Published in final edited form as:
Int J Cancer. 2010 March 15; 126(6): 1283–1290. doi:10.1002/ijc.25014.

miRNA control of tumor cell invasion and metastasis

Somesh Baranwal[1] and **Suresh K. Alahari**[1,2,@]

[1] Department of Biochemistry and Molecular Biology, LSU Health Science Center, New Orleans, LA 70112

[2] Stanley Scott cancer Center, LSU Health Science Center, New Orleans, LA 70112

Abstract

MicroRNAs have emerged as a novel class of non-coding RNAs that regulate gene expression at the post-translational level in almost every biological event. A large body of evidence indicates that microRNAs regulate the expression of different genes that play an important role in cancer cell invasion, migration, and metastasis. In this review, we briefly describe the role of various miRNAs in invasion, migration, and metastasis which are essential steps during cancer progression.

Keywords

miRNA; invasion; epithelial-mesenchymal transition; metastasis suppressor

Introduction

Cell invasion and migration play an important role in embryonic development, homeostasis, and pathobiology and in the process of cancer progression[1, 2]. Cell migration is coordinated by a plethora of cytoplasmic proteins which are involved in transient signaling events with changes in the cellular architecture[3]. MicroRNAs are small noncoding regulatory RNAs ranging in size from 17 to 25 nucleotides. Recently, miRNAs have emerged as major non-coding RNAs that regulate processes of the immune system, differentiation, tumorigenesis, cell death and neurodegeneration[4–7]. There are more than 9500 miRNA entries in the miRbase database including 706 miRNA sequences for Homo sapiens (miR-base version 13.0, March.2009, Cambridge, UK; Wellcome Sanger Trust Inst). Based on the function and location of miRNAs on the chromosomes, miRNAs have been assigned to different families in various biological processes. For instance, the miR-34 family regulates cancer and apoptosis. The miR-200 family has been shown to inhibit the first step of metastasis, epithelial mesenchymal transition (EMT), and the let-7 family of microRNAs regulates cell death in human cancer cells.

In the nucleus, RNase III enzyme, drosha with the help of microprocessor complex, cuts the double stranded RNA segments (pri-miRNAs) into short, hairpin-shaped double-stranded RNA precursor structures (pre-miRNA, 60–70mer). Further processing takes place in the cytoplasm where Dicer cleaves pre-miRNA into mature miRNAs (19–21nt). Mature miRNAs bind the complementary sequence of 3′ untranslated region (3′UTR) of mRNAs and are capable of targeting genes for either degradation of mRNA (which happens when they are complementary or almost complementary) or inhibition of translation (which happens when they are not complementary)[8]. Recently, drosha and dicer expression levels

[@]Corresponding author: Telephone: 504-568-4734, Fax: 504-568-6888, salaha@lsuhsc.edu.

have emerged as important markers for patients with ovarian cancer[9]. Bioinformatics analysis reveals that miRNAs can control the expression of one third of the human proteome[10]. miRNAs participate in keeping the balance of gene regulating networks that determine the cells' fate. Deregulation of miRNAs, which is a frequent outcome in human cancer, weakens this balance, thereby contributing to cancer progression. Several studies have reported that miRNAs affect the expression of genes and pathways involved in cancer pathogenesis from initiation to metastasis disease. In cancer progression, tumor cells originate from their primary site and metastasize to the distant organs. Invasion and metastasis play an important role in the spreading of cancer. The process of invasion involves the directed migration and penetration of cancer cells into surrounding tissue. The process of metastasis is the ability of cancer cells to penetrate into the blood vessel and invade normal tissues to develop cancer at the secondary site. Various cytoplasmic proteins and transcription factors have been identified to mediate this process[11]. In this review, we briefly describe our understanding the role of various miRNAs and their targets in the regulation of tumor cell invasion, migration, and metastasis in different types of cancers.

miRNA regulation of tumor cell invasion and migration

Cell invasion is the first step of cancer progression which involves the movement of tumor cells from their origin and their migration to neighboring tissues. Cell invasion requires the migration of tumor cells and dissolution of the extracellular matrix proteins. miR-21 has emerged as a major microRNA that is overexpressed in a number of cancers such as glioma, breast cancer, colorectal cancer, stomach/gastric cancer, hepatocellular carcinoma, pancreas cancer, lung cancer, leukemia, and prostate cancer[12]. Studies using miR-21 suggest that it post-transcriptionally downregulates tumor suppressor programmed cell death 4 (PDCD4) and tropomyosin 1 (TPM1) genes and stimulates invasion and metastasis in colorectal and breast cancers. Recent studies using prostate cancer cells suggest that miR-21 also targets myristoylated alanine rich protein kinase c substrate (MARCKS), which is involved in cellular processes such as cell adhesion and cell motility through regulation of the actin cytoskeleton, without affecting proliferation[13]. Moreover, a negative correlation between the expression of Bone morphogenetic protein-6 (BMP-6) and miR-21 has been identified in breast cancer tissue samples. The expression of miR-21 is high in MDA-MB231 breast cancer cells that express a very low level of BMP-6 transcripts. Moreover, BMP-6 regulates miR-21 expression in MDA-MB231 cells at the transcriptional level, an effect that is mediated via repression of δEF1 and c-Fos/c-Jun. BMP-6 thus inhibited δEF1 and c-Fos/c-Jun after binding to the miR-21 promoter[14]. Furthermore, miR-21 expression is also correlated with HER2/neu upregulation and is functionally involved in HER2/neu-induced cell invasion that is mediated via the MAPK (ERK1/2) pathway upon stimulation of HER2/neu signaling in breast cancer cells[15]. In addition, it is confirmed that overexpression of other ERK1/2 activators such as RAS V12 or ID-1 are sufficient to induce miR-21 upregulation in HER2/neu negative breast cancer cells[15]. In hepatocellular cancer models, inhibition of miR-21 increases the expression of the phosphatase and tensin homolog (PTEN) tumor suppressor, and decreases tumor cell proliferation, migration, and invasion, while miR-21 precursor overexpression increases. PTEN has been shown to be a direct target of miR-21 and contributes to miR-21 effects on cell invasion. Meng and colleagues (2007) also showed that modulation of miR-21 altered the focal adhesion kinase (FAK) phosphorylation and expression of matrix metalloproteases 2 and 9; both of them are downstream mediators of PTEN and play an important role in tumor cell migration and invasion[16]. A recent study suggests a critical role of miR-21 in regulating the expression of RECK and TIMP3 genes which are suppressors of malignancy and inhibitors of matrix metalloproteinase's (MMPs) in glioma cells. Moreover, repression of miR-21 in glioma cells leads to a decrease in their migratory and invasion abilities, suggesting that miR-21 contributes to glioma malignancy by downregulation of MMP inhibitors such as RECK and

