Members of the microRNA-200 family are promising therapeutic targets in cancer (Review)

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Abstract. MicroRNAs (miRs) are non-coding, single-stranded RNA molecules that regulate gene expression at the post-transcriptional level. Abnormal expression of miR may result in pathophysiological processes occurring that stimulate the development of various diseases. miRs are commonly dysregulated in cancer and may act as either oncogenes or tumor suppressors. Studies have indicated that members of the miR-200 family are involved in different aspects of cancer biology, including the epithelial-to-mesenchymal transition, tumor angiogenesis and chemoresistance by targeting and repressing the expression of several key messenger RNAs. The present review aims to summarize the role of the miR-200 family and its potential mechanism of action in tumor progression, which may advance the development of novel therapeutic drugs against tumor metastasis in clinical cancer treatment.

Contents

1. Introduction

The landmark study of *lin-4* in *Caenorhabditis elegans* identified a novel class of molecules called microRNA (miR), which are small non-coding RNAs consisting of 18-25 nucleotide base pairs (1) These small nucleic acids regulate gene expression by binding to the 3' untranslated region (3'-UTR) of mRNA, resulting in translational repression or transcript degradation (2). Over 2,500 miRs have been identified in the human genome since their discovery in 1993 and it has been determined that 30-50% of genes that code for proteins are controlled by miR in humans (3). Thus, miRs have emerged as integral components of various biological processes, including cell proliferation, migration, differentiation, apoptosis and angiogenesis (4). It has been demonstrated that the altered expression of miR is associated with tumorigenesis and the progression of different types of cancer (5,6). By regulating multiple potential target genes, miR expression may lead to pathological changes in cells, ultimately contributing to the development of cancer (7).

One particular family of miR, the miR-200 family, has been identified to be crucial in tumorigenesis. Members of the miR-200 family are downregulated in aggressive human tumors and target different signaling pathways including the Notch, Wnt and transforming growth factor β (TGF-β) pathways, thus inhibiting migration, tumor cell adhesion, epithelial-to-mesenchymal transition (EMT) and angiogenesis (8,9). The present review will focus on summarizing the roles of the miR-200 family as putative tumor suppressors in tumor progression and propose that the restoration of miR-200 expression may have therapeutic implications for the clinical treatment of metastatic and drug-resistant tumors.

2. Studies of miR-200 family in human cancer

The miR-200 family consists of five members (miR-200a, -200b, -200c, -141 and -429), which are clustered and expressed as two separate polycistronic pri-miR transcripts (miR-200b-200c-429 and miR-200a-141) located on human chromosomes 1 and 12, respectively (10). miR-200b, -200c and -429 contain AAUACUG as their seed sequences, whereas miR-200a and -141 possess AACACUG (11). Due to the similarity of their seed sequences, they may have similar target genes (Table I). A series of studies using superior profiling technologies, such as a combination of miRNA expression arrays, quantitative polymerase chain reaction assays and mass spectrometry DNA methylation analysis, have indicated that the miR-200 family is aberrantly expressed in different types of cancer. Multiple studies utilizing different detection

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Table I. Chromosomal localization and sequences of the miR-200 family.

miR	Sequences (5'-3')	Chromosomal localization
hsa-miR-200b	UAAUACUGCCUGGUAAUGAUGAC	Chromosome 1p36
hsa-miR-200c	UAAUACUGCCGGGUAAUGAUGG	Chromosome 1p36
hsa-miR-429	UAAUACUGUCUGGUAAAACCGU	Chromosome 1p36
hsa-miR-200a	UAACACUGUCUGGUAACGAUGU	Chromosome 12p13
hsa-miR-141	UAACACUGUCUGGUAAAGAUGG	Chromosome 12p13

miR, microRNA.

platforms, which compared extensive sets of tumor tissues and cell lines to large sets of normal control samples, identified that the differential expression of members of the miR-200 family serves an important role in combating tumor cell invasion, EMT and metastasis (8,12). Furthermore, numerous studies have demonstrated that members of the miR-200 family may be associated with pathogenesis and disease prognosis (12-28) (Table II). Therefore, the miR-200 family has the potential to become a novel class of biomarker for tumor prognosis and targets of novel drugs against tumor progression.

