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Control of Metastatic Progression by microRNA Regulatory Networks

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Abstract

Aberrant microRNA (miRNA) expression is a defining feature of human malignancy. Specific miRNAs have been identified as promoters or suppressors of metastatic progression. These miRNAs control metastasis through divergent or convergent regulation of metastatic gene pathways. Some miRNA regulatory networks govern cell-autonomous cancer phenotypes, while others modulate the cell-extrinsic composition of the metastatic microenvironment. The use of small RNAs as probes into the molecular and cellular underpinnings of metastasis holds promise for the identification of candidate genes for potential therapeutic intervention.

Introduction

Epithelial carcinomas are the predominant cancer types that kill humans by metastasizing from their tissues of origin to distal organs. Nevertheless, the cell-biological pathways regulating the multi-step metastatic cascade had not been adequately characterized until recently[1-3]. Several technological advances during the last decade, however, have greatly deepened our molecular and conceptual understanding of this process. The development of whole-genome profiling approaches has permitted global analysis of the cancer transcriptome, whereas the availability of RNA-interference tools and human cancer xenograft models have facilitated functional testing of specific genes in metastasis. A key concept that emerged from early mouse studies was that metastatic colonization requires the actions of many gene products[4-6]. These functional findings were corroborated by transcriptomic studies of human breast cancers, which revealed large sets of genes to be recurrently overexpressed in primary tumours that metastasize[7]. An outstanding question in the field at that time was how such concerted pro-metastatic gene expression states were attained. In recent years, small non-coding RNAs, or miRNAs, have emerged as a class of post-transcriptional regulators that displays a pervasive role in this type of coordinated gene expression control[8-13]. In this review, we provide a conceptual overview of the molecular and cellular processes governed by endogenous miRNAs during metastatic progression.

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Basics of miRNA biogenesis and action

Although the majority of human miRNAs reside in intergenic regions or within the introns of genes, exonic miRNAs have also been described[14]. Transcription of a single or of multiple concatenated miRNA-coding sequences is mediated by RNA polymerase II, and less frequently by RNA polymerase III, yielding a primary miRNA transcript or pri-miRNA[15,16]. Mammalian pri-miRNAs are processed through a series of cleavage steps[15-18]. Initially, the RNA-binding protein DGCR8 recognizes and binds to double-stranded hairpin structures embedded within the pri-miRNA. This interaction allows the ribonuclease Drosha, which forms a complex with DGCR8, to cleave the pri-miRNA at the hairpin junction site, releasing a roughly 70-nt long stem-loop product known as the pre-miRNA[15-17]. Association of the pre-miRNA with Exportin-5 and its co-factor Ran-GTP, facilitates its nuclear export[15,16]. Once in the cytoplasm, the ribonuclease Dicer bound to its co-factor, the RNA-binding protein TARBP2, cleaves the pre-miRNA, yielding a 21-26-nt long miRNA:miRNA* duplex structure[15,16,18]. This miRNA duplex is loaded onto the RNA-induced silencing complex or RISC, where the guide miRNA strand is preferentially incorporated into the complex. The complementary passenger miRNA* strand is typically released and degraded, except for cases in which the miRNA* strand also mediates silencing[14-16,19]. The key effector at the core of RISC is a member of Argonaut (Ago) family of proteins, which directly interacts with single-stranded mature miRNAs and uses them as guides to recognize mRNAs bearing sequences complementary to the bound miRNA[20-22]. Ago binding to target mRNAs results in the recruitment of silencing effector proteins such as GW182[23], ultimately leading to mRNA deadenylation and decay and/or translational repression[13,14]. Although the majority of miRNA regulatory sequences reside in the 3'-untranslated regions of transcripts, interactions of miRNAs with coding sequences have also been mapped[24-26]. The use of computational analyses, gene expression profiling, heterologous reporter assays, and biochemically coupled transcriptomic deep-sequencing approaches[27,28] have provided a toolbox for systematic discovery of miRNA target genes.

Deregulated miRNA expression in human cancer

The link between deregulated miRNA expression and cancer had been well established[29,30] prior to the identification of these small RNAs as regulators of metastasis Calin, Croce, and colleagues reported deregulated miRNA expression in cancer in a study showing that two clustered miRNAs, miR-15a and miR-16, were deleted or downregulated in the majority (> 60%) of B-cell chronic lymphocytic leukaemias tested[31]. Subsequent work demonstrated deregulated miRNA expression also in solid cancers: miR-145 exhibited reduced expression in colon[32] and breast[33] carcinomas, let-7 was found to be silenced in lung cancer[34,35], while miR-155, miR-21, and the miR-17-92 cluster were upregulated in breast and lung cancers[33,35-38]. Interestingly, miR-21, miR-155, and miR-17-92 were also found to be overexpressed in B-cell and Hodgkin's lymphomas[39-43], establishing them as potential oncogenic miRNAs in diverse cancers. Subsequent miRNA-profiling studies revealed distinct expression profiles in various solid and liquid cancers that could inform the diagnosis of these malignancies[29,44-49]. Although a small subset of specific oncogenic miRNAs have been found to be upregulated in cancer, global downregulation of miRNA expression and processing has emerged as a feature of these regulators during

tumorigenesis[50-54]. Consistent with this, a miRNA family (miR-34a-c) was found to be transcriptionally induced by the key tumour suppressor p53, which is frequently mutated and inactivated in several types of cancers[55-58].