TIMP3[17]. Interestingly, miR-21 has multiple targets in different cancers, highlighting the fact that some miRNAs are key regulators of multiple genes that regulate different processes in cancer biology.

In addition to miR-21, recent studies using breast tumor tissues and breast cancer cells by Wu et. al. (2009) indicated the role of miR-205 in cell invasion. miR-205 is highly expressed in normal breast tissues and non-malignant breast epithelial cell line, MCF-10A and ectopic expression of miR-205 in MCF7 significantly reduces cellular proliferation, clonogenic survival and anchorage-independent growth. Also miR-205 is able to suppress invasion and metastasis of MDA-MB231 cells *in vivo*. Furthermore, miR-205 specifically suppresses the expression of ErbB3 and VEGF-A by directly interacting with the putative miR-205 binding site at the 3′-UTR[18]. Prostate cancer studies suggest that miR-205 is highly expressed in normal prostate tissue and RWPE-1 cells, whereas it is almost undetectable in both androgen-dependent (VCaP, LNCaP) and androgen-independent (DU145, PC-3) prostate cancer cells. Overexpression of miR-205 in prostate cancer cells promotes upregulation of E-cadherin and reduction of cell locomotion and invasion, suggesting the mesenchymal epithelial transition (MET). Target prediction analysis indicates that miR-205 targets are N-chimaerin, ErbB3, E2F1, E2F5, ZEB2, and protein kinase-C epsilon, however these targets need to be validated further[19]. Since miR-205 is affecting ErbB3, which is highly expressed in breast cancer, miR-205 may have a great therapeutic value. Similarly, modulating the expression of VEGF will have larger clinical implications since angiogenesis is primarily regulated by VEGF. Additionally, E-cadherin is downregulated in advanced breast cancers, and thus upregulation of E-cadherin will be an attractive strategy in cancer therapeutics.

Malignant gliomas and especially glioblastoma multiforme are the most malignant and prevalent forms of brain tumors. miRNA profiling of normal and glioblastoma tissues revealed an important function for miRNA-146b in human glioblastoma U373 cell migration. Knock-down of miR-146b by anti-miR-146b has no effect on the growth of human glioblastoma U373 cells; however, overexpression of miR-146b significantly reduces invasion and migration of glioblastoma U373 cells, suggesting that cell invasion effects are independent of cell growth effects. Matrix metalloproteinase 16 (MMP16) has emerged as a critical target of miR-146b in regulating the migration of glioblastoma cells[20]. It is not clear from this study whether miR-146 affects other MMPs. Nevertheless, it is very interesting to note that MMP16 has proteolytic activity against collagen, and thus miR-146 has potential clinical implications in gliobostoma multiforme.

A recent report has identified the critical role of miR-1 in lung cancer. miR-1 expression is lost in human primary lung cancer tissues and cell lines while it is highly expressed in cardiac and smooth muscle tissues. Expression of miR-1 in human epithelial cells, A549 and H1299, reversed tumorigenic properties such as growth, replication potential, motility/migration, clonogenic survival, and tumor formation in nude mice. Moreover, expression of miR-1 reduced the expression of MET (a receptor tyrosine kinase), Pim-1 (a Ser/Thr kinase), and FoxP1 (a transcription factor) which are oncogenic molecules frequently upregulated in lung cancer. Furthermore, expression of miR-1 in A549 lung cancer cells induced apoptosis in response to doxorubicin treatment with enhanced activation of caspase 3, caspase 7, and cleavage of PARP-1[21]. These studies suggest miR-1 may function as an inhibitor of migration either by blocking the function of some oncogenes or by activating genes in apoptosis signaling cascades.

Melanoma is a highly aggressive form of cancer, and the mechanism by which melanoma progression occurs is poorly understood. Expression profiling analysis of normal melanocytes and melanoma cell lines identified miR-182 as a most critical miRNA, which is

amplified in melanoma cell line. Moreover, miR-182 is flanked by the *c-MET* and *BRAF* oncogenes in the 7q31–34 region that is frequently amplified in melanoma and often in association with high copy number. Ectopic expression of miR-182 promotes migration potential *in vitro* and metastasis potential *in vivo* using experimental metastasis models. miR-182 downregulation in melanocytes impedes invasion and triggers apoptosis. Overexpression of miR-182 promotes migration and survival by directly repressing microphthalmia-associated transcription factor-M and FOXO3, whereas enhanced expression of either microphthalmia-associated transcription factor-M or FOXO3 blocks miR-182's invasive effects. This suggests that these proteins are potential targets of miR-182[22]. This outstanding study provides an idea that silencing miR-182 could be a therapeutic strategy for melanoma.