3. miR-200 family and tumor metastasis

Different types of miR become deregulated in tumors as a result of various mechanisms, including miR-200c deregulated in ovarian cancer and miR-21 deregulated in breast cancer (29,30). Genomic abnormalities, including deletion, amplification and translocation, are common in tumorigenesis, and miR expression may also be affected by transcriptional and post-transcriptional regulation (7). Many pri-miR are either oncogenes or tumor suppressors and are induced by transcription factors (7). It has been demonstrated that various transcription factors including p53, c-Myc and E2 transcription factor (E2F), are closely associated with miR in cancer (31). The processing and stability of miR are also important factors determining miR expression. Additionally, levels of Dicer or Drosha expression, the miR processing machinery, are altered and transformed in various types of cancer, possibly due to the increase in the copy number of miR (32).

Metastasis is an important characteristic of malignant tumors and EMT is the initial step of metastasis. The miR-200 family inhibits EMT and maintains the epithelial phenotype by directly targeting the transcriptional repressors of E-cadherin [zinc finger E-box-binding homeobox (ZEB)1 and ZEB2] (33).

The primary step and important characteristics of tumor metastasis are the disassembly of tight junctions and loss of apical-basal polarity among cancer cells (8). The loss of epithelial markers and the gain of mesenchymal morphological features in cancer cells contributes to the suppression of the transmembrane adhesion receptor E-cadherin and a gain in the expression of mesenchymal markers, including vimentin, collagen, fibronectin, and the E-cadherin transcriptional repressors ZEB1 and ZEB2 (also known as SMAD-interacting protein 1) (9). These vital molecules cause the extracellular matrix-induced stimulation of the integrin signal pathway, resulting in focal adhesion formation, which facilitates cancer cell migration, invasion and metastasis (8). The transcriptional factors ZEB1 and ZEB2 induce EMT by repressing the expression of E-cadherin and promoting cancer cell migration, invasion and metastasis (9). TGF-β also serves an important role in the EMT in epithelial cells as it commands cell proliferation and differentiation during the process of embryonic development or cancer progression (34).

4. miR-200 family and tumor angiogenesis

It is widely accepted that angiogenesis, the formation of new blood vessels from pre-existing ones, is a fundamental process required for cancer development and growth (26). Without angiogenesis, cancer cells inside the tumor undergo apoptosis. The angiogenesis switch depends on the balance of angiogenesis activators and inhibitors. The activation of angiogenesis is initiated when pre-existing vessels become permeable in response to stimulating factors, including vascular endothelial growth factor (VEGF), placental growth factor and angiopoietin-1 (35). VEGF is considered to be the most well known fundamental factor and modulator of angiogenesis. VEGF combined with its receptors [fms-like tyrosine kinase 1 (flt1) or VEGF receptor (VEGFR)-1 and kinase-insert domain containing receptor (KDR) or VEGFR-2], stimulates endothelial cell migration, proliferation and survival (36). As angiogenesis is essential for tumor growth, inhibiting VEGF signaling using strategies such as small interfering RNA, small molecule inhibitors, antibodies and VEGF-traps is a promising therapeutic approach for cancer treatment. Furthermore, research has demonstrated that miR-200 family members are involved in the regulation of vascular development and angiogenesis by downregulating VEGF signaling (37). By using a clear cell renal cell carcinoma and matched normal kidney sample, Liu *et al* (38) identified a negative correlation between VEGF and the miR-200 family. Choi *et al* (39) demonstrated that VEGF and its receptors, Flt1 and KDR, were the key participants in the VEGF signal pathway and were negatively regulated by miR-200b, which directly targeted the 3'-UTR of those genes. Tube formation and phosphorylation of extracellular signal-regulated kinases 1/2 were inhibited if miR-200b was transected into human umbilical vein endothelial cells, suggesting that miR-200b has anti-angiogenic activity (39).