Metastasis-suppressive miRNA networks

This early work raised the question of whether silencing of specific miRNAs could modulate metastasis by driving pro-metastatic gene expression programs. The search for miRNAs that repress the expression of pro-metastatic genes and consequently suppress metastasis originally focused on breast cancer[6]. Small RNA profiling of highly metastatic sub-lines derived from the *in vivo* selection of a human breast cancer population revealed a subset of miRNAs that were silenced in cells with high metastatic capacity. Functional testing demonstrated that overexpression of miR-335, miR-206, or miR-126 robustly suppressed metastatic colonization by human breast cancer lines. Conversely, antagomir-mediated inhibition of miR-335 enhanced metastasis, providing crucial loss-of-function *in vivo* evidence for endogenous miRNA activity in the regulation of metastasis. Importantly, the expression levels of these miRNAs in a collection of women's breast cancers correlated with these patients' likelihood of metastatic relapse[6]. Several studies have since validated the silenced expression and prognostic capacity of these miRNAs in independent and larger clinical cohorts for diverse cancer types[59-67], as well as their tumor-suppressive and/or metastasis-suppressive functions in other breast cancer models[59,68] and cancer types[67,69-72].

These miRNAs where shown to inhibit metastasis through several mechanisms. Whereas miR-335 and miR-206 suppressed cell invasion[6], endogenous miR-126 inhibited tumour growth, endothelial recruitment, and metastatic initiation by breast cancer cells[73]. Consistent with a role for miRNAs in establishing a metastatic gene expression program, miR-335 silenced a set of pro-metastatic genes, the expression of which was found to correlate with metastatic outcomes in breast cancer patients. Two of these genes, SOX4 and Tenascin-C, were found to drive the miR-335-suppressed phenotypes—cell invasion, migration, and metastasis[6] (Fig. 1a). SOX4 and Tenascin-C have been subsequently validated as promoters of cancer progression in additional models[74-76].

Another well-characterized example of a metastasis-suppressive miRNA network is that of miR-31[77-79] (Fig. 1b). Overexpression of miR-31 in human cancer cells strongly suppressed metastasis, whereas stable miRNA inhibition in otherwise non-metastatic cells led to a robust 10-fold increase in metastatic capacity, consistent with metastasis suppression by endogenous miR-31. Importantly, the expression of miR-31 in patients' primary breast tumors was prognostic of metastatic relapse[77]. Silencing of miR-31 has also been observed in additional cancer types[80-83]. Mechanistically, miR-31 was found to inhibit metastatic progression through coordinated regulation of three genes—integrin α5 (ITGA5), radixin (RDX), and RhoA, each of which was shown to promote anoikis resistance and cell invasion[77-79] (Fig. 1b). Collectively, these findings illustrate that silencing of robust metastasis-suppressor miRNAs (Table 1) in highly metastatic cells results in the lifting of key post-transcriptional regulatory barriers imposed on pro-invasive genes.

Metastasis-promoting miRNA networks

Could a miRNA conversely promote metastasis by targeting a metastasis-suppressive gene? In a bold series of experiments, Ma and Weinberg demonstrated that miR-10b was a driver of metastasis[84]. Overexpression studies in human breast cancer cells demonstrated sufficiency of miR-10b in promoting metastasis. This miRNA was found to enhance cell migration and invasion through targeting of homeobox D10 (HOXD10), which in turn inhibited the metastasis-promoting gene, RhoC[84,85] (Fig. 2a). Subsequent studies revealed a role for endogenous miR-10b in driving metastasis as well[86].

miR-373 and miR-520c, which belong to the same miRNA family, were also identified as metastasis-promoting miRNAs[87]. In an innovative forward genetic screen using human breast cancer cells transduced with a miRNA-expression library and assessed for their migration ability, miR-373 and miR-520c were found to be enriched in cells with enhanced migratory capacity. These miRNAs were shown to promote cell migration and invasion by targeting CD44 (Fig. 2b). Importantly, introduction of miR-373 or miR-520c was sufficient to confer metastatic capacity to otherwise non-metastatic human breast cancer cells[87].

The miR-200 family, which includes miR-200a, miR-200b, miR-200c, miR-141, and miR-429, is another prominent example of a metastasis-regulatory network driven by miRNAs. Early work by several independent groups demonstrated that this miRNA family is important in maintaining the tumor epithelial phenotype and inhibiting the epithelial-to-mesenchymal transition (EMT)[88-91]. This was supported by the strong correlation observed between the levels of miR-200s and the epithelial marker E-cadherin in a collection of cancer lines as well as in clinical samples[88,90]. Mechanistically, miR-200s were found to inhibit cell migration by directly targeting the transcription factors ZEB1 and ZEB2, which suppress E-cadherin (Fig. 2c). Overexpression of miR-200s was shown to decrease metastatic dissemination from primary tumors in a syngenic breast cancer mouse model[92]. In addition to the reports indicating a metastasis-suppressive role for the miR-200 family, recent findings have also demonstrated a role for these miRNA in promoting metastasis[93]. High levels of miR-200s correlated with shorter metastasis-free survival times in breast cancer patients, whereas overexpression of miR-200s enhanced lung colonization by murine breast cancer cells. Interestingly, the metastasis-promoting effects of miR-200s were mediated through downstream targets distinct from the ones implicated in EMT. Targeting of Sec23a, a COPII vesicle component, resulted in decreased secretion of TINAGL1 and IGFBP4—factors shown to suppress metastasis by mouse breast cancer cells (Fig. 2c)[93].

