Cancer Microenvironment (2010) 3:137–147
DOI 10.1007/s12307-010-0037-4

REVIEW PAPER

The Roles of MicroRNAs in the Cancer Invasion-Metastasis Cascade

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Received: 18 November 2009 / Accepted: 3 January 2010 / Published online: 23 February 2010

Abstract Cancer metastasis results from a multi-step cascading process that includes: 1) vascularization of the primary tumor; 2) detachment and invasion of cancer cells; 3) intravasation into lymphatic and blood vessels; 4) survival and arrest in the circulation; 5) extravasation into distant organs; and 6) colonization and growth of metastatic tumors. microRNAs (miRNAs) play critical roles in this multi-step process, both promoting and suppressing metastasis. This review updates the progress made in understanding the roles of miRNAs for invasion and metastasis during cancer progression. A specific miRNA signature of cancer metastasis is also reviewed.

Keywords miRNAs · Cancer metastasis · Invasion · Cancer stem cells and angiogenesis

Introduction

For most solid malignancies, metastasis is the predominant cause of cancer death [1]. Elucidation of the molecular mechanisms that regulate the sequential steps of metastasis is critical for the reduction of mortality by cancer. Cancer metastasis is the process by which cancer cells spread from a primary tumor to other non-adjacent organs and tissues, forming viable secondary deposits of cancer. Cancer cells spread to other organs through a multi-step process that includes: 1) progressive vascularization and growth of the primary tumor; 2) detachment and invasion of cancer cells; 3) intravasation into lymphatic and blood vessels; 4) survival and arrest in the circulation; 5) extravasation into a new microenvironment; and 6) colonization and growth of metastatic tumors [1, 2].

Angiogenesis permits the initial growth of primary tumors and the survival and growth of metastatic tumor colonies [1, 2]. During detachment and invasion, cancer cells secrete matrix metalloproteinases (MMPs), which undergo an epithelial-mesenchymal transition (EMT) and acquire both motility and invasiveness [1, 3]. While EMT has been found to impact cancer cell intravasation, mesenchymal-epithelial transition (MET) may influence cancer cell extravasation. The properties of cancer stem cells (CSC) and organ-specific gene expression may dictate the successful rate of colonization of metastatic tumors.

The microRNAs (miRNAs) are small 19–25 nucleotides non-coding RNAs that can modulate gene expression by hybridizing to complementary target mRNAs, resulting in either translation inhibition or mRNA degradation [4–12]. Discovery of miRNAs has provided a novel mechanism for regulating human gene expression that impacts diverse biological and pathological processes, including development, cell proliferation, differentiation, apoptosis and tumorigenesis [6–11]. Recent data clearly demonstrates that miRNAs can function as both metastasic activators and suppressors by critically regulating various stages of migration and invasion [13, 14]. Several excellent reviews have covered this exciting area [15–18]. In the present review, recent progress made in understanding the roles of miRNAs in regulating the cancer invasion-metastasis

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cascade is highlighted. A specific miRNA signature of cancer metastasis is also reviewed.

miRNAs Modulate Cancer Angiogenesis

The formation of new blood vessels is essential for the initial growth of primary cancers that are more than 1–2 mm in diameter [1]. Once microcirculation is established, tumor-associated blood vessels provide an important route for cancer metastasis [1]. Recent studies suggest that miRNAs can regulate angiogenesis. Several miRNAs have been identified that exert proangiogenic or antiangiogenic effects and these are summarized in Table 1 and Fig. 1. miR-221 and miR-222 are highly expressed in endothelial cells [19]. Through directly regulating downstream targets, such as c-kit, p27Kip1, p57Kip2 and cyclin G1, miR-221 and miR-222 impact migration and proliferation of endothelial cells [20–22]. While c-kit has been well recognized for its importance in stem/progenitor cell proliferation, p27Kip1 has also shown to have oncogenic function to increase the numbers of stem/progenitor cells in the blood, retina, lung, and glial systems [23]. Thus, miR-221 and miR-222 may play direct roles in regulating the circulating endothelial progenitors. The miR-15a-16-1 cluster can promote apoptosis, as well as inhibit cell proliferation and VEGF expression by targeting Bcl-2, cyclin D1, wingless-type MMTV integration site family member 3A (WNT3A), AKT serine/threonine-protein-kinase (AKT3), ribosomal-protein-S6, MAP-kinases, and NF-kappaB activator MAP3-KIP3 [24–27]. miR-122 targets a known promoter of metastasis, a disintegrin and metalloprotease 17 (ADAM17), and inhibits both tumor angiogenesis and cancer cell migration/invasion [28].

