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Review Article

MicroRNAs in cancer treatment and prognosis

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Abstract: Disturbances in microRNA expression by epigenetic alterations and mutations may promote not only tumorigenesis but also tumor aggressiveness, invasion, metastasis, and resistance to chemotherapy and radiotherapy. Several studies have profiled microRNA expression in normal and tumorigenic tissues, demonstrating a unique microRNA signature, which can be used as a marker for cancer diagnosis and prognosis. This review discusses the importance of microRNAs as regulatory biomolecules involved in cancer, focusing on microRNAs related to cancer invasion, metastasis, epigenetic alterations, chemoresistance, and radioresistance. The identification of both differentially expressed microRNAs in tumors and their target genes provides new tools for gene therapy; the re-expression of microRNAs silenced by cancer development or the silencing of oncogenic microRNAs can be effective in the blockade of cancer-related cell proliferation.

Keywords: MicroRNA, epigenetic modifications, metastasis, chemotherapy, radiotherapy

Introduction

MicroRNAs (miRNAs) are small non-coding RNAs of approximately 22 nucleotides (nt) and act as post-transcriptional regulators of gene expression. This type of regulation was first described in *Caenorhabditis elegans* in 1993 [1, 2], and it has since been described in many other organisms. Today, more than 1,400 miRNAs have been described in humans (miRBase - http://www.mirbase.org/cgi-bin/browse.pl), constituting 1 to 3% of the genes in the human genome [3]. It has been estimated that miRNAs regulate 30 to 60% of protein-coding genes [4, 5]. MiRNAs are involved in the regulation of genes related to many biological processes, such as cell proliferation and apoptosis. However, their main function is to establish and maintain the differentiated status of many cell types [6]. They are located in different regions of the genome; 70% are intragenic, and the host gene and miRNA invariably have the same orientation and are expressed together because both are controlled by the same promoter region [7]. Forty percent of all miRNAs are organized in clusters, and the miRNAs in each cluster usually regulate a common pathway [7, 8].

These small RNA molecules are generally processed through the transcription of a primary miRNA (pri-miRNA), which can have one or several secondary structures with 60- to 80-nt loops that are recognized and cleaved in the nucleus by DROSHA, an RNase III endoribonuclease, and its partner, DGCR-8 [9]. Pri-miRNA cleavage generates miRNA precursors (pre-miRNAs), which are hairpin structures of approximately 70 nt with a 2-nt 3' overhang [10]. These are exported to the cytoplasm by exportin 5 and its co-factor, Ran-GTP [11]. Finally, the pre-miRNAs are processed into miRNA duplexes of 21 to 25 nt by DICER, another RNase III endoribonuclease, and its partner, trans-activator RNA-binding protein (TRBP) [12]. Once mature, miRNA duplexes are loaded by DICER into the RNA-induced silencing complex (RISC), which keeps only the strand that is less stable at its 5' end and subsequently initiates the post-transcriptional gene silencing (**Figure 1**). The fate of the unused miRNA strand is not fully understood. However, this unused strand may be incorporated into specific exosomes and