These findings implicate miR-200s as potential pleiotropic regulators of metastatic progression. Whereas silencing of miR-200s may be beneficial at the early steps of metastasis by triggering EMT through ZEB1/2-dependent repression of E-cadherin, upregulation of miR-200s at distal metastatic sites may actually promote metastatic colonization through Sec23 targeting. These seemingly opposing roles of miR-200s in metastasis could be reconciled if one considers the multi-faceted nature of metastasis. It is conceivable that context-dependent miRNA modulation at different steps of the metastatic cascade may drive both promoting and suppressive roles in cancer progression through divergent miRNA targeting of distinct molecular and cellular pathways. Loss-of-function

experiments on miR-200s should provide important insights into their complex roles during metastatic progression.

Whereas miR-200s indirectly promote E-cadherin expression to maintain an epithelial phenotype, miR-9, was found to directly target E-cadherin to drive cancer cell motility, invasion, and metastasis[94] (Fig. 2d). Overexpression of miR-9 enhanced micrometastasis formation by human breast cancer cells, whereas its stable silencing inhibited murine breast cancer metastasis. Subsequent work demonstrated that overexpression of miR-9 in E-cadherin-negative human breast cancer cells enhanced metastasis, suggesting that miR-9 represses additional metastasis-suppressive target genes[95]. This study identified leukemia inhibitory factor receptor (LIFR) as a direct target of miR-9 that mediates its pro-metastatic effects in the absence of E-cadherin. Depletion of LIFR in non-metastatic cells was shown to increase invasion and migration, whereas its overexpression abrogated the metastatic capacity conferred by miR-9. The metastasis-suppressive role of LIFR was found to be mediated through inactivation of YAP, a previously known oncoprotein whose activity is regulated by the Hippo pathway[95] (Fig. 2d).

In addition to breast cancer, metastasis-regulatory miRNAs have been identified in multiple other cancer types (Table 1). At roughly the same time as several reports demonstrating pro-tumorigenic and pro-metastatic roles for miR-21 in breast cancer[96-97], another group showed that miR-21 drives colorectal cancer invasion and metastasis by targeting PDCD4[98] (Fig. 2e). TPM1 and Maspin were two additional miR-21 targets uncovered in breast cancer.[97] Recently, miR-21 was also shown to drive metastasis of squamous cell carcinoma tumors deficient for p53[99]. Metastasis-regulatory miRNAs have also been described in melanoma, a highly intractable malignancy. Overexpression of miR-182 was initially shown to increase metastasis by mouse melanoma cells[100]. Subsequently, miR-30b/30d were found to correlate with human melanoma progression outcomes and their overexpression in human melanoma cells enhanced micrometastasis formation by targeting the GALNT1 and GALNT7 suppressors of cell invasion[101] (Fig. 2f). Finally, miR-199a-3p, miR-199a-5p, and miR-1908 were recently discovered as endogenous miRNA drivers of human melanoma metastasis. Inhibition of these clinico-pathologically correlated miRNAs in a series of human melanoma lines robustly suppressed metastasis[102].

Cell-intrinsic versus cell-extrinsic effects of metastasis-regulatory miRNAs

Early studies ascribed cell-autonomous phenotypes to miRNAs implicated in metastasis. For example, miR-335[6] and miR-31[77] were identified as suppressors of cell migration and invasion (Fig. 1), whereas miR-10b[84], miR-373/miR-520c[87], miR-9[94], and miR-21[96-98] were shown to promote these phenotypes (Fig. 2). Another prominent example of a miRNA regulatory network controlling a cell-intrinsic phenotype is that of the let-7 family[103-105] (Fig. 3a). let-7 overexpression in human breast cancer cells was initially shown to decrease the incidence of metastatic events to the liver and lung[103]. Subsequent work revealed that let-7-mediated repression of the chromatin-remodeling protein HMGA2 and the transcription factor BACH1, both of which promote the transcription of pro-invasive genes, suppressed cell invasion and metastasis to the bone[104-105] . Thus, let-7 halts the cell-intrinsic

phenotype of cell invasion, which is required for efficient metastasis, by coordinately targeting factors that regulate pro-invasive gene expression programs (Fig. 3a).

Recently, miRNAs were shown to regulate metastasis also by cell-extrinsic modulation of the metastatic microenvironment. Combined loss-of-function, gain-of-function, and epistasis experiments demonstrated that miR-126 inhibits the recruitment of endothelial cells by multiple human breast cancer lines *in vitro*. Endogenous miR-126 was also found to suppress metastatic endothelial recruitment (MER), the ability of cancer cells to recruit endothelial cells to incipient metastases *in vivo*[73]. Human breast cancer cells displaying silenced expression of miR-126 were shown to upregulate a set of miR-126 target genes—IGFBP2, MERTK, and PITPNC1—molecules that individually and collectively correlate in expression with human metastatic progression (Fig. 3b)[73]. These previously uncharacterised angiogenesis genes were shown to modulate the cell-extrinsic recruitment of endothelial cells by forming two divergent signalling pathways that emanate from cancer cells and engage endothelial cells. Cancer cell-secreted IGFBP2 was shown to promote endothelial cell migration by driving IGF1-dependent activation of the endothelial IGF1-Receptor (IGF1-R). PITPNC1, a phospho-inositide binding protein[106], was found to promote endothelial cell migration by enhancing extracellular IGFBP2 levels. Finally, the receptor tyrosine kinase MERTK was shown to be proteolytically cleaved from the surface of cancer cells and released into the extracellular space. This MERTK ectodomain promoted endothelial cell migration by acting as a decoy receptor and sequestering circulating GAS6—a molecule shown to inhibit endothelial cell migration through its action on endothelial MERTK receptors (Fig. 3b)[73].