While some miRNAs can inhibit angiogenesis, other miRNAs can stimulate new vessel formation. miR-126 is an endothelial-specific miRNA, which modulates vascular endothelial growth factor (VEGF) levels and endothelial cell proliferation by directly repressing the sprouty-related protein SPRED1 and phosphoinositol-3 kinase regulatory subunit 2 (PIK3R2) [29, 30]. Knockout of miR-126 leads to loss of vascular integrity and neoangiogenesis [29, 30]. Despite its positive role in angiogenesis, miR-126 appears to inhibit cancer cell growth, proliferation, adhesion and invasion, and is downregulated in colon, lung and breast cancers (Table 2 and Fig. 1) [31–33]. miR-296 can modulate the expression of VEGF receptor 2 and platelet-derived growth factor (PDGF) receptor β by directly targeting the hepatocyte growth factor-regulated tyrosine kinase substrate (HGS), which mediates degradation of the growth factor receptors [34]. The miR-17-92 cluster, which contains miR-17, miR-18, miR-19a, miR-19b-1, 20a and miR-92-1, is the first oncogenic miRNAs identified in

Table 1 miRNAs involved in angiogenesis

miRNAs	Targets	Molecular regulation	Deregulation in cancer	Refs
Anti-angiogenic miRNAs				
miR-221/222	c-kit, cyclin G1, p27Kip1, p57Kip2	Endothelial cell proliferation/ migration	Decreased	[19–22]
miR-15a-16-1	Bcl-2, CCND1, WNT3A, AKT3 S6, MAP3KIP3	VEGF level, cell proliferation/ survival	Decreased	[24–27]
miR-122	ADAM17	Unknown	Decreased	[28]
Pro-angiogenic miRNAs				
miR-17–92 cluster	TSP-1, CTGF, SIRT1, Rap-1, $S1P_1$, MKK4, integrin	Endothelial cell proliferation/migration, proangiogenic molecules	Increased or Decreased	[39, 40]
miR-126	SPRED1, PIK3R2	VEGF level, endothelial cell proliferation	Decreased	[29, 30]
miR-296	HGS	VEGFR2 and PDGFRβ	Increased	[34]
miR-378	Sufu, Fus-1	VEGF, Ang-1 and Ang-2	Increased	[41]
miR-210	Ephrin-A3	Endothelial cell proliferation/migration	Increased	[45, 46]
miR-130a	HOXA5, GAX	Endothelial cells proliferation/ migration, tube formation	Increased	[47]
miR-143-145	ACE, Tpm4	VSMCs, vessel wall	Increased	[48]

ACE angiotensin-converting enzyme; *ADAM17* a disintegrin and metalloprotease 17; *AKT3* AKT serine/threonine-protein-kinase 3; *Ang-1/2* angiopoietin-1/2; *CCND1* cyclin D1; *CTGF* connective tissue growth factor; *Fus-1* fusion 1 protein; *GAX* growth arrest homeobox; *HGS* the hepatocyte growth factor-regulated tyrosine kinase substrate; *HOXA5* homeobox protein A5; *MAP3KIP3* MAP-kinases, and NF-kappaB-activator; *MKK4* the mitogen-activated kinase kinase 4; *PDGFRβ* platelet-derived growth factor (PDGF) receptor β; *PIK3R2* phosphoinositol-3 kinase regulatory subunit 2; *Rap-1* the small guanosine triphosphate-binding protein; $S1P_1$ the sphingosine-1-phosphate receptor 1; *S6* ribosomal-protein-S6; *SIRT1* silent mating type information regulation 2 homolog 1; *SPRED1* sprouty-related, EVH1 domain containing 1; *Sufu* suppressor of fused; *Tpm4* tropomyosin 4; *TSP1* thrombospondin-1; *VEGF* vascular endothelial growth factor; *VEGFR2* VEGF receptor 2; *VSMCs* murine vascular smooth muscle cells; and *WNT3A* wingless-type MMTV integration site family member 3A