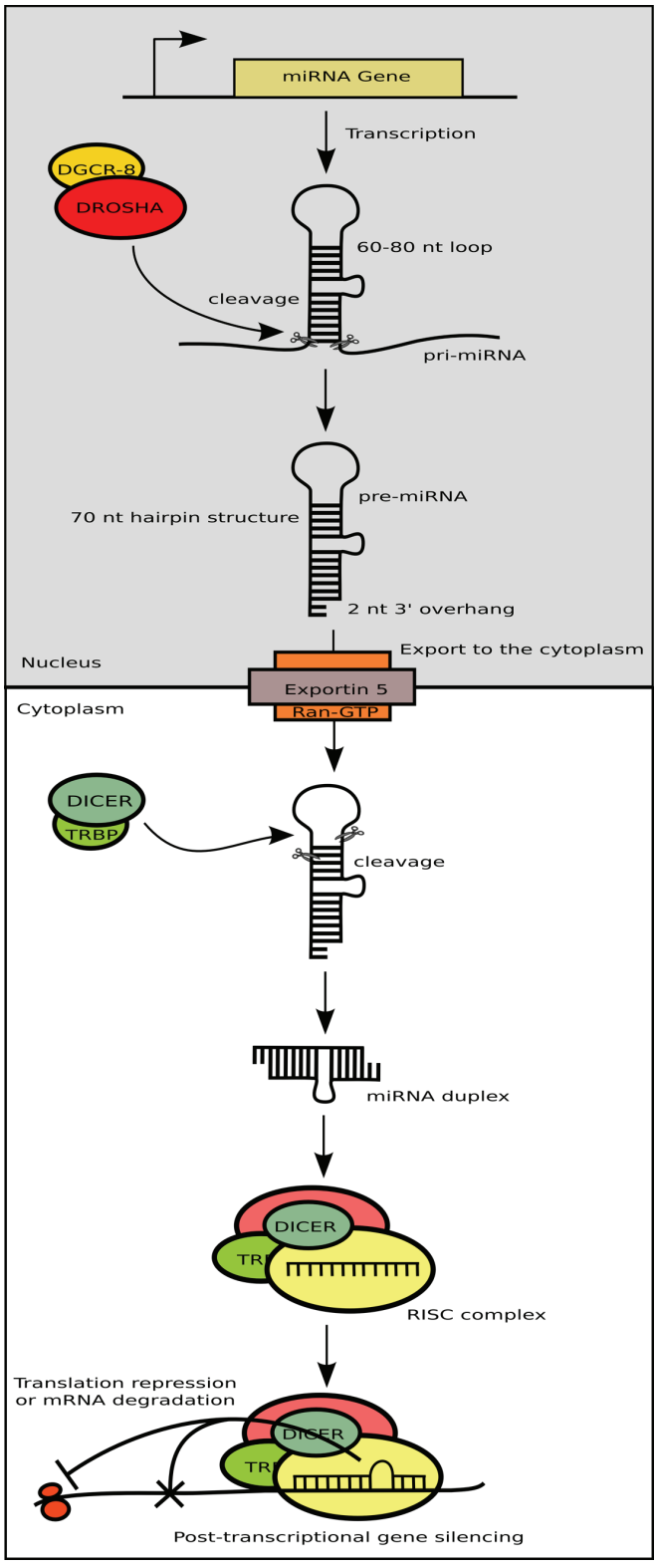

Figure 1. MicroRNA biogenesis, with a special focus on the roles of DROSHA and DICER and the formation of the RISC complex.

extruded from the cell [13]. This biological event results in the presence of extracellular microRNAs in body fluids, primarily blood serum. Accordingly, the possibility of using serum miRNAs for cancer detection and prognosis has received much attention [13].

In mammals, translational repression by miRNAs is usually achieved through partial complementarity to the 3'UTR of target mRNAs [14]. The fine-tuning between coding gene expression and repression is accomplished by the differential expression of miRNAs, which makes their regulation difficult to study. Nevertheless, the identification of new miRNAs and their targets, miRNA expression profiling in different species and tissues [15] (such as in human and mouse embryonic stem cells [16] and tumor cells [17]) and epigenetic regulation of miRNAs [18] have been explored. Many miRNAs have been associated with the development of various cancers; establishment of cancer aggressiveness, invasiveness and metastatic capacity; and resistance to anti-cancer treatments. Addressing these issues is the main aim of this review.

MiRNAs and cancer development

The first cancer-associated miRNAs were miR-15a and miR-16-1, which are located in the human chromosome 13q14 region, between exons 5 and 6 of the *LEU2* gene, a region frequently deleted in chronic lymphocytic leukemia (CLL) that progresses to an aggressive state [19]. In normal cells, these miRNAs induce apoptosis through the regulation of the anti-apoptotic proto-oncogene *BCL2* [20]. Several other miRNAs with altered expression in cancers have been identified, and many causes for their deregulation have been discovered.

A systematic analysis of the locations of miRNAs in the mammalian genome has shown that more than 50% of them are located in fragile genomic sites, regions of loss of heterozygosity (LOH), minimal amplicons, and regions more susceptible to mutations, breaks and rearrangements, all situations frequently found in tumor cells [21]. Thus, changes in the copy number of miRNA genes have an important role in

