

# MicroRNAs (miRNAs) in cancer invasion and metastasis: therapeutic approaches based on metastasis-related miRNAs

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**Abstract** The management of tumor cell invasion and metastasis is instrumental in cancer therapy, since metastases are the prime reason for cancer patient mortality. Various cellular mechanisms and underlying molecular pathways relevant for metastasis have been identified so far, providing a basis for antimetastatic drugs. MicroRNAs (miRNAs) are highly conserved, small noncoding RNA molecules that have been shown to regulate various cellular processes by interfering with protein expression through posttranscriptional repression or mRNA degradation. More importantly, beyond their roles in physiological processes, many miRNAs are aberrantly expressed in various pathologies including cancer and regulate tumor- and metastasis-associated genes. Their pivotal role in metastasis has emerged only recently. After an introduction into the mechanisms of miRNA action, this review article describes the roles of miRNAs in cancer invasion and metastasis. Various miRNAs are discussed with regard to their upstream regulators, downstream target genes, and pro-/antimetastatic effects. A table provides a comprehensive overview of miRNAs that are misregulated/relevant in metastasis and the current knowledge regarding their underlying molecular effects. Furthermore, therapeutic approaches based on miRNAs, either as drugs or as therapeutic targets, are described prior to the discussion of the delivery of miRNA-based therapeutics as novel strategy in antimetastatic treatment.

**Keywords** Metastasis · MicroRNAs · miRNA · Novel miRNA-based therapies

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## Introduction: principles of metastasis

Since mortality in cancer patients is mostly associated with metastasis rather than with the primary tumor itself [1], the clinical management of tumor cell invasion and metastasis plays a pivotal role. This indicates the need for the development of novel treatment strategies to interfere with these processes. Beyond the understanding of the cellular mechanisms relevant to metastasis formation, this will rely on the identification of the relevant molecular pathways and their therapeutic inhibition. In addition to various proteins, microRNAs (miRNAs) have recently emerged as important molecules in metastasis and as possible drugs/drug targets.

Based on the complex multi-step process in metastasis formation (see, e.g., [2, 3] for review), antimetastatic drugs can be designed to act at various levels. Therapeutically attractive approaches include (a) anti-angiogenesis in order to inhibit the initial formation of blood vessels in the primary tumor and/or the blood vessel supply in established micrometastases, (b) anti-invasive or antimigratory strategies in order to inhibit tumor cell invasion into the blood or lymphatic system, (c) sensitization to anoikis in order to induce cell death of detached cells, (d) anti-adhesive strategies in order to inhibit tumor cell attachment and extravasation at the site of metastasis formation, as well as (e) antiproteolytic and antimigratory strategies which inhibit later steps of extravasation by limiting both degradation of the ECM and tumor cell motility. At all steps, critical proteins have been identified which may serve as targets (see, e.g., [4, 5]).

Notably, while the characteristics of the tumor cells largely contribute to the successful formation of metastases, the surrounding microenvironment also plays a major role. This can readily explain the preferential metastasization of certain tumor cells to certain organs (see, e.g., [3, 6] for review). This

“soil and seed” concept, which was already introduced in 1889 by S. Paget (see [7] for review), consequently also identifies the secondary site as a potential therapeutic target, in addition to the tumor cell, to block metastasis.

Another very important process affects the tumor cell itself and relates to the so-called epithelial–mesenchymal transition (EMT), which is characterized by the molecular reprogramming of the cell and the introduction of phenotypic changes. The induction and progression of EMT may involve various pathways, which ultimately lead to the upregulation of mesenchymal genes and the downregulation of epithelial genes, and may include the action of various miRNAs (see below). Notably, several of these pathways, like the Notch and Wnt signaling or the signal transduction of various growth factors (EGF, FGF, PDGF, and TGF- $\beta$ ) mediated through their receptor tyrosine/serine–threonine kinases, are also relevant in the primary tumor (see [8, 9] for review). A key process in the EMT is the change in the cadherin expression pattern by transcriptional repression of E-cadherin, which is expressed in epithelial cells and promotes cell–cell contacts and a parallel increase in N-cadherin expression, which is associated with mesenchymal cells and facilitates tumor cell binding to the stroma [10]. In addition to E-cadherin, other epithelial genes involved in cell–ECM and cell–cell interactions are also downregulated (see [11] for review). E-cadherin is transcriptionally repressed by Snail, Snail2/Slug, the basic helix–loop–helix protein E47, and the zinc finger/homeodomain proteins zinc finger E box-binding homeobox (ZEB)1 and ZEB2, which thus represent metastasis-relevant genes. Consequently, a correlation between metastasis formation/poor prognosis and the expression of Snail, Snail2, Twist as well as ZEB1 and ZEB2 has been described in various tumors (for review, see [12] and references therein). Interestingly, ZEB1 and ZEB2 have been found to be involved in a miRNA network, thus mediating miRNA effects on metastasis (see below). The formation of micrometastases eventually also requires the reverse process, i.e., the reversion of mesenchymal cells to epithelial cells. Thus, this process may represent another drug target for metastasis prevention.