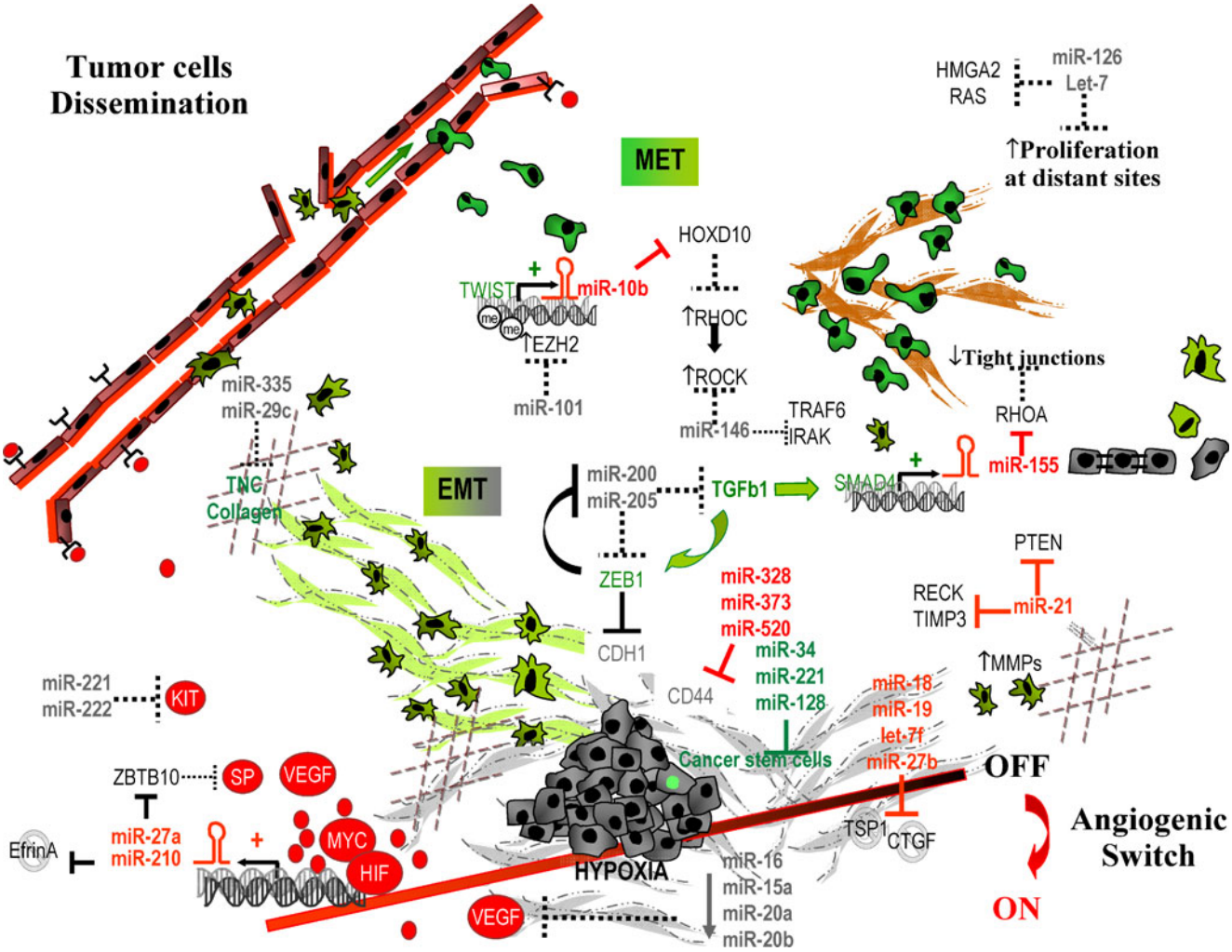

Fig. 1 A network of microRNAs and protein-coding genes involved in the multiple steps of cancer metastasis. For details see text

human [35]. Although the major known function of the miR-17-92 cluster is related to transcriptional factors c-Myc, E2F and their autoregulatory loop [36–38], this cluster also enhances tumor angiogenesis by targeting thrombospondin-1 (TSP1), connective tissue growth factor (CTGF) and a number of proangiogenic targets including the histone deacetylase SIRT1, the small guanosine triphosphate-binding protein Rap-1, the sphingosine-1-phosphate receptor 1 ($S1P_1$), the mitogen-activated kinase kinase 4 (MKK4) and integrin subunits $\alpha 5$ and αv [39, 40]. miR-378 can induce Sonic hedgehog (Shh) signaling, expression of VEGF and angiopoietin-1 (Ang-1) and -2 (Ang-2) and promote large-diameter vessel formation by directly targeting two tumor suppressors, suppressor of fused (Sufu) and Fusion 1 protein (Fus-1) [41, 42]. miR-210, a hypoxia-induced miRNA identified by three independent reports [24, 43, 44], seems to play a crucial role in regulating tube formation and survival of endothelial cells under hypoxic conditions by depressing Ephrin-A3 [45, 46]. Two anti-angiogenic homeobox proteins, HOXA5 and growth arrest homeobox (GAX) in vascular endothelial cells, are regulated by miR-130a [47]. The miR-143-145 cluster promotes the contractile phenotype of murine vascular smooth muscle cells (VSMCs) and improves vascular wall support/integrity by regulating angiotensin-converting enzyme (ACE) and tropomyosin 4 (Tpm4) [48].

miRNAs Modulate Cancer Cell Detachment and Invasion

Cancer cell detachment, migration and invasion represent early steps in the metastatic cascade. Activation of MMPs, breakdown of extracellular matrix, EMT, an increase in cancer cell migration, and motility and invasiveness all occur during these early stages of tumor progression. Table 2 and Fig. 1 list the miRNAs that have been implicated in regulating these events. Several miRNAs have been found to promote cancer cell detachment and invasion. miR-21 is elevated in many tumors and promotes cancer cell proliferation, migration, detachment and invasion while suppressing apoptosis. Expression of miR-21 targets and decreases expression of