tumor formation [19]. Moreover, the deregulation of miRNA expression can have other causes, such as the deregulation of transcription factors, epigenetic modifications, mutations, and polymorphisms in the miRNA or in the binding site of the target mRNA sequences, and the deregulation of proteins that participate in miRNA biogenesis (reviewed in [22]). In full-blown cancer, tumor suppressor miRNAs involved in cell cycle blockage, apoptosis, cell differentiation, and oncogene silencing are suppressed by genetic alterations, mainly the homozygous deletion of miRNA genes, which is a common event in human cancer [21]. However, the silencing of protective miRNAs is an early event during carcinogenesis. Indeed, exposure to environmental mutagens, such as cigarette smoke, results in a trend toward lung miRNA downregulation, as demonstrated in mice [23] and humans [24]. MiRNA downregulation is a reversible event in cases of short-term exposure to cigarette smoke but becomes irreversible after long-term exposure, thus committing a cell to cancer development [25]. For cigarette smoke-induced lung cancer, both DNA damage and miRNA alteration have to occur for full-blown cancer to develop. Cigarette smoke induces early DNA damage by promoting DNA adduct formation [26] and mutations in oncogenes, such as K-ras [27]. However, the expression of mutated oncogenes is silenced by the physiological expression of specific miRNAs, such as those belonging to the let-7 family, which targets K-ras gene products. When let-7 becomes irreversibly downregulated due to long-term cigarette smoke exposure, cells exhibit uncontrolled expression of mutated K-ras and become committed to cancer development.

High-throughput studies have screened the expression of different miRNAs to compare them between normal and cancer tissues. The data obtained show that there is an overall decrease in the expression of these miRNAs in tumor tissues compared with normal tissues [17]. Moreover, there is a direct relationship between the expression of miRNAs and the origin of the tissue. Consequently, each tumor type has a specific expression profile, which could provide information regarding its origin, degree of malignancy, and state of differentiation [17]. This fact has great importance in the diagnosis and prognosis of tumors, given that 3 to 5% of all human malignancies are metastatic cancers of unknown primary origin (CUP) [28].

Although there is an overall decrease in the expression of miRNAs in tumor cells, specific miRNAs can be over-expressed, which causes deregulation of the expression levels of the target genes. MiRNAs that suppress the expression of proto-oncogenes and prevent the development or maintenance of the tumor state are usually down-regulated in tumor cells and are called tumor suppressor miRNAs, as previously reported for let-7. On the other hand, oncomiRs are miRNAs that generally modulate the expression of tumor suppressor genes and are usually over-expressed or amplified in tumor cells, contributing to tumor development. Despite the fact that these terms facilitate the description of the functions of these miRNAs, they reflect a state of activity, and a particular miRNA or cluster can have both functions. This can vary according to the cell type and its specific gene expression pattern. An example is the miR-17-92 cluster, whose members can function as oncomiRs in different tumor types, but one member, miR-17-5p, is down-regulated in breast cancer cell lines, and its ectopic expression has an antiproliferative effect by targeting the oncogene *AIB1* [29]. Deletion of this specific miRNA has been reported in some cases of melanoma and breast and ovarian cancers [29], showing that it acts as a tumor suppressor. Other possible mechanisms for the dual role played by this cluster may include post-transcriptional regulation, as over-expression of all of its members has been seen in lung cancer cells, with the exception of miR-17-5p, which was down-regulated [30].

The miR-17-92 cluster is the most extensively studied cluster that has an oncogenic function. It comprises seven miRNAs, which reside in intron 3 of the C13orf25 gene at 13q31.3 [31]. This cluster is widely expressed in healthy tissues and is important for the regulation of the immune and hematopoietic systems and lung development [32-34]. However, it is situated in a region that is commonly amplified and, therefore, is usually over-expressed in B-cell lymphomas [31], as well as in other tumor types, such as other lymphomas and cancers of the lung, breast, prostate, pancreas, stomach, and colon [30, 35-38]. The ectopic expression of this cluster increases the proliferation of lung cancer cells [30]. Additionally, the introduction of a portion of this cluster into hematopoietic stem cells from transgenic Eμ-myc mice, which over-express the oncogene Myc, promotes tumor development, higher levels of malignancy and