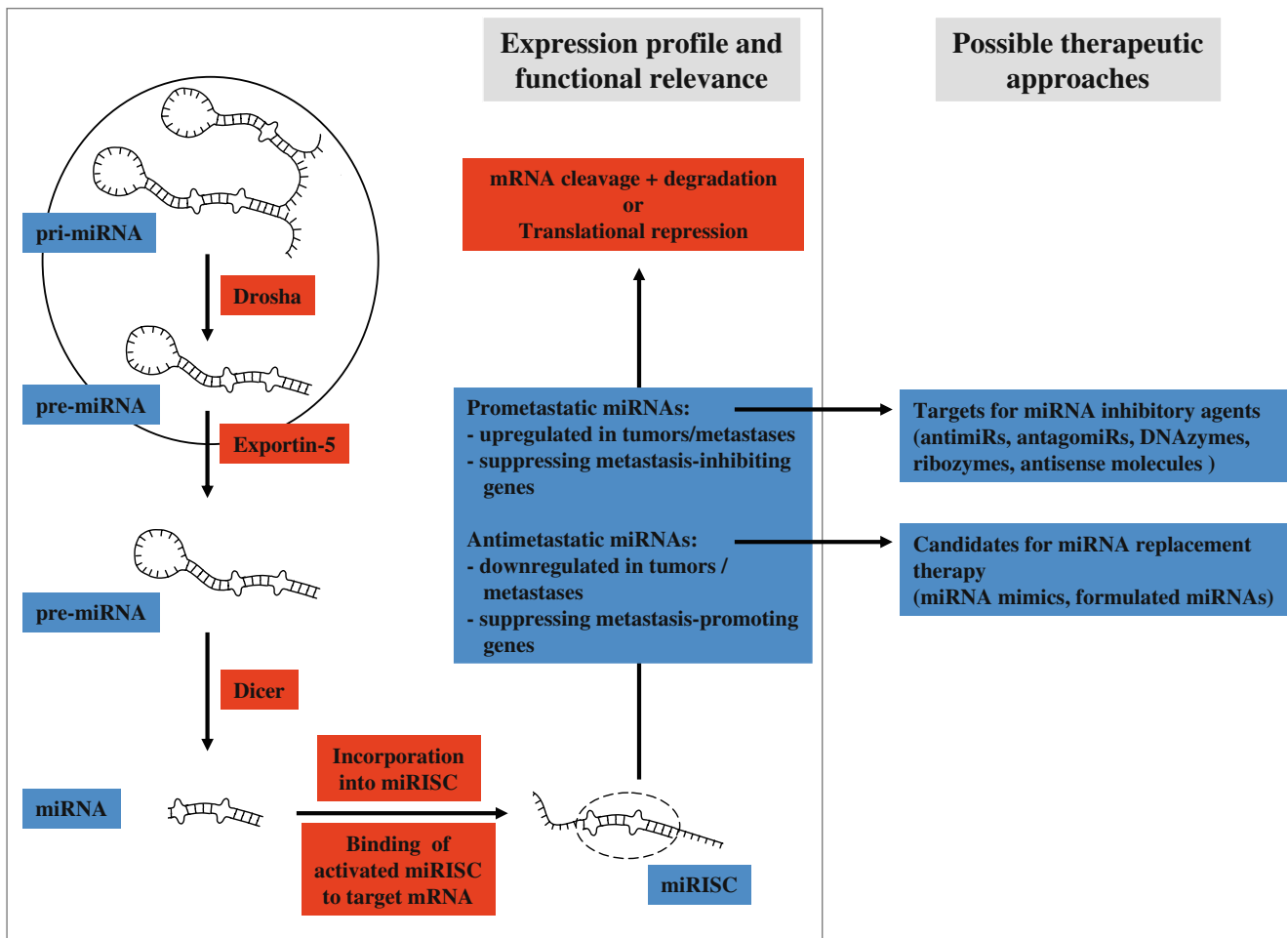
## MicroRNAs

MiRNAs are highly conserved, small 17–25-nt noncoding RNA molecules which are able to control gene expression at the posttranscriptional level by specifically interacting with a target mRNA. More specifically, dependent on the miRNA and its complementarity, miRNAs lead either to the cleavage of the mRNA and its subsequent degradation due to the presence of unprotected ends or to translational repression (see below).

Since their discovery, it has become clear that miRNAs regulate various processes including cell differentiation, proliferation, apoptosis, angiogenesis, and the cell cycle (see, e.g., [13–15]), and it is estimated from *in silico* analyses that miRNAs can control the expression of ~30% of all proteins in humans [16]. Based on their function and location on chromosomes, miRNAs have been assigned to different families and are involved in various physiological and pathophysiological processes.

The mechanism of miRNA expression, processing, and action is shown in Fig. 1. MiRNA transcription occurs in the nucleus, leading to primary miRNAs (pri-miRNAs) of several hundred base pairs in length. They are subsequently processed by the RNase III enzyme Droscha and the protein Pasha/DGCR8 into shorter (60–70 nt), hairpin-shaped double-stranded precursor miRNAs (pre-miRNAs), with a characteristic stem loop structure [17]. By RAN GTPase/exportin-5, the pre-miRNAs are transported into the cytoplasm where they are further processed into mature miRNAs by an enzyme complex containing the TAR binding protein 2 (TARBP2) and the RNase III endonuclease Dicer [18, 19]. This maturation process is aided by Argonaute endonucleases including Ago1 and Ago2, which bind to TARBP2 and Dicer and, in concert with additional proteins, form the so-called multiprotein RNA-induced silencing complex (miRISC). Upon their internalization into miRISC, miRNAs are able to bind to their target mRNA, thus guiding miRISC to the target sequences through binding to imperfect complementary sites within the 3' untranslated regions of the mRNA. This binding of miRISC leads, dependent on the degree of miRNA complementarity, to the inhibition of mRNA translation, or mRNA cleavage and subsequent degradation [20–22]. The so-called seed sequence or seed region (nucleotides 2–8) is crucial for miRNA specificity. Based on