Table II. Studies of miR-200 in various types of human cancer.

Cancer types	Samples	Conclusions made by authors	(Refs.)
Bladder cancer	57 bladder tumors 11 normal bladder urothelium	miR-200c is correlated with early stage T1 bladder tumor progression	(14)
Breast cancer	Breast cancer cell lines (MCF-7, T47D, MDA-MB-231) HC11 mammary epithelial cells	miR-200a and -200b inhibit EMT characteristics in the undifferentiated, non-tumorigenic HC11 cells	(15)
Colorectal cancer	54 primary colorectal cancer tissues 54 matched liver metastasis tissues	miR-200c has an important role in mediating EMT and metastatic behavior in the colon	(25)
Melanoma	23 primary melanomas	Decreased miR-200a, -200c and -203 correlated with increasing thickness in melanomas	(26)
Endometrial cancer	17 endometrial cancer tissues 11 normal endometrial tissues	miR-200 family is highly expressed in endometrial cancer and has an important role in cancer growth	(16)
Gastric cancer	35 gastric neoplastic tissues 35 adjacent non-neoplastic tissues 5 gastric cancer cell lines	miR-141 may be involved in the development of gastric cancer through its inhibitory effect on cell proliferation	(17)
Hepatocellular cancer	55 hepatocellular cancer tissues 30 nontumor liver samples	miR-200 was deregulated in hepatocellular tumors	(18)
Pancreatic cancer	99 pancreatic cancer tissues 14 pancreatic cancer cell lines	miR-200c may have a role in pancreatic cancer biology and may be a novel marker for the prognosis of pancreatic cancer	(27)
Anaplastic thyroid carcinomas	3 anaplastic thyroid cancer tissues 3 normal thyroid sample	Inhibition of TGF-β receptor 1 in thyroid cancer cells induced EMT and caused an increase of the miR-200 family	(13)
Head and neck carcinoma	45 spindle cell carcinomas of head and neck 45 squamous cell carcinomas of head and neck	Downregulation of miR-200 family supports the postulated role of EMT in spindle cell carcinoma of head and neck	(12)
Renal cell carcinomas	33 human renal clear cell cancer tissues human epithelial kidney cell line	Dysregulation of miR-200/EMT confers to immortalized pre-tumoral cells phenotypic traits of metastatic potential in renal cell carcinoma	(24)
Prostate cancer	18 prostate cancer samples with no relapse 18 matched-cancer samples with relapse	Overexpression of miR-200a reduced prostate cancer cell growth	(23)
Malignant pleural mesothelioma	100 mesothelioma tumor samples 32 lung adenocarcinoma tumor samples 4 healthy lung tissue samples	miR-200 was downregulated in malignant pleural mesothelioma compared to lung adenocarcinoma	(22)
Ovarian cancer	20 serous ovarian cancer tissues 8 normal ovarian tissues	Dysregulation of miR-200 is involved in ovarian carcinogenesis and associated with the prognosis of serous ovarian carcinoma	(21)
OSCC	25 OSCC cancer tissues 25 adjacent non-cancer tissues 8 normal control paired oral stroma and epithelium	The suppression of miR-200 may drive tumor expansion and progression in OSCC	(20)

Table II. Continued.

Cancer types	Samples	Conclusions made by authors	(Refs.)
NPC	C666-1 (EBV-positive NPC cell line) CNE-1, CNE-2 and HNE1 (NPC cell lines)	miR-200a as a regulatory factor of NPC carcinogenesis and a potential candidate for miRNA-based therapy against NPC	(28)
NPC	C666-1 (EBV-positive NPC cell line)	miR-200a as a regulatory factor of NPC carcinogenesis and a	(28)
Lung cancer	130 lung squamous cell carcinoma samples Small airway epithelial cells Normal human bronchial/tracheal epithelial cells H1299 (human non-small cell lung cancer cell line) BEAS-2B (human immortalized lung epithelial cell line)	miR-200 family was functionally involved in canonical pathways of immune response, molecular mechanisms of cancer, metastasis signaling, cell-cell communication, proliferation and DNA repair in lung cancer	(19)

miR, microRNA; EMT, epithelial-to-mesenchymal transition; HEK, human epithelial kidney; OSCC, oral squamous cell carcinoma; NPC, nasopharyngeal carcinoma; TGF-β, transforming growth factor β.