Crk (v-crk sarcoma virus CT10 oncogene homolog) is a member of a family of adaptor proteins that are involved in intracellular signal pathways and plays an important role in cell adhesion, proliferation, and migration. Elevated levels of Crk expression has been implicated in lung cancer and are associated with increased tumor invasiveness. In a screen to identify the target for Crk, miR-126 has emerged as a putative target. Overexpression of miR-126 in lung cancer cells increases the expression level of Crk and knock-down studies revealed an important role of miR-126 in migration, invasion and adhesion of non-small cell lung carcinoma cells[23]. Ezrin is a member of the ERM (Ezrin, Radixin, and Moesin) group of proteins which have been identified as critical mediators for cell migration and metastasis. Ezrin is recognized as a *bona fide* target of miR-183 in lung cancer. Overexpression of miR-183 suppresses the expression of Ezrin. Furthermore, microarray analysis revealed that genes associated with migration, invasion, and metastasis are significantly changed in the presence of excess miR-183. This reveals a novel role of miR-183 in metastasis of lung cancer [24].

A recent study has described the mechanistic insight into the role of miRNAs in nasopharyngeal carcinoma (NPC), a highly invasive tumor. miRNA microarray analysis of 31 laser capture micro-dissected NPCs and 10 normal healthy nasopharyngeal epithelial tissues identified that miR-29c is down regulated in nasopharyngeal carcinoma samples. Most of the miR-29c-targeted genes encode extracellular matrix proteins, including multiple collagens and laminin γ1, which are associated with tumor cell invasiveness and metastatic potential[25]. Here, identification of the factors that modify the expression of miR-29c will be important to develop novel clinical strategies to suppress NPC.

Our recent data show that Suppression of Tumorigenicity 14 (ST14) is a novel target for miR-27b, and analysis of human breast tumors revealed that miR-27b expression increased during cancer progression, paralleling a decrease in ST14 expression. Furthermore, we show that miR-27b promotes breast cancer cell growth, migration and invasion, while ST14 suppresses all these processes. Blocking the miR-27b/ST14 interaction, rescuing ST14 function or silencing miR-27b may be effective therapeutic approaches in advanced breast cancers[26].

miRNA regulation of cancer metastasis

Epithelial mesenchymal transition (EMT) is a prerequisite for cancer metastasis, and one of the functional consequences of EMT is the loss of E-cadherin. E-cadherin is a cell adhesion protein and a major constituent of adherens junctions; it seems to function as a suppressor of invasion/metastasis during carcinoma progression[27]. Several transcription factors of the zinc finger family such as Snail1, Slug, ZEB1 and ZEB2 have been shown to mediate the process of epithelial-mesenchymal transition. In the context of miRNA regulation by EMT, the miR-200 family has emerged as a key mediator in regulating the expression of E-cadherin[28].

ZEB family transcription factors are highly expressed in invasive mesenchymal cells. In addition, expression of ZEBs is directly repressed by the miR-200 family in non-invasive epithelial cells. ZEBs repress the transcription of miR-200 genes; thus, forming a double-negative feedback loop to ensure the repression of epithelial genes in ZEBs expressing mesenchymal cells suggesting miR-200 is a major regulator of EMT[29–31] (Figure 2a). As described above, modulation of E-cadherin expression is an attractive idea for cancer therapies, and thus both ZEBs and miR-200 are good targets.

In addition to miR-200, miR-101 plays a major role in EMT through Enhancer of Zeste homolog (EZH2). EZH2 is a histone methyl transferasewhich contributes to the epigenetic silencing of E-cadherin and other target genes, and it regulates the survival and metastasis of cancer cells[32]. miRNA-101 has emerged as a repressor for EZH2, which is found to be elevated in a subset of aggressive, clinically localized prostate cancers and almost all metastatic prostate cancers. Moreover, the miR-101 locus is lost in metastatic prostate cancer which leads to upregulation of EZH2 and concomitant deregulationof epigenetic pathways and results in cancer progression[33]. This seminal work reveals that miRNA-101 can directly regulate epigenetic pathways.

Additional microRNA profiling studies of human mammary epithelial cells (HMEC) and metastatic breast cancer cells (MDA-MB231) revealed that miR-10b plays a critical role in breast cancer metastasis. This report shows that miR-10b is the only microRNA which is highly expressed in MDA-MB231 cells. Knockdown approaches indicated that miR-10b function is required for *in vitro* invasiveness but not for viability of MDA-MB231 cells. Ectopic expression of miR-10b did not affect cell proliferation but did increase invasion and migration of immortalized HMECs and SUM149 breast cells. Xenograft studies indicated that miR-10b has no effect on the primary tumor growth, while the invasion fronts of miR-10b-overexpressing tumors exhibited very high levels of both cell proliferation and angiogenesis as measured by immunohistochemistry with Ki-67, a proliferation marker and MECA-32, an endothelial cell marker protein. A direct role of Twist1 (a EMT promoter) in regulating the expression of miR-10b has also been shown by chromatin immunoprecipitation (CHIP) assays. Furthermore, overexpression of Twist1 in HMEC increases the expression of miR-10b, while snail1 (another EMT regulator) overexpression does not have any effect on miR-10b. Two functional targets for miR-10b have been identified; these include homeoboxD10 (HOXD10) and RB1CC1 (also called FIP200) and both were implicated in the suppression of cell migration and/or invasion. HOXD10 downregulates the expression of genes that are involved in cell migration and extracellular matrix remodeling, including RHOC, α3 integrin, matrix metalloproteinase-14, and urokinase-type plasminogen activator receptor. This study indicates that miR-10b plays a role specifically in the metastatic process but not in primary tumor formation, and thus it is possible to develop miRNA based drugs specific to metastasis[34].