their only limited complementarity (as opposed to small interfering RNAs, siRNAs, which induce RNA interference), it becomes obvious that miRNAs may simultaneously target different genes. In fact, it is estimated that some miRNAs may potentially have 100 or more target genes, which translates into a complex pattern of control of gene expression. Likewise, a given gene can be controlled by different miRNAs, again increasing the complexity of posttranscriptional control (see examples below).

Beyond their roles in physiological processes, many miRNAs have been shown to be aberrantly expressed in various pathologies including cancer (see, e.g., [23–26] for review). In fact, >50% of the genes encoding human miRNAs are located in fragile chromosomal regions that are prone to deletion, amplification, or translocation, e.g., during the development of tumors [27]. This has led to the identification of “miRNA signatures” that are characteristic for certain tumors and allow their further classification [24,



**Fig. 1** Schematic representation of the mechanisms of miRNA expression, processing, and action and of possible therapeutic approaches related to miRNAs in metastasis. MiRNA transcription occurs in the nucleus (*circle*), leading to primary miRNAs (*pri-miRNAs*) which are subsequently processed by Drosha and Pasha/DGCR8 into shorter hairpin-shaped, double-stranded precursor miRNAs (*pre-miRNAs*). By RAN GTPase/exportin-5, the pre-miRNAs are transported into the cytoplasm where they are further processed into mature miRNAs by the TAR binding protein 2 and the RNase III endonuclease Dicer. A miRNA and the Argonaute endonucleases Ago1 and Ago2, in concert with additional proteins, form the so-

called multiprotein RNA-induced silencing complex (miRISC). The miRNA guides miRISC to the target sequences by binding to imperfect complementary sites within the 3' untranslated regions of the target mRNA. This binding of miRISC leads, depending on the degree of miRNA complementarity, to the inhibition of mRNA translation or mRNA cleavage and subsequent degradation. Depending on their expression levels (i.e., up- or downregulated in tumors and/or metastases) and their role in metastasis, miRNAs can be considered as prometastatic or antimetastatic, thus representing targets for miRNA inhibition or candidates for miRNA replacement therapy (*right panel*)