invasiveness, lower levels of apoptosis and accelerated formation of malignancies compared with tumors from transgenic animals carrying an Eμ-myc oncogene and lacking the miRNA cluster [35]. This is due to the fact that the proto-oncogene *Myc* encodes a transcription factor responsible for miR17-92 activation [36]. Similarly, mice that over-express miR-17-92 in their lymphocytes develop lymphoproliferative disease and autoimmunity [34]. Several targets are regulated by this cluster, including E2F1, which is regulated by both miR-17-5p and miR-20a [36]. This transcription factor is responsible for the activation of many genes involved in cell-cycle regulation [39], such as *Myc*, and, as a dual-function protein, can stimulate both cell proliferation and apoptosis (reviewed in [40]). Additionally, under the effects of MYC, members of the miR-17-92 cluster, such as miR-18 and miR-19, promote an increase in tumor neovascularization by targeting connective tissue growth factor (CTGF) and the anti-angiogenic thrombospondin-1 (TSP-1), respectively [37]. Moreover, Xiao et al. [34] and Ventura et al. [33] showed that the tumor suppressor gene *PTEN* and the pro-apoptotic gene *BIM* are also regulated by the miR-17-92 cluster.

Among the tumor suppressor miRNAs, the let-7 family is one of the better studied. Let-7 was one of the first miRNAs identified, from studies in heterochronic nematode mutants [41], and is involved in the regulation of the developmental timing of tissues in *C. elegans* [41]. Over the years, many other functions have been associated with this family in different organisms, such as limb development in mice [42], neuromusculature development in flies [43], and the regulation of cell differentiation and proliferation in mammals [44, 45]. Several additional members of this family have been identified. In humans and mice, they are located in 13 genomic loci and generate 10 mature miRNAs [46], having a highly conserved position and grouping pattern between different species, from flies to higher organisms [47, 48]. Their expression is temporal in several animals, correlating with the cellular differentiation state, showing that this family may have an important role in the development of many organisms [44, 45]. The let-7 miRNA in *C. elegans* leads to the terminal differentiation of seam cells in the last larval stage, initiating the formation of the adult organism [41]. In mutants of let-7, which have defects in this transition, the seam cells maintain the characteristics of stem cells, with a high proliferation capacity and lack of differentiation, which are also characteristics of cancer cells. The first evidence of the involvement of let-7 in cancer was through the observation that this miRNA was down-regulated in lung cancer cells, which was related to a lower survival of patients with non-small-cell lung cancer (NSCLC). Additionally, the over-expression of this miRNA was capable of inhibiting cancer cell growth *in vitro* [49]. Moreover, members of the let-7 family are located in fragile sites of the human genome that are frequently deleted in lung cancer cells [21]. The *RAS* gene, commonly deregulated in lung cancer, and *HMGA2*, an oncogene over-expressed in several tumors, including benign mesenchymal and lung tumors, are regulated by members of the let-7 family [50, 51]. In some tumors, increased levels of HMGA2 can be located even in microenvironments with normal let-7 levels. However, it frequently occurs when the mRNA of *HMGA2* has a truncated 3'UTR region that lacks let-7 binding sites [51].

Other examples of miRNAs with tumor suppressor functions are those belonging to the miR-29 family, which are down-regulated in several tumor types [52, 53]. Its members regulate the expression of the anti-apoptotic protein MCL-1 [54], the oncogene TCL-1 [55], and two DNA methyltransferases, DNMT3a and DNMT3b [52], and indirectly repress the expression of DNMT1 by targeting the transcription factor SP1 [56]. MiR-34 family members also bear a tumor suppressor function, as they are the main p53 effectors and thus are downregulated in a variety of cancer cells [57].

The downregulation of onco-protective miRNAs is not only achieved during the final stages of carcinogenesis but also during its early stages. Indeed, the most important lung carcinogen in humans, cigarette smoke, is able to down-regulate a variety of onco-suppressive miRNAs that inhibit the expression of specific oncogenes, including let-7 (targeting *KRAS*), miR-26a (targeting *TGF*), miR-30a (targeting *EGF*), and miR-125a (targeting *ERBB2*). In parallel, in the lung, cigarette smoke down-regulates the expression of miRNAs that activate onco-suppressors, mainly miR-34 (targeting *TP53*) [23]. Accordingly, the downregulation of onco-protective miRNAs due to cigarette smoke may represent an additional mechanism that worsens the prognosis of smoking-related lung can-

cer patients compared with the prognosis of non-smoking-related cancer patients. Indeed, miRNA alterations greatly affect lung cancer prognosis [58].

MiRNAs and epigenetic modifications in cancer

Aberrant epigenomic patterns in tumor cells are frequently seen, including hypermethylation of CpG islands located next to gene promoter regions, causing the silencing of tumor suppressor genes [59], global hypomethylation [60], and reactivation of oncogene expression and creating chromosome instability. Moreover, histone post-translational modifications, such as deacetylation and methylation, are also common (reviewed in [61]). These modifications have important roles in tumor initiation and maintenance (reviewed in [62]). A recent study has estimated that approximately half of the miRNA genes are associated with CpG islands, which could be an important mechanism for the transcriptional regulation of miRNAs [63].