The metastasis-suppressive role of miR-126 and its clinical association with human metastatic relapse were recently validated also in a mouse xenograft model of breast cancer metastasis[107]. This report found that, in addition to its ability to suppress angiogenesis which was previously demonstrated for human breast cancer lines of triple-negative[73], luminal[73,108], and HER-2 positive[73] backgrounds, as well as for a variety of additional cancer types such as lung[72] and oral[109] carcinomas, miR-126 overexpression can reduce mesenchymal stem cell (MSC) and monocyte content in primary xenograft tumors established by mouse 4T1 cells[107]. Interestingly, miR-126 silencing did not enhance monocyte or leukocyte recruitment by human breast cancer cells *in vivo*[73], suggesting that the inflammatory cell recruitment observed by these investigators may be limited to the 4T1 mouse system or be the consequence of overexpression effects.

Additional metastasis-regulatory miRNAs likely govern other non-cell-autonomous phenotypes by altering the tumor microenvironment. For instance, a recent report suggested a cell-extrinsic role for miR-29b, a miRNA transcriptionally driven by GATA3 whose expression is reduced in breast cancer[110]. This miRNA, which was also shown to inhibit prostate[111] and liver cancer metastasis[112] in addition to breast cancer metastasis[110], was found to target a set of genes that had been previously implicated in cell-extrinsic processes such as angiogenesis and collagen remodelling[110] (Table 1). Some miRNAs may even directly impact neighbouring cell types, as is the case for miR-9, which was shown by Ferrara and colleagues to regulate endothelial cell migration through its secretion from

cancer cells inside microvesicles[113]. Such microvesicles or exosomes have also been found to contain multiple proteins that could modulate the tumour microenvironment[114-115].

Convergent control of metastatic progression by multiple miRNAs

Nearly all previously described metastasis-regulatory miRNAs act through divergent targeting of multiple genes. However, recent work has delineated a network of miRNAs that drive metastasis through convergent targeting of functionally coupled genes[102] (Fig. 3c). Gain- and loss-of-function approaches demonstrated that miR-199a-3p, miR-199a-5p, and miR-1908 promote metastasis of multiple human melanoma lines of diverse mutational backgrounds. The combined expression of the three miRNAs exhibited stronger prognostic capacity in predicting metastatic outcomes than each individual miRNA. These findings, which are suggestive of miRNA cooperativity in human metastatic progression, were corroborated by *in vivo* loss-of-function studies. Inhibition of each miRNA suppressed metastasis by roughly 4-fold, while concurrent silencing of the three miRNAs decreased metastatic colonization by more than 70-fold, attesting to their remarkable functional cooperativity[102].

miR-199a-3p, miR-199a-5p, and miR-1908 were shown to convergently target two phenotypically related genes, the heat-shock factor DNAJA4 and the metabolic protein apolipoprotein-E (ApoE). Consistent with these two genes acting as the key downstream effectors of the miRNAs, the metastasis-suppressive effects observed following miRNA inhibition were rescued by depletion of either DNAJA4 or ApoE. Interestingly, DNAJA4 was shown to suppress metastasis through positive regulation of ApoE expression, establishing ApoE as the central node in this convergent miRNA regulatory network. The metastasis-suppressive role of ApoE was corroborated by the correlation observed between ApoE expression levels and human melanoma progression outcomes; by the ability of ApoE to abrogate metastasis of multiple human melanoma lines; and by the robust enhancement of metastasis following genetic deletion of ApoE in immunocompent mice. ApoE, which is secreted by melanoma cells, was shown to suppress both melanoma cell-intrinsic and cell-extrinsic metastatic phenotypes. The cell-autonomous targeting of melanoma LRP1 receptors by ApoE decreased melanoma cell invasion, whereas its non-cell-autonomous engagement of endothelial LRP8 receptors inhibited MER[102] (Fig. 3c). These findings provide an example of a convergent miRNA regulatory network, with miRNAs playing dual cell-intrinsic/cell-extrinsic roles in metastatic progression. Interestingly, MER has emerged as a common feature driving both breast cancer and melanoma metastasis[73,102].

Divergent versus convergent metastasis regulation by miRNAs

Initial findings on the connectivity of miRNA regulatory networks suggested that they control metastasis through their ability to coordinately target multiple genes. Divergent metastasis control was demonstrated for miR-335[6] and subsequently shown to be the pervasive mode of regulation for many other miRNAs, including miR-31[77], the miR-200 family[88-93], miR-9[94-95], miR-21[96-98], let-7[104-105], and miR-126[73]. The advantage that such divergent gene targeting confers to metastasis is conceptually intuitive: a single miRNA can coordinately regulate the expression of multiple genes, all of which participate in a common

metastatic phenotype. The concurrent silencing of a set of genes by a given miRNA leads to more profound modulation of the phenotype than independent silencing of individual genes.