28]. Thus, miRNAs may serve as potential biomarkers for cancer diagnosis and prognosis (see [25] and references therein). Notably, miRNA analysis is also feasible in formalin-fixed tissue, and this finding supports the validity and utility of approaches aimed at global miRNA profiling in (archived) formalin-fixed paraffin-embedded samples [29, 30]. Furthermore, the maintenance of miRNA integrity also applies to circulating miRNAs in the bloodstream, and recent studies have established the quantitation of tumor-derived miRNAs in serum/plasma as a promising approach in cancer diagnostics (see [31, 32] for review). The screening of blood for miRNAs may well also have prognostic value with regard to the presence of

metastases or the potential of a given tumor for metastasis formation. In fact, several miRNAs have been shown to promote or suppress metastasis (see below; [33]), and, for example, the downregulation of miR-221 or the methylation of miR-9-1 has already been described as markers for metastasis [34, 35].

Beyond analytical purposes, miRNA expression profiles are functionally relevant since many miRNAs have been shown to act as tumor suppressors or, contrarily, as oncogenes. Thus, they contribute to the process of tumorigenesis when down- or upregulated, respectively, or influence the sensitivity of tumor cells to cytostatics or radiotherapy (see, e.g., [36, 37] for review). For many

miRNAs, target genes have been identified which are relevant in tumorigenesis, tumor growth, tumor angiogenesis, and metastasis (e.g., p53 [38] as a target for miR-125b; p63 as a target for miR21, miR-92, and miR-302 [39–41]; HMGA2, myc, and ras as targets for let-7 [42]). Likewise, several upstream regulators of miRNA expression are known which are also tumor relevant, like EGFR regulating miR-125a-5p [43], myc regulating miR-9 [44] and let-7 [45, 46], or NF- $\kappa$ B regulating miR-143 [47]. Beyond the direct involvement in miRNA transcription, this regulation can also involve the processing of miRNAs. In fact, p53/p63/p73 have been shown recently to interfere with the processing of miRNAs let-7, miR-16, miR-21, miR-107, miR-134, miR-143, miR-145, miR-220c, miR-449a, and miR-503, by regulating the major components of the miRNA processing machinery including Drosha-DGCR8, Dicer-TRBP2, and Ago proteins [48]. Finally, in some cases, autoregulatory feedback loops have been found, like for let-7 which inhibits metastasis by downregulating myc, with myc in turn transcriptionally and posttranscriptionally inhibiting let-7. A similar double-negative feedback loop is observed for the miR-200 family, with ZEB1 and ZEB2 being a target of miR-200 and, at the same time, a transcriptional repressor of both miR-200 gene clusters [49, 50]. Since ZEB1/ZEB2 are relevant in the EMT transition, this directly affects the epithelial vs. mesenchymal status of tumor cells and thus their migratory potential. Taken together, this introduces a complex picture of miRNA function and regulation in pathophysiological processes including metastasis.

### MiRNAs in metastasis

A wide variety of miRNAs have been identified with putative or established relevance in metastasis (see Table 1 for a comprehensive overview). Several miRNAs, however, also play pivotal roles in other tumor-relevant processes (see, e.g., [25, 26] for an overview on molecular mechanisms), thus making them attractive targets or tools for the treatment of the primary tumor and simultaneously for a therapeutic intervention in metastasis. Examples include, but are by no means limited to, miR-21 as one of the most intensively studied miRNAs whose metastasis-promoting role has been established in a wide variety of cancers (see Table 1 for references). Simultaneously, it exerts anti-apoptotic, pro-proliferative, and chemoprotectant effects and is linked to aggressiveness and hormone receptor status (see, e.g., [51] for review; [52]). The same is true for members of the let-7 family which comprises 13 miRNA members located on nine different chromosomes. They are aberrantly expressed in many human cancers and, in addition to suppressing metastasis (see Table 1 for

references), have been shown to influence chemo- and radiosensitivity and, more generally, the development of poorly differentiated, aggressive cancers ([52]; see, e.g., [53] for review). Likewise, miRNAs encoded by the miR-15/16 cluster act as tumor suppressors by inhibiting tumor cell proliferation and promoting apoptosis. Their function in suppressing tumorigenicity is impaired by their downregulation in various tumors including prostate carcinoma [54]. Consequently, the systemic delivery of synthetic miRNA-16 offers an approach for therapeutic intervention ([55]; see below).