The first evidence of the altered expression of miRNAs and epigenetic modifications in tumor cells was reported by Scott et al. [64]. These authors demonstrated that the treatment of SKBr3 breast cancer cells with a pro-apoptotic dose of the histone deacetylase inhibitor (HDACi) LAQ824 alters miRNA expression, resulting in 22 down-regulated and 5 up-regulated miRNAs after treatment [64]. However, Diederichs and Harber [65] found no significant alterations in the expression of miRNAs in A549 lung cancer cells treated with a DNMT inhibitor (5-AZA-dC or 5-AZA-C) or HDACi (TSA or sodium butyrate), suggesting that different cell lines may have different responses, as well as different dose-dependent effects [65].

The direct correlation between miRNAs and epigenetic mechanisms in cancer was first demonstrated by Saito et al. [18]. These authors found 17 overexpressed miRNAs in T24 bladder cancer cells treated with both 5-AZA-dC and PBA (an HDACi). Among them, miR-127 was particularly interesting because it was highly expressed after treatment. This miRNA is a member of a cluster located on chromosome 14q32.31 and is included in a CpG island [18, 66]. It is expressed in normal tissues but was down-regulated or silenced in the majority of the tumor cell lines analyzed [18]. The treatment led to the demethylation of its promoter region, which reactivated its expression, resulting in a decreased expression of its target proto-oncogene *BCL6* [18]. This work has opened up new pathways for the treatment of cancer through the correction of miRNA expression with drug treatments.

Lujambio et al. [67] compared the expression of miRNAs in HCT116 colon cancer cells with their double-knockout counterpart DNMT3b/DNMT1 (DKO). The authors found that miR-124a is the only miRNA out of 320 that is embedded in a CpG island, upregulated in DKO compared with the parental HCT116 cell line, methylated in HCT116 cells and demethylated in normal colon cells. They also discovered that the proto-oncogene *CDK6* is a target of miR-124a and that this gene is therefore overexpressed in the HCT116 tumor cells compared with normal controls. Its expression is also decreased in cells treated with demethylating agents and in DKO cells compared with non-treated HCT116 parental cells.

Indeed, the silencing of miRNAs with a tumor suppressor role by aberrant DNA hypermethylation is common in tumors, such as hsa-miR-9-1 in breast cancer [68]; miR-370 in cholangiocarcinoma [69]; miR-342 and its host gene, EVL (Ena/VASP- Like), in colorectal cancer [70]; miR-137 and miR-193 in oral cancer [71]; and miR-203 in hematopoietic malignancies [72].

On the other hand, the overexpression of oncomiRs is also seen as a result of epigenetic changes in cancer cells. One example is the *let-7a-3* gene. Its promoter region is highly methylated in HCT116 cells, it has a lower degree of methylation in HCT116-DNMT1 (1KO) or -DNMT3B (3bKO) knockout cells, and it is almost completely demethylated in DKO cells, indicating that this region is regulated by both DNMT3b and DNMT1 [73]. Additionally, in several normal tissues, a strong methylation of this miRNA is seen. Because miRNAs of the let-7 family have important roles in lung cancer [49, 50], one study compared the methylation statuses of lung adenocarcinoma cells and non-neoplastic lung cells from the same patients, showing that the promoter region of let-7a-3 was highly methylated in normal tissues and demethylated in lung cancers [73]. Moreover, the introduction of the let-7a-3 miRNA in A549 lung adenocarcinoma cells increased the capacity of anchorage-independent growth, demon-

strating an oncogenic function [73]. Because the let-7 family is well known for its tumor-suppressive role, the function of let-7a-3 is atypical, demonstrating the diversity of functions of similar miRNAs in cancer. In agreement with these observations, Lu et al. [74] analyzed the methylation of the let-7a-3 promoter in 214 clinical samples of ovarian epithelial cancer and found a significant correlation between hypermethylation and a lower risk of death. In that work, they also observed an inverse correlation between the level of let-7a-3 methylation and the expression of insulin-like growth factor II (IGF-II), which is indirectly regulated by let-7a-3 and is highly expressed in tumors with poor prognosis. In addition, a positive correlation between the methylation of let-7a-3 and IGFBP-3 expression was observed [74].