Table 2 miRNAs involved in detachment and invasion

miRNAs	Targets	Molecular regulation	Deregulation in cancer	Refs
miR-29c	collagen, laminin γ1, TDG, FUSIP1, SPARC	↓ cancer cell invasion	Decreased	[84]
miR-31	frizzled3, MMP16, integrin a5, radixin, RhoA	↓ cancer cell adhesion/ migration/invasion	Decreased	[85]
miR-122	ADAM17	↓ cancer cell migration/ invasion	Decreased	[28]
miR-126	Crk, VCAM-1, PIK3R2,IRS-1	↓ cancer cell proliferation/ adhesion/ migration/ invasion	Decreased	[31–33]
miR-128	Reelin, DCX, E2F3a	↓ cancer cell migration/ proliferation	Decreased	[86, 87]
miR-146a	ROCK1	↓ cancer cell migration/ invasion	Decreased	[62]
miR-146b	MMP16	↓MMP, cancer cell invasion	Decreased	[61]
miR-200 family	ZEB1, ZEB2	↑E-cadherin, ↓TGFβ, ↓EMT, cancer cell migration/invasion	Decreased	[74–79]
miR-205	ZEB2, PKCε	↑E-cadherin, ↓vimentin, ↓EMT, cancer cell migration/invasion	Decreased	[76, 80]
miR-335	SOX4, TNC	↓ cancer cell migration/ invasion	Decreased	[82]
miR-10b	HOXD10	↑Rho C, ↑cancer cell adhesion/migration/invasion	Increased	[13]
miR-21	Cdc25A, BTG2, LRRFIP1, TPM1, maspin, SPRY2, TAp63, HNRPK, PDCD4, MARCKS, TIMP3, PTEN, RECK	↑MMPs, ↑cancer cell migration/invasion	Increased	[49–56, 58]
miR-29a	TTP	↑EMT, cancer cell migration/invasion	Increased	[81]
miR-155	RhoA	↑TGF-EMT, ↑cancer cell adhesion/migration/ invasion	Increased	[47]
miR-328	CD44	↑Cancer cell adhesion/ migration/invasion	Increased	[64]
miR-373	LATS2, CD44	Oncogene, ↑cancer cell adhesion/migration/ invasion	Increased	[65, 66]
miR-520c	CD44	↑Cancer cell adhesion/ migration/invasion	Increased	[65, 66]

ADAM17 a disintegrin and metalloprotease 17; *BTG2* BTG family, member 2; *Crk* v-crk sarcoma virus CT10 oncogene homolog (avian); *DCX* doublecortin; *FUSIP1* FUS-interacting protein; Fzd3, frizzled3; *HNRNPK* heterogeneous nuclear ribonucleoprotein K; *HOXD10* homeobox D10 gene; *IRS1* insulin receptor substrate 1; *ITGA5* integrin a5; *LATS2, LATS* large tumor suppressor, homolog 2 (Drosophila); *LRRFIP1* leucine rich repeat (in FLII) interacting protein 1; *MARCKS* myristoylated alanine-rich protein kinase c substrate; *MMP16* matrix metallopeptidase 16 (membrane-inserted); *PDCD4* programmed cell death 4; *PIK3R2* phosphoinositol-3 kinase regulatory subunit 2; *PKCε* protein kinase C epsilon; *PTEN* phosphatase and tensin homolog deleted on chromosome 10; *RDX* radixin; *RECK* reversion-inducing-cysteine-rich protein with kazal motifs; *RhoA* ras homolog gene family, member A; *RhoC* ras homolog gene family, member C; *ROCK1* Rho-associated, coiled-coil containing protein kinase 1; *SPARC* secreted protein, acidic, cysteine-rich; *SPRY2* sprouty2; *Tap63* tumor protein p63; *TDG* thymine-DNA glycosylase; *TGFβ* transforming growth factor β; *TIMP3* TIMP metallopeptidase inhibitor 3; *TNC* tenascin C; *TPM1* tropomyosin 1; *TTP* tristetraprolin; *VCAM-1* vascular cell adhesion molecule 1; *ZEB1/2* zinc-finger E-box binding homeobox ½

multiple tumor suppressor genes including reversion-inducing-cysteine-rich protein with kazal motifs (RECK) [49], TIMP metallopeptidase inhibitor 3 (TIMP3) [49], phosphatase and tensin homolog deleted on chromosome 10 (PTEN) [50], tropomyosin 1 (TPM1) [51], programmed cell death 4 (PDCD4) [52, 53], heterogeneous nuclear ribonucleoprotein K (HNRPK) [54], tumor protein p63 (TAp63) [54], Sprouty2 (SPRY2) [55], maspin [56], myristoylated alanine-rich protein kinase C substrate (MARCKS) [57], leucine rich repeat (in FLII) interacting protein 1 (LRRFIP1) [58], BTG family, member 2 (BTG2) [59], and Cdc25A [60]. While miR-146b inhibits cancer cell migration and invasion by targeting MMP16 [61], miR-146a decreases cancer cell migration and invasion by targeting Rho-associated, coiled-coil containing protein kinase 1 (ROCK1) [62]. CD44 is a cell-cell and cell-extracellular matrix adhesion glycoprotein, which modulates adhesiveness, motility, matrix degradation, proliferation and cell survival [63]. Several miRNAs such as miR-328, miR-373 and miR-520c have been reported to regulate CD44 and promote cancer cell motility or invasion [64–66]. In addition, CD44 has recently been recognized as a cell surface marker of CSC and mesenchymal stem cells (MSC) [67–72]. Therefore, these CD44-regulating miRNAs may function in CSC and MSC. miR-373 has been shown to act as an oncogene to repress the tumor-suppressor LATS2 (large tumor suppressor, homolog 2, Drosophila) in human testicular germ cell tumors [73]. miR-10b, which can be induced by the pro-metastatic transcription factor Twist, can indirectly increase Ras homolog gene family, member C (Rho C), as well as motility and invasion by directly targeting homeobox D10 gene (HOXD10) [13].