TGFβ signaling plays an important role in advanced forms of malignancy. MicroRNA microarray profiling of normal murine mammary gland (NMuMG) epithelial cells and TGFβ treated NMuMG cells have identified a critical role of miR-155. TGF-β induces miR-155 expression and promoter activity through Smad4. Knockdown of miR-155 suppressed TGF-β-induced EMT and tight junction dissolution, as well as cell migration and invasion. Furthermore, the ectopic expressionof miR-155 reduced RhoA protein and disrupted tight junction formation. The elevated levels of miR-155 have been shown in invasive breast cancer tissues. This study indicates that miR-155 is regulated by the TGF-β/Smad pathway and plays a rolein mammary epithelial cell plasticity through RhoA signaling cascade[39]. It has been known for quite some time that Rho GTPases play a pivotal role in different cancers, and thus the miRNAs that affect these proteins will have therapeutic advantages to many cancers.

In an elegant study of microRNA array profiling of metastatic breast cancer cells, MDA-MB231 and its derivative cells specific for lung metastasis (LM2 cell line) and for bone metastasis (BoM1 cell line) identified an important role for miR-335, miR-206 and miR-126, whose expressions are lost in both metastatic cell lines. Overexpression of miR-335, miR-206 or miR-126 inhibits metastatic activity as demonstrated by bioluminescent imaging. It was also shown that expression of miR-335 and miR-126 is lost in most of the primary breast tumor patients, and loss of miRNAs is associated with poor metastasis free survival. The targets for miR-335 are identified to be tenascin C (TNC) and the SRY-box containing transcription factor SOX4. In conclusion, these studies suggest the metastatic suppressor function for miR-335, miR-126 and miR-206 in breast cancer[35]. Since the loss of the micoRNAs has strong association with metastatic relapse, these molecules may be used as prognostic markers for advanced breast cancer.

Using a reverse screen approach, Huang *et. al*, (2008) identified the role of miR-373 and miR-520c in promoting tumor invasion and metastasis by performing bioluminescent imaging using MCF7 cells expressing these miRNAs. Furthermore, it was demonstrated that these miRNAs induce migration and invasion partly through the direct suppression of CD44[36].

Tumor metastasis suppressors are the natural regulators of cancer metastasis. Metastasis suppressor genes either prevent the progression of tumor cells to metastasize or are able to reverse the metastatic phenotype. Valastyan et. al (2009) have identified a pleiotropic function of miR-31 in breast cancer metastasis. miR-31 was discovered as an anti-metastatic human miRNA whose expression is attenuated in metastatic breast cancer cells, acts at multiple steps of the invasion-metastasis cascade and represses a cohort of pro-metastatic targets. Over-expression of miR-31 in MCF7-Ras cells has no effect on primary tumor growth and cell proliferation, however, lung metastasis is enhanced. MDA-MB231 cells expressing miR-31 repressed 3′ UTRs of myosin phosphatase-Rho interacting protein (M-RIP), matrix metallopeptidase 16 (MMP16), radixin (RDX), frizzled3 (Fzd3), integrin α5 (ITGA5), and RhoA; genes all are known to play an important role in metastasis. Furthermore, overexpression of Fzd3, ITGA5, RDX, and RhoA reverses miR-31-dependent metastasis in breast cancer cell lines. These data suggest that miR-31 is a novel miRNA that inhibits metastasis through several mechanisms affecting different genes[37]. The miRNAs that have pleiotropic effects are particularly interesting because modulation of one miRNA affects several genes that would have cumulative effect from all genes, which is needed to attack multifactorial diseases such as cancer.

Breast cancer metastasis suppressor 1 (BRMS1) is a nuclear protein which regulates expression of multiple genes leading to suppression of metastasis without affecting orthotopic tumor growth in murine and human cancer cells. miR-146 exerts an important role in regulating the nuclear factor- B pathway and is more abundantly expressed in BRMS1-expressing cells. It was found that BRMS1 upregulates expression of miR-146 in metastatic breast cancer cells. Transduction of miR-146a or miR-146b into MDA-MB231 downregulated expression of epidermal growth factor receptor, inhibited invasionand migration *in vitro*, and suppressed experimental lung metastasis. In summary, this result supports the theory that modulating the expression level of miR-146 may have a therapeutic function for breast cancer metastasis[38]. This is another good example by which one can develop miRNA therapies that can selectively attack advanced metastasis. These types of approaches are especially important because most of the patients die as a result of metastasis and not because of primary tumor growth

Raf kinase inhibitory protein (RKIP; also called PEBP1) is a member of the evolutionarily conserved phosphatidylethanolamine binding protein family and negatively regulates G

protein-coupled receptor kinase-2, the MAP kinase (MAPK), and NF- B signaling cascades. RKIP has been shown as a suppressor of metastatic progression without affecting the primary tumor growth in an orthotopic murine model of androgen-independent prostate tumor cells[40]. A recent report has identified the critical role of RKIP in induction of let-7/miR-98. RKIP represses invasion, intravasation and bone metastasis of breast tumor cells through a signaling cascade involving inhibition of MAPK, Myc, and LIN28 which leads to induction of the microRNA *let-7* and downregulation of its target genes[40, 41]. Thus understanding the biogenesis of miRNAs such as let-7 will be useful in identifying novel therapeutic targets.