Thus, an increasing number of studies have recently associated aberrant methylation and alterations in chromatin structure with the deregulated expression of miRNAs and, consequently, with the onset of cancer.

The opposite is also seen; namely, miRNAs can regulate the expression of components of the epigenetic machinery. In several types of cancer, DNMTs show high levels of expression [75, 76]. Thus, miRNA activities may be good candidates with which to control the aberrant methylation of tumor suppressor genes in cancer. The first evidence that miRNAs regulate the expression of genes associated with the epigenetic machinery was reported by Chen and colleagues [77]. They observed the regulation of the enzyme histone deacetylase 4 (HDAC4) by miR-1, which promotes myogenesis. Members of the miR-29 family have been frequently associated with cancer, being generally downregulated, which indicates a tumor suppressor role [52, 56]. Fabbri and colleagues [52] demonstrated that members of the miR-29 family directly regulate the mRNAs of DNMT3a and DNMT3b in lung cancer cells, and the ectopic expression of these miRNAs inhibits the expression of these DNA methyltransferases, causing increased global methylation and the re-expression of tumor suppressor genes. Furthermore, the ectopic expression of miR-29 inhibits cellular growth and induces apoptosis *in vitro*, as well as reduces the growth of tumors in nude mice. Next, Garzon and colleagues [56] observed an indirect regulation of DNMT1 by miR-29b in leukemia. This miRNA targets SP1, a transcription factor that regulates the expression of DNMT1. In another study, Duursma et al. [78] observed that miR-148 inhibits the expression of DNMT3b by binding to its ORF; this is one of the few studies showing this type of silencing, which is common in plants. Interestingly, this miRNA targets the DNMT3b1 isoform, but not DNMT3b3, revealing a splicing variant-specific regulation. The interplay between miRNAs and changes in DNA methylation in tumor cells is represented in **Figure 2**.

The regulation of epigenetic machinery gene expression by miRNAs also has an important role in embryonic development, as observed by Sinkkonen et al. [79]. They demonstrated that miRNAs from the miR-290 cluster regulate the expression of *de novo* DNA methyltransferases in an indirect manner, through the inhibition of their transcriptional repressor, Rbl2. On the other hand, the DNA methyltransferases have an important role in the initiation of embryonic development, contributing to embryonic stem cell differentiation.

MiRNAs play a major role in maintaining the stemness of stem cells. Indeed, various stem cell-specific miRNAs have been identified [80]. Furthermore, miRNA expression in lung physiologically shifts from the staminal status, which is characteristic of the prenatal and postnatal stages, to a differentiated pattern, which is characteristic of adults [81]. Because of the pivotal role of stem cells in cancer maintenance, recurrences after treatment, and metastasis formation [82], the maintenance of stemness by miRNAs plays a physiological role during intrauterine life but may play an oncogenic role when expressed in adult organisms.

MiRNAs in tumor invasion and metastasis

Metastasis is the ability of tumor cells to reach sites far from the primary site, and it is the main cause of death in cancer patients. To metastasize, tumor cells must cross several barriers, such as migration of the primary tumor and tissue invasion, entrance into and survival in the bloodstream or lymphatic system and, finally, extravasation and complete colonization of the tissue (reviewed in [83]). MiRNAs are involved in the development of such processes and could be prognostic markers and therapeutic targets in metastatic tumors. The most important miRNAs related to this topic are reported in