Several miRNAs regulate EMT either negatively or positively (Table 2 and Fig. 1). Five members of the miR-

200 family (miR-200a, miR-200b, miR-200c, miR-141 and miR-429) and miR-205 are able to increase E-cadherin expression, decrease vimentin expression, inhibit EMT, and prevent migration and invasion of cancer cells through post-transcriptional repression of zinc-finger E-box binding homeobox 1 (ZEB1) and ZEB2 (ZFHX1B) [74–78]. On the other hand, ZEB1 can directly suppress transcription of miR-200 family members and stabilize EMT and promote cancer cell invasion [79]. miR-205 has a function similar to members of the miR-200 family [76]. Additionally, miR-205 represses protein kinase C epsilon (PKCε) and prevent EMT [80]. miR-155 expression is increased in cancer cells and plays a positive role in transforming growth factor (TGF) β-induced EMT and cell migration and invasion by targeting RhoA [47]. miR-29a can promote EMT and cancer metastasis in cooperation with oncogenic Ras signaling by repressing the expression of tristetraprolin (TTP), a protein involved in the degradation of messenger RNAs with AU-rich 3′-untranslated regions [81].

A number of miRNAs negatively regulate the process of cancer cell detachment and invasion (Table 2). miR-126 appears to have multiple functions. As describe above, it supports neoangiogenesis. Yet, miR-126 is downregulated in cancers and inhibits cancer cell growth, adhesion, migration, and invasion through suppressing PIK3R2, insulin receptor substrate 1 (IRS-1), adhesion adaptor protein Crk, and vascular cell adhesion molecule 1 (VCAM-1) [31–33, 82, 83]. miR-335 can suppress cancer cell migration and invasion through targeting of the progenitor cell transcription factor SOX4 and extracellular matrix component tenascin C (TNC) [82]. miR-29c, which is inhibited in nasopharyngeal carcinomas, directly regulates a variety of genes that constitute extracellular matrix such as collagen 3A1, 4A1, 15A1, laminin γ1, thymine-DNA glycosylase (TDG), FUS-interacting protein (FUSIP1) and secreted protein, acidic, cysteine-rich (SPARC) [84]. miR-31 is able to repress a group of metastasis-promoting genes including frizzled3 (Fzd3), integrin a5 (ITGA5), MMP16, radixin (RDX), and RhoA [85]. miR-128 inhibits migration and proliferation of neuroblastoma or glioma cells by down-modulating the expression of Reelin, DCX [86] and E2F3a [87].

miRNAs Modulate Cancer Cell Intravasation

As a part of the process of metastasis, cancer cells intravasate by dissociating from other epithelial cancer cells, invading through the basement membrane and entering into blood vessels (not necessary for all types of tumors, e.g. lymphomas) [1, 3, 88]. The exact mechanisms that regulate this process, which requires collegenases [88], MMPs [89], urokinase plasminogen activator (uPA), uPA receptor (uPAR) [89] and EMT, are not clear. Therefore, some of the miRNAs that modulate detachment and invasion are also involved in regulating cancer cell intravasation. Oncogenic miR-21, for example, targets a number of tumor suppressor genes (Table 2) and is able to enhance cancer cell intravasation in addition to migration and cell proliferation [53].

Recently, miR-23b was reported to directly target uPA and c-Met and decrease migration and proliferation of human hepatocellular carcinoma (HCC) cells [90]. Raf kinase inhibitory protein (RKIP) as a metastasis suppressor inhibits breast tumor cell intravasation and bone metastasis by a signaling cascade that includes inhibition of MAP kinase (MAPK), decrease in transcription of LIN28 by c-Myc, expression of let-7, repression of high mobility group A2 (HMGA2, a chromatin remodeling protein), and activation of pro-invasive genes (Snail) [91]. It has been demonstrated that Tetraspanin CD151 (Tspan 24), an important regulator of laminin-binding integrins (alpha[6] beta[4], alpha[6]beta[1], and alpha[3]beta[1]), specifically regulates intravasation of the epidermoid carcinoma cells and fibrosarcoma cells in vivo without affecting cancer cell motility, proliferation and extravasation [92]. The Targetscan algorithm [93] has predicted that miR-124 and miR-506 regulate CD151, but this has yet to be confirmed. As describe above, miR-29c targets collagens [84] and miR-31 targets MMP16 [85]. It is likely that both miR-29c and miR-31 may also be involved in regulation of cancer cell intravasation.