Furthermore, it is understood that intratumoral ligands establish a complex network of cell-cell interactions within the tumor microenvironment. Roybal *et al* (40) determined that Flt1/VEGFR1 was a candidate miR-200 target gene. Overexpression of miR-200 inhibited angiogenesis in metastasis-prone lung adenocarcinoma cells derived from *K-ras*/*p53*-mutant mice by inhibiting angiogenesis, thus decreasing Flt1 levels (40). These results indicate that lung adenocarcinomas with low levels of miR-200 expression may be responsive to treatment with anti-VEGF agents (40). In some chronic non-cancer non-healing diseases, Chan *et al* (41) reported that hypoxia inhibited miR-200b expression in human dermal microvascular endothelial cells, thus promoting angiogenesis. Furthermore, it was identified that down-regulation of endothelial miR-200b is crucial in stimulating cutaneous wound angiogenesis by attenuating the repression of GATA binding protein 2 and VEGFR2 expression (42). These results identified that GATA2 was a target of miR-200b, which provided novel insight regarding the regulation of wound angiogenesis by miR-200 and the significance of such regulation in the context of chronic cutaneous wounds (42). Additionally, it was demonstrated that in lung, renal, ovarian and basal-like breast cancer, miR-200 inhibits angiogenesis via direct and indirect mechanisms by targeting interleukin-8 and C-X-C motif chemokine ligand 1 secretion by tumor endothelial cells (43). Thus, the miR-200 family may provide a potential anti-angiogenesis therapy to treat cancer and other diseases dependent on angiogenesis, as inhibition of VEGF signaling interferes with angiogenesis.

5. miR-200 family and chemosensitivity

Chemotherapy drug resistance often inhibits the clinical treatment of cancer, resulting in a poor prognosis for patients with cancer. MiR-200 family members are involved in maintaining sensitivity to microtubule targeting agents and DNA damaging drugs, which are two classes of chemotherapeutics (44). Gibbons *et al* (34) identified an association between miR expression and cancer chemosensitivity in cholangiocarcinoma. It has been demonstrated that miR-21 and -200b are involved in the regulation of tumor cell sensitivity to gemcitabine by targeting specific genes, including Circadian Locomotor Output Cycles Kaput, phosphatase and tensin homolog and protein-tyrosine phosphatase 1B, as well as downstream oncogene products, including c-Abl, Src and Ras (34,45). Furthermore, it has been suggested that there are similarities between drug-resistant cancer cells and enhanced invasiveness or metastasis, which is consistent with the dysregulation of miR-200 in drug resistant cancer cells (34,44,45). For example, decreased expression of miR-200b and -200c has been observed in the acquired cisplatin resistant phenotype of MCF-7 human breast adenocarcinoma cells (46). It was reported that aberrant miR expression participated in the regulation of cell survival, cell conductive signaling, invasiveness and DNA methylation, implying that abnormal miR expression was associated with the unusual activity of cellular processes in cisplatin-resistant breast cancer cells (46). In a study investigating miR expression profiles, the expression of miR-200b was significantly downregulated in a docetaxel-resistant human non-small cell lung cancer (NSCLC) cell line (SPC-A1/docetaxel) compared