In contrast, the pervasiveness of the convergent target regulation by multiple unique miRNAs in additional metastasis models remains to be explored. The advantage of convergent miRNA regulation stems from the maximum silencing of a single gene, such as ApoE, that is the central determinant of the metastatic phenotype[102]. Cooperative targeting by multiple miRNAs ensures more robust gene expression control than that afforded by a single miRNA and may have an added advantage in cases where genetic inactivation of a key metastasis-suppressive factor may not be tolerated by cells. The divergent and convergent models of connectivity that characterize miRNA regulatory networks in metastasis are unlikely to be coincidental. Instead, they could have arisen from evolutionarily conserved connectivity that likely serves important roles during development and physiology, making them vulnerable to co-option during pathogenesis.

Mechanisms underlying aberrant miRNA expression in metastasis

Altered miRNA expression results from diverse mechanisms operating during metastatic progression. For instance, the metastasis-suppressor miR-335 (Fig. 1a) was found to be inactivated in human breast cancer through both genetic deletion of the miR-335 locus and through epigenetically-driven transcriptional silencing of the locus by promoter hypermethylation[59]. Moreover, several metastasis-promoting miRNAs have been shown to be direct targets of well-known oncogenic transcription factors. miR-10b and miR-9 were found to be transcriptionally activated by TWIST1[84] and MYC/MYCN[94], respectively. Conversely, miR-200s have been reported to be transcriptionally repressed by ZEB1/2, allowing for negative feedback regulation of miR-200s by their metastasis-promoting target genes[91,116]. The metastasis-suppressor miR-34 was also shown to form a double-negative feedback loop with one of its targets, the oncogenic transcription factor SNAIL[117].

The components of the miRNA processing machinery have also been implicated in cancer progression. Consistent with expression analyses indicating downregulation of Dicer and Drosha in breast cancer progression[54,118], direct repression of Dicer by miR-103/107 enhanced breast cancer metastasis[119], whereas transcriptional induction of Dicer by Tap63 suppressed metastasis[120]. Additionally, inactivating mutations in Exportin-5 have been identified in a small subset of human colorectal tumors, leading to defective miRNA nuclear export[121]. Finally, small RNA-binding proteins that regulate the processing of a specific miRNA have also been implicated in metastasis. For instance, LIN-28, which binds and destabilizes the let-7 pre-miRNA, was shown to promote breast cancer metastasis[104]. Taken together, these findings reveal a diversity of inactivating and activating molecular mechanisms governing the expression of metastasis-regulatory miRNAs, which underscores their importance amongst the regulatory molecules expressed by cancer cells.

Translational potential for basic discoveries

The ability of small RNAs to regulate gene expression has generated much hope for exploiting them as potential therapeutic molecules or therapeutic targets of human diseases. The two main problems that have hindered the clinical translation of miRNAs are their

limited half-lives in serum and their limited delivery into target tissues. Although there is no broadly accepted or applied methodological approach for systemic *in vivo* delivery of therapeutic miRNAs, two recent reports raise renewed hope by using an alternative approach to "naked" nucleic acid delivery, which involves viral-based delivery methods[122-123]. Adeno-associated viral delivery of miR-26a, for instance, was found to significantly suppress tumorigenesis in a murine model of hepatocellular carcinoma—a cancer known to display reduced miR-26a expression[122].

In vivo inhibition of miRNAs through the systemic administration of locked nucleic acids (LNAs) has progressed to a much greater degree in the pre-clinical and clinical arenas than miRNA delivery[124]. For instance, systemic delivery of an LNA targeting miR-122—a hepatic miRNA that regulates cholesterol—led to significant decreases in plasma cholesterol in non-human primates[125]. Therapeutic targeting of this miRNA, which is also required for hepatitis C virus (HCV) replication, was additionally shown to successfully suppress HCV viremia[126]. These successes have motivated studies involving therapeutic LNA delivery in pre-clinical cancer models. To this end, systemic delivery of an LNA targeting the breast cancer metastasis-promoter miR-10b was shown to suppress metastasis[86]. Recently, silencing of miR-199a-3p, miR-199a-5p, and miR-1908 using a cocktail of LNAs was found to dramatically suppress melanoma metastasis[102], a condition that currently lacks effective therapeutic options.

Summary and future directions

miRNAs have been established as key metastasis regulators by numerous investigators working on diverse cancer types, with concepts such as divergent and convergent control by miRNA regulatory networks emerging more recently. Metastasis-regulatory miRNAs have been found to govern both cell-intrinsic processes and cell-extrinsic features of the tumour microenvironment. The prognostic capacity of these non-coding RNAs in predicting human metastatic outcomes suggests an active role for these molecules in human cancer progression. Most importantly, miRNAs have accelerated the discovery of genes and signalling pathways mediating metastasis. These basic explorations will expedite translational efforts aimed at reducing cancer mortality.