miRNAs Modulate Cancer Cell Survival and Arrest in the Circulation

It is known that cancer cells can attach to capillaries in significantly greater numbers than normal fibroblasts [94]. Cells with high metastatic potential attach to the capillaries in greater numbers than cells with low metastatic potential [94]. The green fluorescent protein (GFP)-expressing cancer cells proliferate inside the blood vessels of the liver and the lung to express Ki-67 and MMP [95]. However, most of cancer cells that intravasate and enter into the circulation are still destroyed in the bloodstream by shear stress to the cells, as well as attack from the immune system [1–3]. It is possible that miRNAs that regulate cell proliferation and apoptosis [7] have some roles in this stage of cancer metastasis. miR-126 expression is often downregulated in cancers and is able to decrease leukocyte and possibly cancer cell adherence to endothelial cells by targeting VCAM-1 on endothelial cells [83]. Additionally, a number of miRNAs have been discovered to play critical roles in modulation of T and B lymphocytes activation, innate and adaptive immune responses [96, 97]. For example, miR-155 is required in conventional and regulatory T lymphocyte differentiation/activation, B lymphocyte development, mac-

rophage response and toll-like receptors (TLR) response through targeting suppressor of cytokine signaling 1 (SOCS1), activation-induced cytidine deaminase (AID) and transcription factor c-Maf [96–98]. miR-223 is found to have crucial roles in regulating granulocyte proliferation and activation by regulating myeloid ELF-1-like factor (Mef2c) [99]. Cancer suppressing miR-146a can regulate TLR and cytokine signaling through a negative feedback regulation of TNF receptor-associated factor 6 (TRAF6) and IL-1 receptor-associated kinase 1 (IRAK1) genes [100]. miR-181, miR-17~92 cluster and miR-150 are important regulators of the immune system and therefore could participate in cell survival and arrest in circulation [96, 97].

miRNAs Modulate Cancer Cell Extravasation

The ability of cancer cells to exit the capillaries and enter the parenchyma of organs is closely related to the ability of cancer cells to establish viable colonies in new sites as described in next section. Although a particular miRNA that specifically regulates cancer cell extravasation has not yet been identified, it is still believed that this step may also be regulated by miRNAs. Indeed, two miRNAs have been shown to be involved in this process. miR-31, which inhibits cancer cell detachment and invasion through repressing several metastasis-promoting genes (Fzd3, integrin a5, MMP16, radixin and RhoA) [85], can also impair extravasation of GFP-labeled breast cancer cells [85]. The precise mechanism by which miR-31 suppresses extravasation is not known, but may be associated with the repression of its targeted genes (Table 2). In addition to their effects in EMT, miR-200 family members are reported to promote a mesenchymal to epithelial cell transition by inhibiting ZEB2 expression, which enhances macroscopic metastases in mouse breast cancer cell lines [101]; however, the relationship between mesenchymal to epithelial cell transition and cancer cell extravasation is not clear yet.

miRNAs Modulate Metastatic Colonization

The last step of cancer metastasis is the establishment of macroscopic tumors at distant sites [1, 3]. According to the cancer “seed and soil” hypothesis [1], the properties of the “seed”, or cancer cells, and the “soil”, or cancer environment, determine the outcome of metastasis in a new organ. Consistent with this hypothesis, cancer stem cells could provide the “seed” [102]. Expression of metastasis progression genes at organ-specific locations could provide the “soil” [103]. Successful metastatic colonization may depend on 1) stem cell properties that ensure proliferation of cancer cells or dormancy in a new environment and/or 2) expression of site-specific genes that ensure cancer cells interact with and survive in a particular environment.