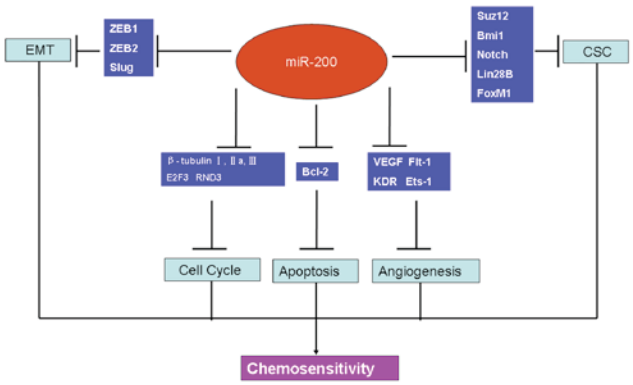

Figure 1. Targets of miR-200 involved in cancer chemoresistance. The verified targets of miR-200 are associated with cancer chemosensitivity through EMT, the cell cycle, apoptosis, angiogenesis and CSC maintenance. miR, microRNA; EMT, epithelial-to-mesenchymal transition; CSC, cancer stem cell; ZEB, zinc finger E-box-binding homeobox; E2F3, E2F transcription factor 3; RND3, Rho family GTPase 3; Bcl-2, B-cell lymphoma 2; VEGF, vascular endothelial growth factor; Flt-1, fms-like tyrosine kinase 1; KDR, kinase-insert domain containing receptor.

with a control SPC-A1 cell line (47). The results suggested that measuring miR-200b expression may provide an explanation for drug sensitivity or resistance in docetaxel-resistant cells in patients with NSCLC (47). Additionally, Chen *et al* (48) demonstrated that restoring expression of miR-200b was able to reverse chemoresistant phenotypes of docetaxel-resistant cells in human lung adenocarcinoma (LAD) by targeting E2F transcription factor 3 using histone deacetylase (HDAC) inhibitors. These findings suggest that the HDAC1/miR-200b/E2F3 signal pathway may be responsible for chemoresistance in docetaxel-resistant LAD cells (48). Furthermore, members of the miR-200 family may be potential therapeutic targets for disseminated or chemoresistant cancer cells in ovarian cancer (49).

miR-200 family members serve a vital role in regulating cancer chemosensitivity. The association between miR-200 and cancer chemoresistance may be explained by a number of factors, including EMT, cancer stem cell (CSC) maintenance, apoptosis, angiogenesis and cell cycle distribution (Fig. 1). It has been demonstrated that miR-200 family members are critical determinants in the EMT process via targeting ZEB transcription factors (11,50,51). Following this, the expression of clusters of genes, including E-cadherin and vimentin, were controlled (11,50,51). CSCs are defined as a small population of cells that possess the capacity of self-renewal and generate differentiated progeny in tumors. The acquisition of an EMT phenotype and the induction of CSC phenotypes have biological functions in common and these similarities synergistically contribute to cancer chemoresistance. Thus, the properties of CSCs partly gave rise to EMT in cancer metastasis.

It is widely understood that angiogenesis is essential for tumor progression. VEGF is a fundamental regulatory molecule in tumor angiogenesis and VEGF function may be reduced by the use of antiangiogenic agents to inhibit VEGFR, which means that reducing VEGF function may result in a decrease in interstitial fluid pressure and improved blood vessel morphology recovery (52). Therefore, decreased tumor blood supply combined with lower chemotherapy drug delivery may reduce the sensitivity of cancer cells to chemotherapy, resulting in the development of chemoresistance. The B-cell lymphoma 2 (Bcl-2) protein family is associated with multi-drug resistance (53). One of the most important and common features of tumor cells is resistance to apoptosis signaling, which generates multi-drug resistance (53). The miR-200 family is able to reduce resistance to apoptosis and drugs in cancer cells by targeting Bcl-2 (54). Furthermore, Bcl-2 expression was reduced by miR-200 in gastric carcinoma, which led to enhanced apoptosis (54). Chemotherapy resistance is mediated by the cell cycle, and sensitivity to drugs or agents in cancer treatment may be attributed to the stage of the cell cycle in which the cells are (55). The miR-200 family may affect cell cycle distribution by targeting β-tubulin, E2F3 or Rho family GTPase 3 (55,56).