Conclusion and future perspective

Discovery of regulating the expression of proteins by non-coding RNAs is a breakthrough of the 21st century. miRNAs are emerging as a novel class of therapeutic molecules/targets to treat various diseases including cancer. It is clear that the number of miRNAs is much smaller than the number of protein coding genes in the genome; however, the miRNAs have specific functions in different biological processes. Many discoveries opened the floodgates of information to the understanding that miRNAs function as key players in cancer progression. Depending on the targets, miRNAs can function as either tumor suppressors or oncogenes. miRNAs play different roles in cancer and can be involved in migration, invasion, metastasis, proliferation and the cell cycle. The studies discussed here show that miRNAs have distinct functions in regulating invasion and metastasis. miRNA, unlike mRNA remains highly intact in the routinely collected formalin fixed and embedded cancer specimens, and hence miRNA based markers may emerge as prognostic markers in the realms of cancer diagnosis. Recently, using bead-based flow cytometry miRNA expression profiling of 217 mammalian miRNAs from 334 samples has provided compelling evidence to classify human cancers. This highlights the potential of miRNA profiling in cancer diagnosis[42]. The *let-7* miRNA is one of the natural miRNA tumor suppressors in lung tissues that may be useful in treating lung cancer or enhancing current treatments for lung cancer[43]. A recent interesting report has identified a small molecule inhibitor for miRNA-21 for the first time for any microRNA which may be of therapeutic use[44]. Several *in vitro* and *in vivo* studies have identified the critical role for miRNAs in regulation of tumor cell invasion, migration and metastasis. Various miRNA functions are validated by either overexpressing or knocking down in various tumor cell lines and by performing xenograft and metastasis experiments in nude mice models. Since one miRNA can regulate several target proteins, and current studies of miRNA targets are based on computer algorithms, the daunting challenge of the next generation biologists would be to validate and confirm the role of miRNAs in transgenic animal models. Studies with miRNAs may be useful for early prognosis and may serve as markers for therapy indicators. Further, studies with miRNAs may lead to development of miRNA based therapy either by miRNA silencing or by using miRNA mimics. In the last decade, miRNA research has grown tremendously, and thus we are not too far in developing miRNA targeted therapies and using miRNAs as diagnostic and prognostic markers.

Acknowledgments

We would like to express our apologies to all researchers whose work could not be cited in this review as a result of space constraints. This work is supported by National Cancer Institute grant, CA115706 with additional support from Susan G Komen foundation grant (BCTR0600278) and Louisiana Board of Regents (LEQSF-RD-A-14) to S.K. Alahari.