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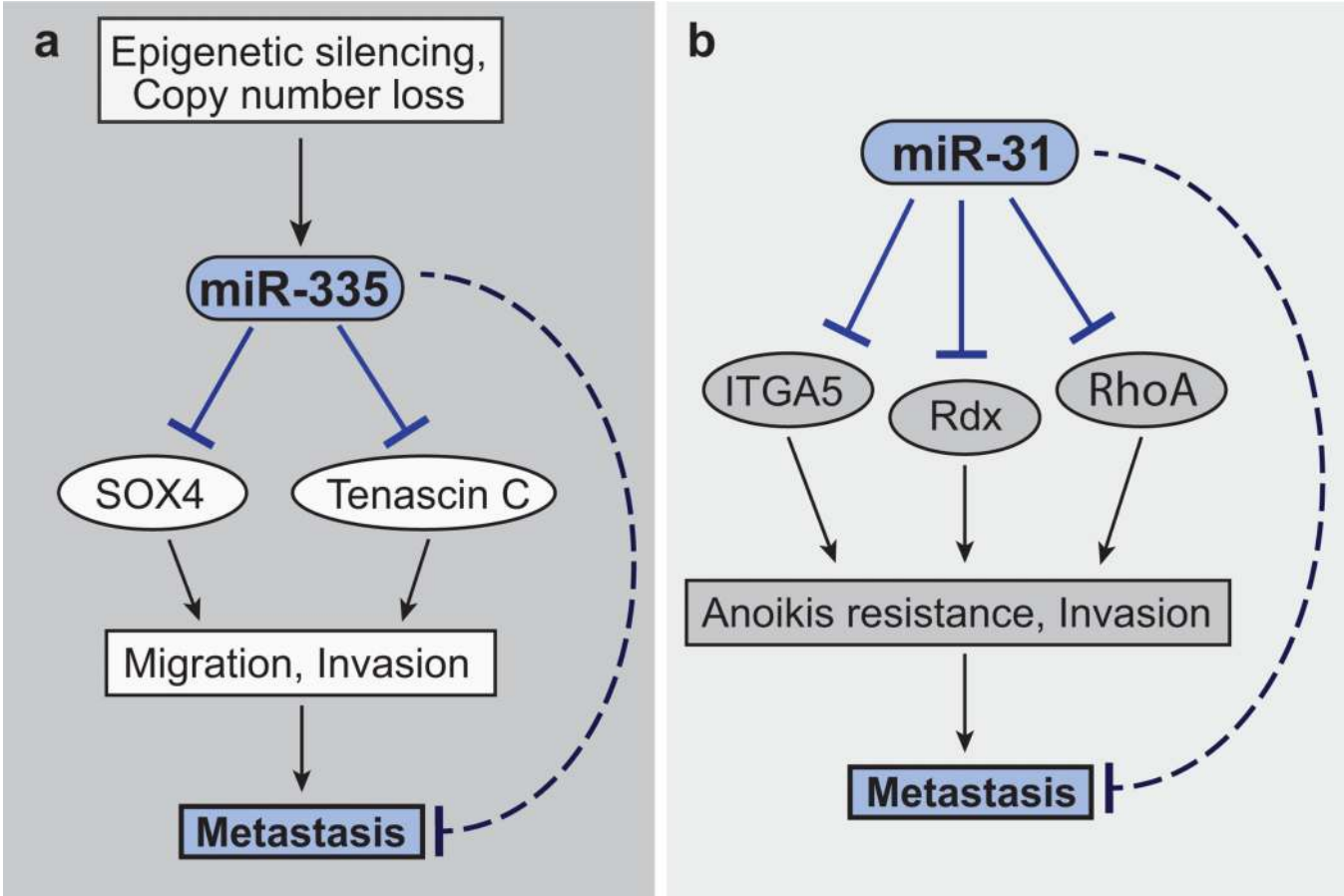

Figure 1.

Metastasis suppression by divergent miRNA regulatory networks. (**a**) miR-335 suppresses breast cancer metastasis by targeting SOX4 and Tenascin-C, which in turn promote cancer cell migration, invasion, and ultimately metastasis[6]. miR-335 is silenced in metastatic breast cancer cells through genetic copy number loss and epigenetic promoter hypermethylation[59]. (**b**) miR-31 inhibits breast cancer cell invasion, anoikis resistance, and metastasis by coordinately targeting ITGA5, RDX, and RhoA[77-79].