CSC, also called tumor-initiating cells represent a small subpopulation of cells identified in a variety of cancers that have the ability to self-renew and to develop into multilineage cells [104]. Dysregulation of stem cell self-renewal and differentiation is not only a likely requirement for the initiation of cancer, but also a likely requirement for cancer metastasis, especially in the stage of metastatic colonization [1, 3, 104]. Several signaling molecules and pathways have been shown to regulate the self-renewal, differentiation and survival of CSC, such as Notch, Hedgehog, Wnt/β-catenin, HMGA2, Bcl-2, Bmi-1, c-Myc and c-Met [102, 104, 105]. miRNA regulation is also essential in maintaining the stem cell population since depletion of Dicer-1 function results in early embryonic death and depletion of stem cells in mouse embryos [106]. Other evidence to support the roles of miRNAs in CSC is that a number of miRNAs have been found to critically regulate stem cell molecules and pathways as described above. For example, the miR-34 family (miR-34a, miR-34b and miR-34c) directly targets Notch, HMGA2, Bcl-2, CDK6, c-Met and c-Myc genes involved in the self-renewal and survival of cancer stem cells [66, 68, 107]. Expression of miR-34 family members inhibits cancer growth and metastasis [66, 68, 107]. Table 3 lists the important miRNAs that regulate CSC. Tumor suppressor let-7 can repress Ras and reduce self renewal of CSC [14, 108]. let-7 can also repress HMGA2 and enhance differentiation and transformation [14, 109]. The miR-15a-16-1 cluster can act as a negative regulator of cancer angiogenesis as described in the Angiogenesis section. Additionally, the miR-15a-16-1 cluster also suppresses the self-renewal and survival of cancer stem cells by targeting the oncogene Bcl-2 and WNT3A [24–27]. miR-199b-5p is downregulated in medulloblastoma and is regarded as tumor suppressor gene [110]. miR-199b-5p can reduce stem-cell-like (CD133+) subpopulation of medulloblastoma cells through its targeting of the transcription factor HES1 (hairy and enhancer of split 1) and the Notch pathway [110].

Bmi-1, a polycomb ring finger family member, is essential for self-renewal of CSC [111, 112]. Beside its roles in cell proliferation and migration as discussed above [86, 87], miR-128 can specifically block the self-renewal of glioma by targeting the Bmi-1 oncogene [113]. miR-125b and miR-326 are able to inhibit proliferation and to enhance differentiation of medulloblastoma cells through repressing Smoothened (Smo), an activator of the Hedgehog pathway [114]. miR-324-5p can also inhibit proliferation and enhance differentiation of medulloblastoma cells by repressing transcription factor Gli-1 (Gli family zinc finger 1) [114]. Conversely, a number of miRNAs can positively regulate CSC functions. The oncogenic miR-17–92 cluster is not only induced by transcriptional factors c-Myc, E2F [36–38], but also by Hedgehog signaling [115, 116], which suggests that this cluster may play a role in promoting self

Table 3 miRNAs involved in stem/progenitor cells phenotype

miRNAs	Targets	Molecular regulation	Refs
Let-7	Ras, HMGA2	↓CSC	[14, 108, 109]
miR-15a-16-1	Bcl-2, WNT3A	↓CSC	[24–27]
miR-34a/b/c	Notch, Bcl-2, HMGA2, CDK6, c-Met, c-Myc	↓CSC	[66, 68, 107]
miR-128	Bmi-1	↓CSC	[113]
miR-199b-5p	HES1/Notch	↓CSC	[110]
miR-125b, miR-326, miR-324-5p	Smo Gli-1 Hedgehog	↓CSC	[114]
miR-328	CD44	↑CSC and MSC	[64, 67–72]
miR-373	CD44	↑CSC and MSC	[65, 67–72]
miR-520c	CD44	↑CSC and MSC	[65, 67–72]

Bmi1 BMI1 polycomb ring finger oncogene; *CSC* cancer stem cells; *Gli1* GLI family zinc finger 1; *HES1* hairy and enhancer of split 1, (Drosophila); *HMGA2* high mobility group A2; *MSC* mesenchymal stem cells; *SMO* smoothened homolog (Drosophila); *WNT3A* wingless-type MMTV integration site family, member 3A

renewal of cancer stem cells. miR-328, miR-373, and miR-520c have been reported to regulate CSC surface marker CD44 and may promote CSC proliferation and motility [67–72].

Metastases from certain primary cancers display specific organ preference. For example, pancreatic and colorectal cancers often metastasize to the liver and lungs while prostate cancers frequently metastasize to bone [103]. Certain tumor types such as breast cancer remain dormant for long periods before developing metastasis, indicating that 'speciation' of cancer cells in microenvironments of a particular organ is needed [103]. This kind of organ-specific colonization of cancers may require expression of metastasis progression genes [103]. Indeed, pro-metastatic genes, such as cyclooxygenase 2 (COX2) and the epidermal growth factor receptor (EGFR) ligand HBEGF, are the mediators of cancer cell colonization in both brain and lung tissues [117]. The alpha2,6-sialyltransferase ST6GALNAC5 is specifically expressed in cancer cells that are able to pass through the blood-brain barrier [117]. miRNAs are well suited to play roles in expression of these organ-specific genes. Although no miRNA has been indicated in regulating organ-specific genes, it is likely that miRNAs' roles in organ-specific cancer metastasis will be confirmed in the near future. Recently, miRNA profiling showed that downregulation of miR-30e-3p and miR-514 and upregulation of miRNAs, such as miR-199a*, miR-515-3p, miR-519d, miR-302c*, miR-517b, and miR-520f, miR-30a-5p, miR-518b, miR-523, miR-425-3p, and miR-519b, are important for metastatic colonization in melanoma cells [118].