References

1. Vicente-Manzanares M, Webb DJ, Horwitz AR. Cell migration at a glance. J Cell Sci. 2005; 118:4917–9. [PubMed: 16254237]
2. Yamaguchi H, Wyckoff J, Condeelis J. Cell migration in tumors. Curr Opin Cell Biol. 2005; 17:559–64. [PubMed: 16098726]
3. Le Roy C, Wrana JL. Signaling and endocytosis: a team effort for cell migration. Dev Cell. 2005; 9:167–8. [PubMed: 16054022]
4. Gangaraju VK, Lin H. MicroRNAs: key regulators of stem cells. Nat Rev Mol Cell Biol. 2009; 10:116–25. [PubMed: 19165214]
5. Pauley KM, Chan EK. MicroRNAs and their emerging roles in immunology. Ann N Y Acad Sci. 2008; 1143:226–39. [PubMed: 19076353]
6. Fontana L, Sorrentino A, Condorelli G, Peschle C. Role of microRNAs in haemopoiesis, heart hypertrophy and cancer. Biochem Soc Trans. 2008; 36:1206–10. [PubMed: 19021526]
7. Bushati N, Cohen SM. MicroRNAs in neurodegeneration. Curr Opin Neurobiol. 2008; 18:292–6. [PubMed: 18662781]
8. Pillai RS, Bhattacharyya SN, Filipowicz W. Repression of protein synthesis by miRNAs: how many mechanisms? Trends Cell Biol. 2007; 17:118–26. [PubMed: 17197185]
9. Merritt WM, Lin YG, Han LY, Kamat AA, Spannuth WA, Schmandt R, Urbauer D, Pennacchio LA, Cheng JF, Nick AM, Deavers MT, Mourad-Zeidan A, et al. Dicer, Drosha, and outcomes in patients with ovarian cancer. N Engl J Med. 2008; 359:2641–50. [PubMed: 19092150]
10. Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell. 2005; 120:15–20. [PubMed: 15652477]
11. Nguyen DX, Bos PD, Massague J. Metastasis: from dissemination to organ-specific colonization. Nat Rev Cancer. 2009; 9:274–84. [PubMed: 19308067]
12. Krichevsky AM, Gabriely G. miR-21: a small multi-faceted RNA. J Cell Mol Med. 2009; 13:39–53. [PubMed: 19175699]
13. Li T, Li D, Sha J, Sun P, Huang Y. MicroRNA-21 directly targets MARCKS and promotes apoptosis resistance and invasion in prostate cancer cells. Biochem Biophys Res Commun. 2009; 383:280–5. [PubMed: 19302977]
14. Du J, Yang S, An D, Hu F, Yuan W, Zhai C, Zhu T. BMP-6 inhibits microRNA-21 expression in breast cancer through repressing deltaEF1 and AP-1. Cell Res. 2009; 19:487–96. [PubMed: 19308091]
15. Bodendorf MO, Haas V, Laberke HG, Blumenstock G, Wex P, Graeter T. Prognostic value and therapeutic consequences of vascular invasion in non-small cell lung carcinoma. Lung Cancer. 2009; 64:71–8. [PubMed: 18790545]
16. Meng F, Henson R, Wehbe-Janek H, Ghoshal K, Jacob ST, Patel T. MicroRNA-21 regulates expression of the PTEN tumor suppressor gene in human hepatocellular cancer. Gastroenterology. 2007; 133:647–58. [PubMed: 17681183]
17. Gabriely G, Wurdinger T, Kesari S, Esau CC, Burchard J, Linsley PS, Krichevsky AM. MicroRNA 21 promotes glioma invasion by targeting matrix metalloproteinase regulators. Mol Cell Biol. 2008; 28:5369–80. [PubMed: 18591254]
18. Wu H, Zhu S, Mo YY. Suppression of cell growth and invasion by miR-205 in breast cancer. Cell Res. 2009; 19:439–48. [PubMed: 19238171]
19. Gandellini P, Folini M, Longoni N, Pennati M, Binda M, Colecchia M, Salvioni R, Supino R, Moretti R, Limonta P, Valdagni R, Daidone MG, et al. miR-205 Exerts tumor-suppressive functions in human prostate through down-regulation of protein kinase Cepsilon. Cancer Res. 2009; 69:2287–95. [PubMed: 19244118]
20. Xia H, Qi Y, Ng SS, Chen X, Li D, Chen S, Ge R, Jiang S, Li G, Chen Y, He ML, Kung HF, et al. microRNA-146b inhibits glioma cell migration and invasion by targeting MMPs. Brain Res. 2009; 1269:158–65. [PubMed: 19265686]
21. Nasser MW, Datta J, Nuovo G, Kutay H, Motiwala T, Majumder S, Wang B, Suster S, Jacob ST, Ghoshal K. Down-regulation of micro-RNA-1 (miR-1) in lung cancer. Suppression of tumorigenic

property of lung cancer cells and their sensitization to doxorubicin-induced apoptosis by miR-1. J Biol Chem. 2008; 283:33394–405. [PubMed: 18818206]

22. Segura MF, Hanniford D, Menendez S, Reavie L, Zou X, Alvarez-Diaz S, Zakrzewski J, Blochin E, Rose A, Bogunovic D, Polsky D, Wei J, et al. Aberrant miR-182 expression promotes melanoma metastasis by repressing FOXO3 and microphthalmia-associated transcription factor. Proc Natl Acad Sci U S A. 2009; 106:1814–9. [PubMed: 19188590]
23. Crawford M, Brawner E, Batte K, Yu L, Hunter MG, Otterson GA, Nuovo G, Marsh CB, Nana-Sinkam SP. MicroRNA-126 inhibits invasion in non-small cell lung carcinoma cell lines. Biochem Biophys Res Commun. 2008; 373:607–12. [PubMed: 18602365]
24. Wang G, Mao W, Zheng S. MicroRNA-183 regulates Ezrin expression in lung cancer cells. FEBS Lett. 2008; 582:3663–8. [PubMed: 18840437]
25. Sengupta S, den Boon JA, Chen IH, Newton MA, Stanhope SA, Cheng YJ, Chen CJ, Hildesheim A, Sugden B, Ahlquist P. MicroRNA 29c is down-regulated in nasopharyngeal carcinomas, up-regulating mRNAs encoding extracellular matrix proteins. Proc Natl Acad Sci U S A. 2008; 105:5874–8. [PubMed: 18390668]
26. Wang Y, Rathinam R, Walch A, Alahari SK. ST14 (suppression of tumorigenicity 14) gene is a target for miR-27b, and the inhibitory effect of ST14 on cell growth is independent of miR-27b regulation. J Biol Chem. 2009; 284:23094–106. [PubMed: 19546220]
27. Baranwal S, Alahari SK. Molecular mechanisms controlling E-cadherin expression in breast cancer. Biochem Biophys Res Commun. 2009; 384:6–11. [PubMed: 19379710]
28. Korpal M, Kang Y. The emerging role of miR-200 family of microRNAs in epithelial-mesenchymal transition and cancer metastasis. RNA Biol. 2008; 5:115–9. [PubMed: 19182522]
29. Gregory PA, Bert AG, Paterson EL, Barry SC, Tsykin A, Farshid G, Vadas MA, Khew-Goodall Y, Goodall GJ. The miR-200 family and miR-205 regulate epithelial to mesenchymal transition by targeting ZEB1 and SIP1. Nat Cell Biol. 2008; 10:593–601. [PubMed: 18376396]
30. Park SM, Gaur AB, Lengyel E, Peter ME. The miR-200 family determines the epithelial phenotype of cancer cells by targeting the E-cadherin repressors ZEB1 and ZEB2. Genes Dev. 2008; 22:894–907. [PubMed: 18381893]
31. Bracken CP, Gregory PA, Khew-Goodall Y, Goodall GJ. The role of microRNAs in metastasis and epithelial-mesenchymal transition. Cell Mol Life Sci. 2009; 66:1682–99. [PubMed: 19153653]
32. Cao Q, Yu J, Dhanasekaran SM, Kim JH, Mani RS, Tomlins SA, Mehra R, Laxman B, Cao X, Kleer CG, Varambally S, Chinnaiyan AM. Repression of E-cadherin by the polycomb group protein EZH2 in cancer. Oncogene. 2008; 27:7274–84. [PubMed: 18806826]
33. Varambally S, Cao Q, Mani RS, Shankar S, Wang X, Ateeq B, Laxman B, Cao X, Jing X, Ramnarayanan K, Brenner JC, Yu J, et al. Genomic loss of microRNA-101 leads to overexpression of histone methyltransferase EZH2 in cancer. Science. 2008; 322:1695–9. [PubMed: 19008416]
34. Ma L, Teruya-Feldstein J, Weinberg RA. Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. Nature. 2007; 449:682–8. [PubMed: 17898713]
35. Tavazoie SF, Alarcon C, Oskarsson T, Padua D, Wang Q, Bos PD, Gerald WL, Massague J. Endogenous human microRNAs that suppress breast cancer metastasis. Nature. 2008; 451:147–52. [PubMed: 18185580]
36. Huang Q, Gumireddy K, Schrier M, le Sage C, Nagel R, Nair S, Egan DA, Li A, Huang G, Klein-Szanto AJ, Gimotty PA, Katsaros D, et al. The microRNAs miR-373 and miR-520c promote tumour invasion and metastasis. Nat Cell Biol. 2008; 10:202–10. [PubMed: 18193036]
37. Valastyan S, Reinhardt F, Benaich N, Calogrias D, Szasz AM, Wang ZC, Brock JE, Richardson AL, Weinberg RA. A pleiotropically acting microRNA, miR-31, inhibits breast cancer metastasis. Cell. 2009; 137:1032–46. [PubMed: 19524507]
38. Hurst DR, Edmonds MD, Scott GK, Benz CC, Vaidya KS, Welch DR. Breast cancer metastasis suppressor 1 up-regulates miR-146, which suppresses breast cancer metastasis. Cancer Res. 2009; 69:1279–83. [PubMed: 19190326]
39. Kong W, Yang H, He L, Zhao JJ, Coppola D, Dalton WS, Cheng JQ. MicroRNA-155 is regulated by the transforming growth factor beta/Smad pathway and contributes to epithelial cell plasticity by targeting RhoA. Mol Cell Biol. 2008; 28:6773–84. [PubMed: 18794355]