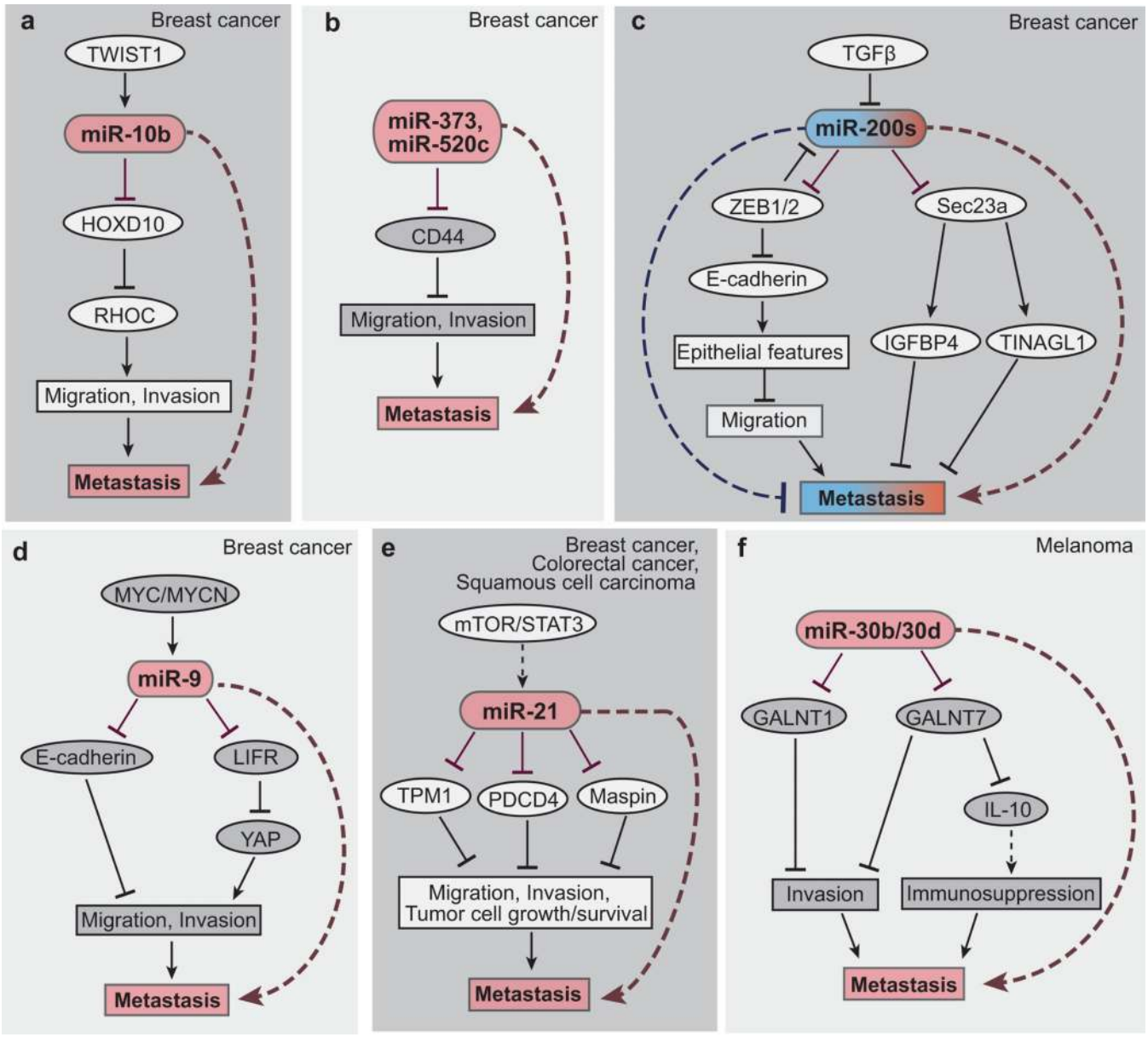

Figure 2.

Examples of miRNA regulatory networks that promote metastasis. (**a**) miR-10b drives metastatic breast cancer progression by direct targeting and suppression of HOXD10, which in turn inhibits RhoC—a promoter of migration, invasion, and metastasis. TWIST1 transcriptionally induces miR-10b in breast cancer cells[84]. (**b**) miR-373 and miR-520c promote breast cancer metastasis by directly targeting CD44, which leads to increased cancer cell invasion and migratory capacities[87]. (**c**) miR-200s, which are negatively regulated by TGFβ[127], display a pleiotropic role in metastatic progression. miR-200-mediated targeting of ZEB1/2 results in de-repression of E-cadherin expression, which maintains the epithelial tumor phenotype and suppresses migration and metastasis[88-92] (left blocking arrow), while miR-200-mediated silencing of Sec23a promotes metastatic colonization by inhibiting secretion of TINAGL1 and IGFBP4[93] (right blocking arrow). (**d**)

miR-9 enhances breast cancer metastasis by two independent pathways. Direct repression of E-cadherin by miR-9 leads to enhanced cell invasion and migration[94] (left blocking arrow), whereas miR-9 targeting of LIFR results in activation of the Hippo signalling component YAP[95] (right blocking arrow). MYC/MYCN drives transcription of miR-9 in breast cancer cells[94]. (**e**) miR-21 promotes cancer cell invasion and metastasis through coordinate suppression of TPM1, PDCD4, and Maspin[96-98]. The miR-21 target genes have also been shown to suppress cancer cell growth and survival[96,134-136]. Active mTOR and STAT3 signaling has been shown to correlate with miR-21 upregulation in cancer cells[99]. (**f**) miR-30b/30d increases melanoma invasion and metastasis by targeting GALNT1 and GALNT7. GALNT7 reduces the levels of IL-10—a putative immunosuppressive factor[101].