6. Signals regulated by the miR-200 family in cancer

The signaling of EMT is considered to be active throughout tumorigenesis and tumor progression. It has been demonstrated that overexpression of miR-200 inhibits the capacity of tumor cells to undergo invasion and metastasis, highlighting the role of miR-200 in the regulation of EMT and subsequent metastasis (57). Notch signaling is a crucial pathway involved in embryogenesis and is responsible for the self-renewal abilities of embryonic stem cells (58). In various solid tumors, including lung, pancreatic, breast carcinoma and malignant melanoma, aberrant activation of Notch signaling is frequently observed (58). Mechanistic studies supporting that Notch signaling serves a crucial role in regulating EMT and metastasis during cancer progression have also identified that miR-200 decreases expansion of human metastatic prostate cancer cells by targeting the Notch ligand, jagged (JAG) 1, and the mastermind-like transcriptional coactivators (Maml) 2 and 3 (58,59) Additionally, the Notch ligand JAG2 was able to inhibit miR-200 family expression at the transcriptional level by inducing GATA transcription factors, thus stimulating tumor progression (60).

It has been determined that TGF-β, a profibrotic cytokine, is the primary pathogenic driver in tubular epithelial cells and is able to induce EMT (61). The pathway of TGF-β/SMAD signaling is considered to be a classical pathway that induces EMT. This inhibits tumor development at the early stage of cancer, yet promotes tumor progression at the advanced stages of cancer (61). miR-200 family members suppress TGF-β/SMAD signaling, promote epithelial gene expression and suppress cell invasion by regulating a network of genes (61). In a study on gastric cancer, miR-200 was downregulated by CpG island methylation and TGF-β signaling, which increased Zeb1/2 expression and decreased E-cadherin expression to promote cancer cell migration and invasion, providing powerful evidence supporting the application of decitabine in clinical cancer treatment as decitabine inhibits methylation (62).

The complicated mutual transcriptional regulation of feedback ZEB/miR-200 loops and TGF-β signaling is involved in the interaction between ZEB and TGF-β protein (13,63). The mechanisms of the autocrine TGF-β/ZEB/miR-200 loop signaling regulatory network to control cell plasticity between the epithelial and mesenchymal states may include: i) The regulation of ZEB2 transcription induced by TGF-β

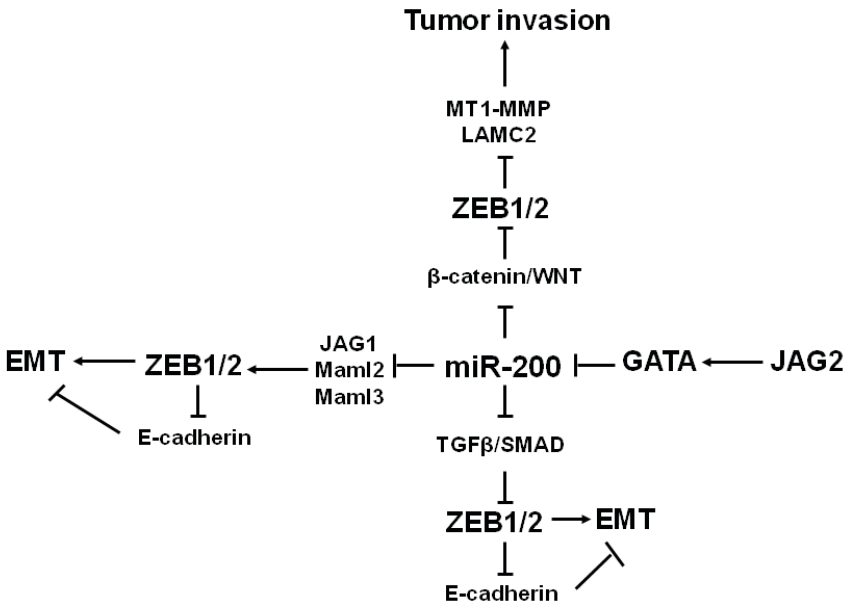

Figure 2. miR-200/ZEB and cancer-associated signaling pathways. miR, microRNA; ZEB, zinc finger E-box-binding homeobox; EMT, epithelial-to-mesenchymal transition; Maml, mastermind-like transcriptional coactivators; TGF, transforming growth factor; JAG, jagged; MT1-MMP, membrane-type 1 matrix metalloproteinase; LAMC2, laminin gamma chain 2.