For many miRNAs relevant in metastasis, target genes with an established role in tumor cell invasion, migration, and other steps in the metastatic process have been identified. Examples are MMPs, HER receptors, BMPs, PTEN, ZEB1, ZEB2, or E-cadherin (see Table 1). Some metastasis-relevant genes are simultaneously regulated, either directly or indirectly, by several miRNAs. For example, while members of the miR-200 family (miR-141, miR-200a,b,c, miR-429) have been shown to regulate the epithelial character of cells through silencing of ZEB proteins, ZEB1/ZEB2 are also regulated by miR-205 [56] or miR-192 [57]. ZEB1 and ZEB2 are regulators of the mesenchymal phenotype and transcriptional inhibitors of E-cadherin [58, 59]. E-cadherin, however, is also regulated by several other miRNAs including miR-101 via EZH2 [60] or miR-9 [44], and EZH2 interferes with metastasis through other pathways, e.g., by activating Ras and NF- $\kappa$ B [61]. On the other hand, any given miRNA might regulate a repertoire of metastasis-related genes rather than only a single one (see, e.g., Table 1). This can lead to additive effects as a result of interference with multiple pathways, which may also compensate for the sometimes rather subtle regulation of a given gene. Thus, it is necessary to elucidate the individual contribution of various regulatory pathways to metastasis as well as their complex interplay. The multiple functions of a given miRNA may indeed also lead to seemingly contradictory effects. For example, miR-200 was found to be inversely correlated with tumor invasion and metastasis in NSCLC, forced expression of miR-200 in lung carcinoma cells abrogated EMT, invasion, and metastasis [62, 63], and its effect on E-cadherin/N-cadherin expression also suggests antimetastatic effects (see above, [63]). However, other studies have demonstrated a miR-200-mediated increase in metastasis of mammary carcinoma cell lines [58]. An approach to reconcile those seemingly contradicting findings focuses on the different steps during metastasis. In fact, dependent on the cellular context, the rate-limiting step in metastasis formation may either lie in tumor cell invasion for the initiation of metastasis or in tumor cell colonization as its final step, and therefore, the enforcement of the epithelial character may either inhibit or favor metastasis, respectively. Thus,

miR-200 is able to promote or inhibit metastasis formation while acting through the same molecular pathway (i.e., through silencing of ZEB1 and ZEB2).

Conversely, miR-31 has clearly been demonstrated to exert antimetastatic effects, and in breast cancer metastasis, three target molecules, integrin- $\alpha$ 5 (ITGA5), radixin (RDX), and RhoA, have been identified [64]. As a consequence, miR-31 simultaneously inhibits different steps in the metastatic process, i.e., invasion, extravasation, survival, and colonization [64–66], which may explain the high impact of this miRNA on metastasis. Interestingly, while miR-31 is predicted to modulate >200 mRNAs, this study also demonstrates that its antimetastatic effect can be entirely explained by the regulation of the three targets [65].

### Therapeutic approaches based on miRNAs

Since miRNAs have been shown to play pivotal roles in tumorigenesis and metastasis, they may well serve as targets for their therapeutic inhibition or as therapeutic drugs. In the case of aberrant upregulation, the direct targeting of miRNAs participating in tumor pathogenesis by “anti-miRNA” molecules can silence miRNAs. In contrast, in the case of aberrant downregulation of miRNAs which directly target the mRNA of genes involved in tumor pathogenesis, so-called miRNA mimics or similar agents can be employed as drugs to substitute for the pathologically downregulated miRNAs. The fact that miRNAs exert only moderate effects on their target genes and that they are already present and functional under normal physiological conditions may account for the absence of unwanted side effects. Indeed, the literature on miRNA replacement approaches so far does not provide evidence for adverse effects in other organs [55, 67–69]. Careful monitoring will still be required to firmly exclude unwanted effects. Oncogenic lesions are commonly characterized by a widespread miRNA deregulation as shown, for example, for c-Myc [46], suggesting that more than one miRNA could be needed for a successful therapeutic intervention. Interestingly, however, previous studies have shown that the delivery of just one miRNA already exerts tumor-inhibitory effects [55, 67–71]. This may well also apply to the therapeutic intervention in metastasis. Yet, the development of more personalized treatment strategies may be required by precisely analyzing aberrant miRNA expression profiles prior to selecting one or more miRNAs relevant for therapy. Apart from miRNA-related agents, various RNA inhibiting agents have been explored *in vitro* and *in vivo* and introduced into the clinics so far, including antisense oligonucleotides, ribozymes/DNAzymes, and siRNAs/shRNAs (see, e.g., [72–75] for review). In contrast to these, the major advantage of miRNAs as therapeutic