40. Fu Z, Smith PC, Zhang L, Rubin MA, Dunn RL, Yao Z, Keller ET. Effects of raf kinase inhibitor protein expression on suppression of prostate cancer metastasis. J Natl Cancer Inst. 2003; 95:878–89. [PubMed: 12813171]

41. Dangi-Garimella S, Yun J, Eves EM, Newman M, Erkeland SJ, Hammond SM, Minn AJ, Rosner MR. Raf kinase inhibitory protein suppresses a metastasis signalling cascade involving LIN28 and let-7. EMBO J. 2009; 28:347–58. [PubMed: 19153603]

42. Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, Mak RH, Ferrando AA, Downing JR, Jacks T, et al. MicroRNA expression profiles classify human cancers. Nature. 2005; 435:834–8. [PubMed: 15944708]

43. Johnson CD, Esquela-Kerscher A, Stefani G, Byrom M, Kelnar K, Ovcharenko D, Wilson M, Wang X, Shelton J, Shingara J, Chin L, Brown D, et al. The let-7 microRNA represses cell proliferation pathways in human cells. Cancer Res. 2007; 67:7713–22. [PubMed: 17699775]

44. Gumireddy K, Young DD, Xiong X, Hogenesch JB, Huang Q, Deiters A. Small-molecule inhibitors of microrna miR-21 function. Angew Chem Int Ed Engl. 2008; 47:7482–4. [PubMed: 18712719]

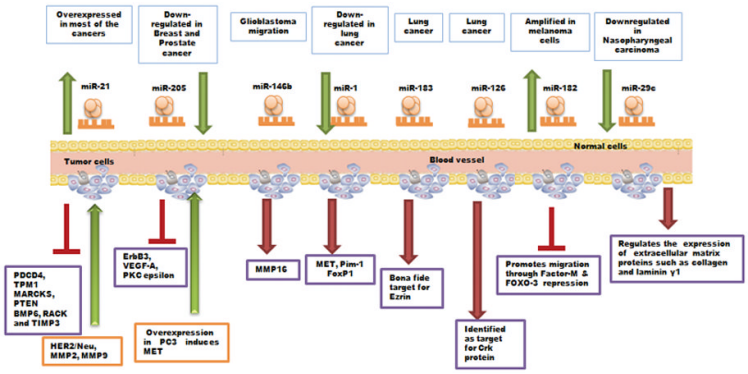

Figure 1. Schematic illustration of role of miRNAs in regulation of tumor cell invasion and migration

miR-21 is upregulated in cancer, and represses the expression of various tumor suppressor proteins such as PDCD4, TIMP1, MARCK5, BMD6, RACK, TIMP3 and PTEN. Also, miR-21 upregulates the expression of HER2, MMP2 and MMP9. MiR-205 is downregulated in several cancers, and it represses kinases such as ErbB3, VEGF and PKC epsilon. miR-146b modulates MMP16 in glioblastoma migration. miR-1 is downregulated in lung cancer, and targets of miR-1 are MET, Pim-1 and FoxP1. Ezrin is a target for miR-183 and Crk is a target for miR-126. miR-182 is upregulated in melanoma, and it promotes migration through its effects on Factor-M and FOXO-3. miR-29c is downregulated in nasopharyngeal carcinoma, and it regulates the expression of collagen and laminin. Arrows in the upper part of the figure represents upregulation (↑) and downregulation (↓) of respective miRNAs in cancer. Also shown in the lower part of the figure are the targets of those miRNAs which are either repressed (shown by reverse T) or upregulated (↓).