miRNA Signature of Cancer Metastasis

The previously reviewed data clearly indicates that miRNAs critically regulate the process of cancer metastasis. Are there specific miRNA signatures for cancer metastasis? Based on available data, there indeed are a number of miRNAs strongly associated with cancer metastasis. In a prototypical mouse model of multistage tumorigenesis that involves the stepwise transformation of pancreatic β cells into pancreatic neuroendocrine carcinomas, down-regulation of the miR-200 family (miR-200a, miR-200b, miR-200c, miR-141 and miR-429) is found to be a metastasis-specific feature [119]. As described in a previous section, the miR-200 family negatively controls expression of ZEB1/2 (Table 2) and inhibits EMT [74–78]. As such, it makes sense that down-regulation of this family permits cancer cells to acquire aggressive EMT, invasion and metastasis. This finding is further supported by another report that illustrates a metastatic cancer miRNA signature in 43 metastatic lymph nodes of primary tumors (including

Table 4 miRNA signature of cancer metastasis

miRNAs	Molecular regulation	Deregulation in cancer	Refs
miR-9 family	↓NF-kappaB1 ↓DNA methylation,	Down-regulated	[120, 121, 124]
miR-148a, miR-148b	↓DNMT3B, ↓TGIFB	Down-regulated	[107, 119, 121]
miR-200 family	↑E-cadherin, ↓EMT, ↓cancer cell migration/ invasion	Down-regulated	[119, 120]
miR-210	hypoxia-induced, ↑angiogenesis	Up-regulated	[119, 128]

DNMT3B DNA (cytosine-5-)-methyltransferase 3 beta; *EMT* epithelial-mesenchymal transition; *TGIFB* TGFB-induced factor homeobox 2

colon, bladder, breast, and lung cancers) [120]. Down-regulation of miR-148a and miR-148b seems to be a common metastasis feature in HCC and pancreatic cancer [119, 121]. This observation is supported by the fact that miR-148 is specifically hypermethylated in metastatic cancers [107]. miR-148 is known to target DNMT3B and TGFB-induced factor homeobox 2 (TGIFB, a transcriptional repressor) [107, 122, 123]. The mechanism by which down-regulation of miR-148a and miR-148b promote cancer metastasis is not well known. Down-regulation of the miR-9 family (including miR-9-1, miR-9-2, and miR-9-3) also seems to be a common metastasis feature in a number of solid tumors [120, 121, 124]. This observation is also supported by the report that the miR-9 family is specifically hypermethylated in metastatic cancers [107]. Downregulation of the miR-9 family members is shown to activate NF-kappaB1, recruit DNA methyltransferase 1 and increase in DNA methylation [125–127]. Lastly, expression of the proangiogenic miR-210 (Table 1), a hypoxia-induced miRNA [24, 43, 44], has been shown to increase metastatic capability in both breast and pancreatic cancers [119, 128]. In addition, some miRNAs are reported to link to metastatic cancers at specific organs of origin [120]. For example, up-regulated miR-21 is reported to be involved in metastatic colon cancer; whereas, up-regulated miR-10b is reported to be involved in metastatic bladder cancer [120]. Nevertheless, further validation of these organ-specific miRNA signatures is needed. Taking together, three down-regulated and one up-regulated miRNAs have been confirmed to have a strong association with cancer metastasis (Table 4).

Future Perspectives

Each of the steps in cancer metastasis can be regulated by different miRNAs. Five key issues remain to be addressed in the future studies. The first issue is to validate which miRNAs are critically involved in cancer metastasis (Tables 1~3 and Fig. 1). Identification of miRNA targets is just the beginning of this exploration. For example, more attention needs to be paid to exploring the molecular mechanisms by which miRNAs regulate their targets and associated signaling pathways. The second is to explore which miRNAs play important roles in the regulation of cancer stem cells. Cancer stem cells have been found to be critically involved in intravasation, extravasation and organ-specific metastasis. The third is to further identify the cancer type-specific miRNA signatures of metastasis. The forth is to translate laboratory observations regarding miRNA-regulated cancer metastasis into the development of markers for prognosis, as well as new approaches for treatments. And the last is to develop new techniques and technology for miRNA detection, whereby, it will be useful to develop a reliable in situ hybridization method for detecting miRNAs on formalin-fixed paraffin embedded tissues. In addition, the efficiency of in vivo delivery of miRNA remains to be improved, but the future looks promising.

Acknowledgements The authors thank Dr. Milena Nicoloso for drawing the Fig. 1 and Sherri De Jesus for her technical editing expertise. George A. Calin, a Fellow, is supported by The University of Texas M. D. Anderson Research Trust, The University of Texas System Regents Research Scholar Program, and by the Ladjevardian Regents Research Scholar Fund. This study was supported in part by a grant from the Anne and Henry Zarrow Foundation and by NIH grant 1R01CA135444. The authors acknowledge that all related articles are not cited in this article due to the space limit.

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