targets or drugs may lie in the fact that a given miRNA targets several genes at the same time with the possibility of enhanced biological effects (or, on the other hand, unwanted additional effects).

For the inhibition of miRNAs, anti-miRNA oligonucleotides (AMOs) have been introduced, which bind to the miRNA and thus impair its interaction with the target mRNA. Chemical modifications were developed to protect AMOs from degradation and to prevent cleavage by the RISC nuclease. In a nontumor-related study *in vivo*, this anti-miRNA approach elucidated the role of miR-122 regulation of lipid metabolism [76]. Likewise, it was shown that locked nucleic acid (LNA)-modified anti-miRNA oligonucleotides [77] were able to inhibit miR-21 in glioblastoma or breast cancer cells, leading to antitumor effects *in vitro* and *in vivo* [78, 79]. Upon systemic delivery in nonhuman primates, LNA-modified, unconjugated “AntimiRs” also effectively antagonized miR-122 in the liver [80]. Furthermore, so-called antagomirs, comprising chemically modified single-stranded 23-nt RNA molecules complementary to their target miRNA and cholesterol as their covalently coupled binding partner for delivery, have been shown upon *i.v.* injection in mice to specifically silence their target miRNA, e.g., miR-122, in liver and other organs [81]. Finally, the transcription of miR-21 has been blocked by a low molecular weight compound, thus demonstrating the possibility to interfere with miRNA expression at an early stage [82].

The relevance of various miRNAs in metastasis clearly indicates an avenue toward novel therapeutic strategies based on the blocking of metastasis-promoting miRNAs. Indeed, a stable “miRNA sponge strategy” for competitive inhibition of miRNAs by providing transcripts with multiple tandem binding sites for the target miRNA was employed for the inhibition of miR-31 *in vivo*. Since miR-31 represses metastasis formation, this approach induced the metastatic potential of otherwise nonaggressive breast cancer cells and thus only aimed at the functional analysis of miR-31 [66]. In a similar approach, the expression of multiple complementary binding sites for the targeted miRNA through a lentiviral vector (“anti-miRNA decoy”) was shown to efficiently inhibit miRNAs. The targeting of miR-223 resulted in a functional knockdown similar to the corresponding knockout mice [83, 84]. Finally, Ma et al. demonstrated that the administration of miR-10b antagomirs to mice bearing highly metastatic breast carcinoma cells did not reduce primary tumor growth but led to marked suppression of the formation of lung metastases [85].

On the other hand, so-called miRNA mimics have been introduced to substitute for pathologically downregulated miRNAs (“miRNA replacement therapy”). Intranasal let-7 administration led to decreased tumorigenesis in a mouse