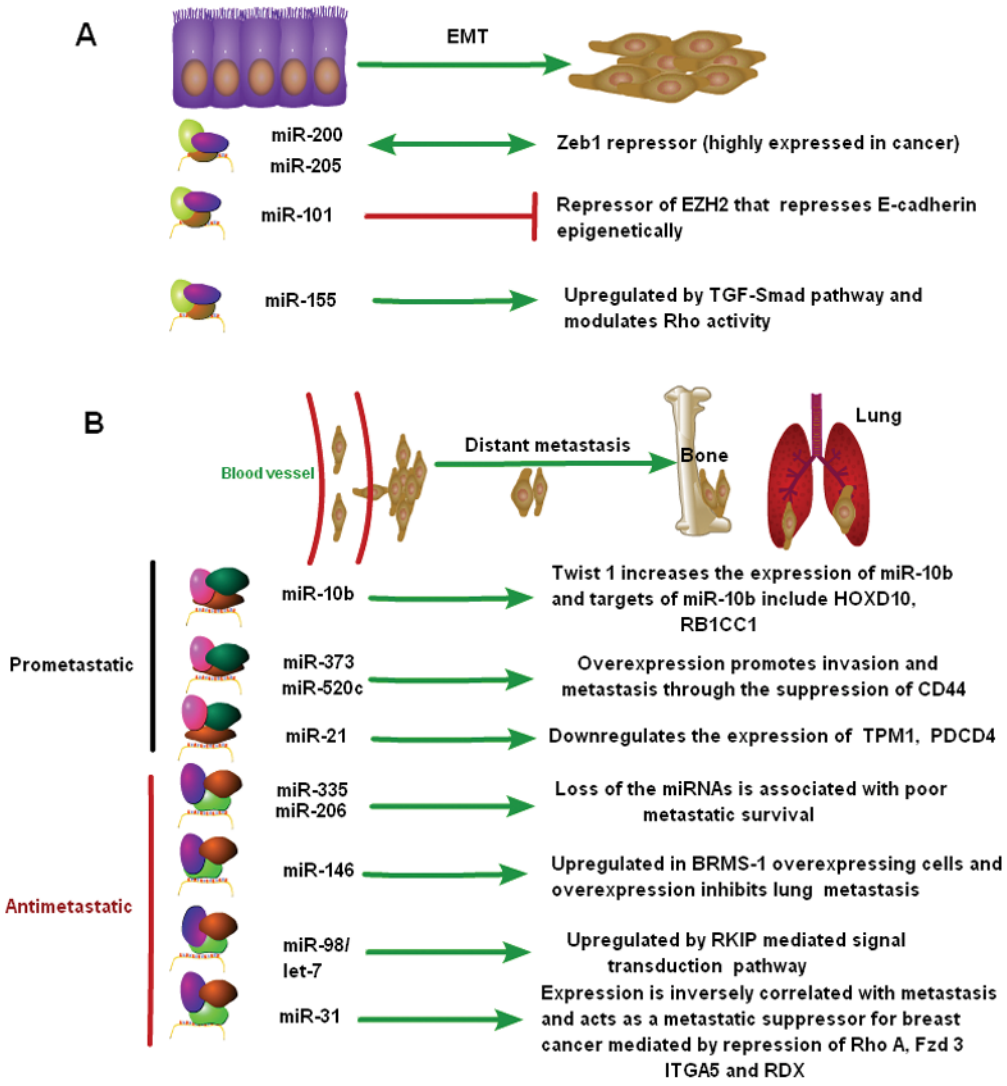

Figure 2. Role of miRNAs in metastasis

(A) EMT regulation by miRNAs: miRNAs such as miR-200 and miR-205, modulate the ZEB family of transcription factors and regulate epithelial-mesenchymal transition. miR-101 modulates E-cadherin expression epigenetically via targeting Zeste Homolog 2 (EZH2) in prostate cancer. miR-155 modulates TGFβ pathway, which plays an important role in cancer metastasis. (B) Suppression of metastasis by miRNAs:. miR-10b is the first miRNA identified to be upregulated in breast cancer that is mediated by Twist1, MMPs, urokinase plasminogen activator(uPA), and various integrins. miR-373 and miR-520c are pro-metastatic miRNAs that serve as metastasis promoters (partly mediated by modulation of CD44). miR-335, miR-206 and miR-31 are identified as antimetastatic miRNAs that target RhoA, Fzd3, RDX, and integrin α_5. miR-146 and miR-98/let-7 are shown to be upregulated in response to metastatic suppressor proteins BRMS-1 and RKIP respectively. miR-10b, miR-373, miR-520c and miR-21 are pro-metastatic miRNAs, while miRNAs such as miR-335, miR-206 and miR-31 are anti-metastatic. Prometastatic miRNAs are depicted pink, dark green and red, while anti-metastatic ones are shown in violet, red and light green.