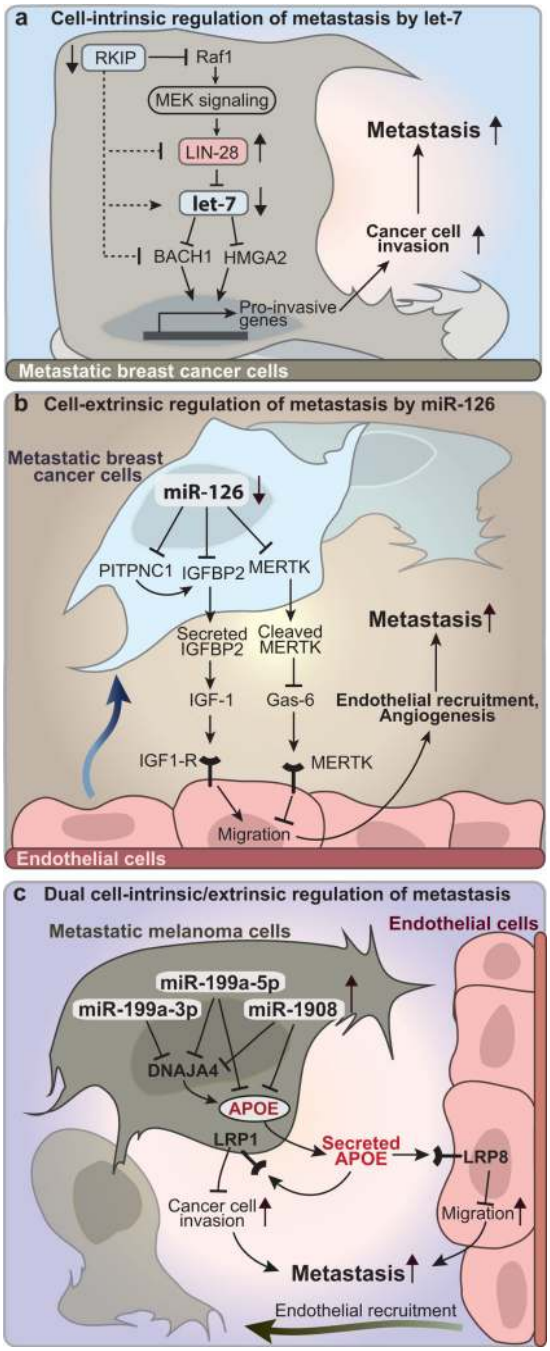

Figure 3.

Cell-intrinsic versus cell-extrinsic control of metastatic progression by miRNAs. (**a**) The let-7-regulatory network suppresses metastasis by inhibiting the cell-intrinsic phenotype of cancer cell invasion. Direct targeting of HMGA2 and BACH1 by let-7 leads to transcriptional suppression of a set of pro-invasive genes. Let-7 is regulated by LIN-28, a miRNA-binding protein that destabilizes the let-7 pre-miRNA and ultimately promotes metastasis. RKIP, an indirect repressor of LIN-28, suppresses metastasis through upregulation of let-7[104-105]. (**b**) miR-126 halts metastasis by impairing the cell-extrinsic

ability of breast cancer cells to recruit endothelial cells into the metastatic niche. Cancer-expressed miR-126 coordinately targets PITPNC1, IGFBP2, and MERTK. Cancer cell-secreted IGFBP2 promotes endothelial cell migration by binding to endothelial IGF1-R, while PITPNC1 induces IGFBP2 expression. In parallel to this IGFBP2-driven pathway, cancer cell-cleaved MERTK ectodomain increases endothelial cell migration by binding and sequestering Gas-6—an extracellular factor that inhibits endothelial cell migration through its action on endothelial MERTK receptors[73]. (**c**) Three miRNAs—miR-199a-3p, miR-199a-5p, and miR-1908—promote melanoma metastasis by convergent targeting of DNAJA4 and ApoE. DNAJA4 suppresses metastasis by inducing ApoE expression. Melanoma cell-secreted ApoE halts metastatic progression by both cell-autonomous and non-cell-autonomous mechanisms. ApoE suppresses melanoma cell invasion by targeting melanoma LRP1 receptors, while its inhibition of endothelial cell migration results from its engagement of endothelial LRP8 receptors[102].

Table 1

Examples of metastasis-regulatory miRNAs

miRNA	Cancer type	Targets	Phenotype	Refs
Metastasis suppressor miRNAs				
miR-335	Breast cancer	SOX4, Tenascin C	Invasion, Migration	6
miR-126	Breast cancer	IGFBP2, PITPNC1 MERTK	Endothelial recruitment	73
miR-200s	Breast cancer	ZEB1, ZEB2	EMT, Migration	88-92
miR-31	Breast cancer	RhoA, RDX, ITGA5	Invasion, Anoikis resistance	77-79
let-7	Breast cancer	BACH1, HMGA2	Invasion	103-105
miR-34a	Prostate cancer, Breast cancer, Colorectal cancer	CD44, Fra-1, SNAIL	Invasion, Migration EMT	117, 128-129
miR-29b	Breast cancer, Prostate cancer, HCC	ANGPTL4, LOX, MMP2, MMP9, VEGFA, PDGF	EMT, Invasion	110-112
miR-139	HCC	ROCK2	Invasion, Migration	130
Metastasis promoter miRNAs				
miR-10b	Breast cancer	HOXD10	Invasion, Migration	84,86
miR-373/520c	Breast cancer	CD44	Invasion, Migration	87
miR-200s	Breast cancer	Sec23a	Colonization	93
miR-9	Breast cancer	E-cadherin, LIFR	Invasion, Migration	94-95
miR-21	Breast cancer, Colorectal cancer, Squamous carcinoma	PDCD4, TPM1, Maspin	Invasion, Tumor growth, Tumor cell survial	96-99, 134-136
miR-103/107	Breast cancer	Dicer	Migration, EMT	119
miR-182	Melanoma	FOXO3, MITF	Invasion, Apoptosis	100
miR-30b/30d	Melanoma	GALNT7, GALNT1	Invasion	101
miR-199a-3p, miR-199a-5p, miR-1908	Melanoma	DNAJA4, ApoE	Invasion, Endothelial recruitment	102
miR-214	Melanoma	TFAP2C, ITGA3	Migration, Extravasation, Anoikis resistance	131
miR-223	Gastric cancer	EPB41L3	Migration, Invasion	132
miR-143	HCC	FNDC3B	Invasion	133

HCC = hepatocellular carcinoma