by SMAD, which may be directly targeted by the ZEB2 gene promoter *in vivo* (13,63) and ii) the suppression of miR-200 loci was recovered by reversible DNA methylation of ZEB proteins participating in the process of recruitment of histone-modifying complexes (64).

Furthermore, it has been demonstrated that Wnt signaling is functionally correlated with the ZEB/miR-200 loop in tumors (65). A functional conserved miR-200a-binding site is contained in the 3' UTR of β-catenin mRNA, meaning that miR-200a is able to directly target and bind to this position to suppress the β-catenin/Wnt signaling pathway. This was observed in tumorigenesis for human solid tumors, including hepatocellular carcinoma, melanoma, colon, ovarian and prostate cancer (66). Nuclear accumulation of β-catenin is linked with EMT in invasive colorectal tumors, and the association between the Wnt pathway and EMT has been investigated (67). Levels of ZEB1 expression have no influence on β-catenin/transcription factor 4 transcriptional signaling in colon cancer cell lines, although ZEB1 immunopositivity has been confirmed in non-invasive colon cancer cells and in tumor-associated fibroblasts (67). Furthermore, ZEB1 influenced the expression of other key proteins mediated by the Wnt signaling pathway, including membrane-type 1 matrix metalloproteinase and laminin gamma chain 2 (LAMC2) (59). Therefore, indicating that LAMC2 and all proteins mediated by the Wnt signaling pathway were implicated in the invasion and dissection of colorectal cancer cells (67). Additionally, in human meningioma tumor tissues, the expression of miR-200a was negatively associated with β-catenin and cyclin D1 (68). The aforementioned signals regulated by members of the miR-200 family in cancer are summarized in Fig. 2.

7. Future directions and concerns

Although much insight has been gained regarding the role of the miR-200 family in tumorigenesis and cancer progression, there is still a long way to go to fully comprehend and take advantage of miR utilizations in tumor therapeutics. Identifying unique patterns of deregulated expression of miR-200 family members may provide more significant information on the involvement of miR-200 family members in cancer. For example, miR-200 family members may act as molecular tumor markers for cancer initial diagnosis, confirm the degree of risk for cancer patients and to predict cancer prognosis and clinical responses to certain therapeutic strategies.

Furthermore, at the mechanistic level, despite some understanding of the miR-200/ZEB loop and signaling, further studies are required to define the elusive role of the miR-200 family in cancer pathogenesis, particularly in appropriate cellular and animal models. At the clinical level, further studies based on statistically valid experimental designs and selection of highly characterized case materials are required, which may identify the appropriate tumor marker and novel therapeutic strategies for improved cancer diagnosis and treatment.

In conclusion, tumor invasion and metastasis are responsible for cases of carcinoma-associated mortality. It is understood that EMT and tumor angiogenesis are critical steps in tumor invasion and metastasis. Therefore, targeting these processes may be a promising therapeutic strategy to treat cancer. Encouragingly, the miR-200 family members are key regulators of the epithelial phenotype, with targets involved in many aspects of EMT. In the future, improving understanding of the regulation and function of miR-200 family members in EMT, tumor angiogenesis and metastatic processes may aid

in the development of a more effective method of attenuating cancer metastasis. Further understanding regarding the role of miR-200 in cancer progression and the development of more efficient miR regulatory molecules to treat cancer may vastly improve the clinical treatment of tumors.

Acknowledgments

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