, 200b, 200c, 141, 429	Lung cancer	[43; 59]
miR-200a	Nasopharyngeal carcinoma	[44]
miR-200a, 200b, 200c, 141, 429	Oral squamous cell carcinoma	[45]
miR-200a, 200b, 200c, 141, 429	Ovarian cancer	[46-51]
miR-200a, 200b	Pancreatic cancer	[52; 60]
miR-200b, 200c, 141, 429	Pleural mesothelioma	[53]
miR-200a	Prostate cancer	[54]
miR-200a, 200b, 200c, 141	Renal cell carcinoma	[55]
miR-200a, 200c, 141, 429	Spindle cell carcinoma of the head and neck	[56]
miR-200a, 200b, 200c, 141	Thyroid carcinoma	[57]