**Table 1** miRNAs relevant in tumor cell invasion and metastasis

miRNA	Impact on metastatic processes	Upstream regulators	Target genes	Tumor types (misregulation/relevance in metastasis)	References
miR-7	Suppressing	HoxD10	Pak1	Breast, urothelial	[93, 94]
miR-9	Suppressing		TrkC	Colorectal	[34]
miR-16	Suppressing		CDK1, CDK2	Prostate	[55]
miR-17/20	Suppressing		IL8, Cyclin D1	Breast	[95]
miR-22	Suppressing			Ovarian	[96]
miR-23	Suppressing		c-met, uPA	Hepatocellular	[97]
miR-29b	Suppressing	MBP-1	Mcl-1, COL1A1, COL4A1, MMP-2	Prostate	[98]
miR-29c	Suppressing		Laminin gamma1, collagens	Nasopharyngeal	[99]
miR-30	Suppressing			Breast	[100]
miR-31	Suppressing		ITGA5, RDX, RhoA	Breast	[64–66]
miR-34a	Suppressing		c-met	Hepatocellular	[101]
miR-106b	Suppressing			Renal	[102]
miR-122	Suppressing	HNF1A, HNF3A, HNF3B	ADAM17	Hepatocellular	[103, 104]
miR-125b	Suppressing		LIN28B	Hepatocellular	[105]
miR-126	Suppressing		Crk	Breast, lung, gastric	[106–108]
miR-130b	Suppressing	TAp63		HNSCC	[109]
miR-138	Suppressing			HNSCC	[110]
miR-139	Suppressing		ROCK2	Hepatocellular	[111]
miR-145	Suppressing		c-myc, mucin1		[112]
miR-146a/b	Suppressing	BRMS1	EGFR, IRAK-1, MMP16, NF-κB	Breast, glioma, pancreatic	[113–117]
miR-183	Suppressing		Ezrin	Breast, lung	[118, 119]
miR-193b	Suppressing		uPA	Breast	[120]
miR-194	Suppressing		N-cadherin, DNMT3A, HBEGF, IGF1R, PTPN12, PTPN13, ITGA9, SOCS2, RAC1	Liver	[121]
miR-204	Suppressing		APRC1B, CTSC, FAP, MMPs, BMP1, CDH11, ITGB4	HNSCC	[122]
miR-205	Suppressing		ZEB1, ZEB2, LRP1	Bladder, breast, glioblastoma, lung	[123–127]
miR-206	Suppressing		NOTCH3	Breast, lung	[128, 129]
miR-211	Suppressing	MITF	KCNMA1	Melanoma	[130]
miR-218	Suppressing		Robo1	Gastric	[131]
miR-221	Suppressing		c-kit	Prostate	[35]
miR-222	Suppressing		MMP1, SOD2	Tongue SCC	[132]
miR-335	Suppressing	BRMS1	MERTK, PTPRN2, SOX4, TNC	Breast	[108, 114]
miR-338	Suppressing			Hepatocellular	[133]
miR-339-5p	Suppressing			Breast	[134]
let-7	Suppressing	LIN28, MYC	HMGA2, MYC, NOTCH, RAS, COL1A2	Breast, lung, pancreatic, hepatocellular	[135–137]
miR-10b	Promoting/suppressing	BRMS1, Twist	HoxD10, KLF4, RhoC, Tiam1	Breast, hepatocellular, ovarian, AML, esophageal, nasopharyngeal, SCC	[85, 114, 138–140]
miR-125a-3p/5p	Promoting/suppressing	EGFR	ARID3B	Lung, ovarian	[43, 141, 142]
miR-141/miR-200a,b,c/miR-429	Promoting/suppressing	ZEB1, ZEB2	ZEB1, ZEB2, E-cadherin, N-cadherin	Breast, lung, pancreatic	[58, 63, 124, 125, 143, 144]
miR-661	Promoting/suppressing	c/EBPalpha	MTA1, Nectin-1, StarD10	Breast	[145, 146]

**Table 1** (continued)

miRNA	Impact on metastatic processes	Upstream regulators	Target genes	Tumor types (misregulation/relevance in metastasis)	References
mir-9	suppressing Promoting	MYC	E-cadherin	Breast, brain, hepatocellular	[44, 147, 148]
miR-10a	Promoting	Retinoid acid	HOXB1, HOXB3	Pancreatic	[149]
miR-17-92	Promoting	Myc	CTGF, TSP-1	Leukemia, breast, colon	[150, 151]
miR-21	Promoting	AP-1, BMP2, BMP4, BMP6, EGF/HER2, TGF-beta, ZEB1	ARCKS, HNRPK, NF1B, PDCD4, PTEN, TPM1, MARCKS, Maspin, RECK, SPRY2, TAp63, TIMP3, TPM1	Breast, bladder, colorectal, gastric, lung, ovarian, cholangiocarcinoma, pancreatic, prostate, hepatocellular, glioblastoma, uterine leiomyoma, cervical, B cell lymphoma, lymphocytic leukemia, esophageal	[41, 123, 152–171]
miR-27a	Promoting			Gastric	[172]
miR-27b	Promoting		ST14	Breast	[173]
miR-29a	Promoting		Tristetraprolin	Breast	[174]
miR-30d	Promoting		GNAI2	Hepatocellular	[175]
miR-107	Promoting		DRCE1	Gastric	[176]
miR-130a	Promoting		GAX, HOXA5		[177]
miR-143	Promoting	NF-κB	FNDC3B	Hepatocellular	[47]
miR-151-5p	Promoting		RhoGDIA	Hepatocellular	[178]
miR-155	Promoting	Smad4	RhoA	Breast	[179]
miR-182	Promoting		FOXO3, Mitf	Melanoma	[180]
miR-210	Promoting			Breast	[181]
miR-224	Promoting		CD40	Pancreatic	[182]
miR-373	Promoting	BRMS1	CD44	Breast	[114, 183]
miR-378	Promoting		Fus-1, SuFu	Breast, glioblastoma	[184]
miR-486	Promoting		CD40	Pancreatic	[182]
miR-520c	Promoting	BRMS1	CD44	Breast	[114, 183]
miR-520h	Promoting	E1A	PP2A/C	Breast	[185]
miR-532	Promoting		RUNX3	Melanoma	[186]

model of lung cancer [67] and lentiviral vector-induced overexpression of let-7g resulted in significant growth reduction of NSCLC tumors in mice [86]. This also indicates that the therapeutic introduction of let-7 could act as a preventive therapy approach against lung cancer. Likewise, lentivirus-mediated re-introduction of miR-15a/miR-16 into prostate carcinoma xenografts led to growth arrest and volume reduction of the tumors [87], and adeno-associated virus 8-mediated delivery of miR-26a through i.v. injection led to a significant regression of tumors in a murine liver cancer model [68]. Finally, the forced expression of miRNAs has also been shown to increase the sensitivity to chemo- or radiotherapy, indicating that the combination of miRNA-based strategies and established treatment regimens may provide attractive future approaches [37, 88, 89]. Again, this study also included miRNAs which have been shown to be relevant in metastasis like miR-200c [89], thus indicating that a therapeutic intervention may simultaneously act at

different levels like chemosensitizing the solid tumor mass and blocking metastasis.

**Delivery of miRNA-based therapeutics**

The therapeutic application of miRNAs or any miRNA-related molecule will rely on the development of efficient delivery strategies based on viral vectors or nonviral nanoparticles. While viral systems have shown high efficacy, they have major limitations with regard to immunogenicity, risk of insertional mutagenesis, large-scale production, loading capacity, and often poor pharmacokinetics (see, e.g., [90, 91] and references therein). Alternatively, nonviral vectors have been developed with the aim of condensing small RNA molecules or DNA expression vectors, thus mediating protection against degradation, cellular uptake, and intracellular release (see, e.g., [92] for review). While the nonviral

delivery of expression plasmids may lead to significant effects, they may suffer from issues including nonspecific toxicity, low transfection efficacy, and the unsolved safety concerns mentioned above. Thus, the direct delivery of unmodified or chemically modified RNAs (miRNAs, miRNA mimics, AMOs, etc.) may be more promising but critically relies on the protection of the RNAs against degradation, efficient cellular internalization, and intracellular release, as well as on the correct subcellular localization and release from its formulation. Various systems based on the formation of nanoscale particles have been developed for the delivery of unmodified or chemically modified siRNA molecules in vivo, including the covalent conjugation to lipids; the encapsulation in liposomes; the complex formation with liposomes/lipoplexes; the covalent conjugation to peptides, aptamers, or antibodies; or the complex formation with cationic polymers (see, e.g., [91] for review). They may now also be explored with regard to miRNA drugs or miRNA inhibition. Indeed, the systemic application of polyethylenimine-complexed miRNAs has led to antitumor effects in various mouse tumor xenograft models (Ibrahim et al., submitted), and in a therapeutic bone metastasis model, the i.v. injection of miR-16, formulated with atelocollagen, has already been shown to significantly inhibit the growth of metastatic tumors in bone [55].

Taken together, in addition to the analysis of their functional relevance, this clearly demonstrates the potential of miRNAs, either as drugs or as therapeutic targets, in the treatment of primary tumors as well as in the prevention or treatment of metastases. The therapeutic exploration of miRNAs requires the development of sophisticated delivery vehicles and the identification of optimal (modified) miRNAs/inhibitors of miRNAs with high relevance in metastasis. This in turn also calls for the availability or the establishment of appropriate animal models of metastasis for basic research and compound testing.

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