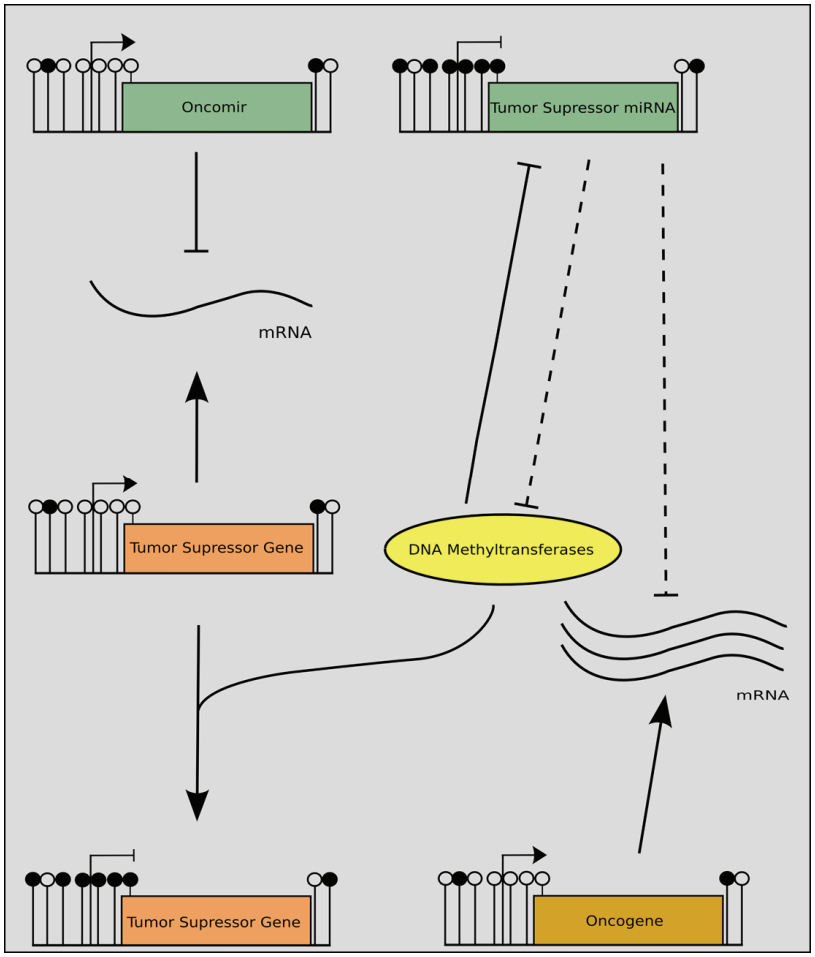

Figure 2. Overview of the interplay between miRNAs and DNA methylation in tumor cells. Dashed lines denote the original function in normal cells that is lost in tumor cells. Open circles: unmethylated CpG sites; filled circles: methylated CpG sites.

Figure 3. A complete list of these metastasis-related miRNAs is provided as **Table 1**. The first observation of a miRNA involved in metastasis was made by Ma et al. [84]. The authors found that miR-10b was overexpressed in breast cancer cells with metastatic potential compared with non-metastatic breast cancer cells. However, in a previous study, primary breast tumors exhibited miR-10b down-regulation compared with normal breast tissue, regardless of their aggressiveness [85]. In fact, Ma and colleagues observed that the expression of this miRNA was down-regulated in tumor samples of patients free of metastasis compared with normal breast tissue. On the other hand, half of the patients with metastatic tumors showed an increased expression of miR-10b in tumor cells compared with normal tissue [85]. When silenced by an antagomiR, a decrease in the invasive properties of these cells was observed *in vitro*, with no alterations in their viability or motility. The ectopic expression of miR-10b had no effect on cell proliferation *in vitro*, but it increased motility and invasive capacity. In an *in vivo* assay, human breast cancer cells were transduced with a miR-10b-encoding retroviral vector and inoculated in mice. All animals developed tumors, including the control group. However, those who received cells overexpressing miR-10b showed higher invasion rates into adjacent tissues, higher tumor growth and proliferative rates, and greater angiogenesis and metastases at distant sites. Chromatin immunoprecipitation (ChIP) showed that TWIST1, a transcription factor that plays an essential role in tumor metastasis [86], binds to the miR-10b promoter, indicating that it controls the expression of this miRNA [84]. Indeed, TWIST1 expression correlates with that of miR-10b, and a target of this miRNA, *HOXD10* [84], encodes a protein that inhibits the expression of several genes involved in cellular migration and extracellular matrix remodeling [87].

Comparing miRNA expression profiles between parental lineages of human breast cancer and their metastatic derivatives, which have high bone- and lung-targeting capacities, Tavazoie and colleagues [88] characterized miRNAs downregulated in the latter, including miR-126, miR-206, and miR-335. These miRNAs are important for the metastatic phenotype because their ectopic expression significantly reduced lung metastases in mice. The authors also found that in humans, patients with primary breast cancer with down-regulation of miR-126

Table 1. A complete list of these metastasis-related miRNAs (Table 1 references reported between square brackets are available as Supplementary Material)

MicroRNA	Metastasis-suppressing miRNA (MSM) or metastasis-promoting miRNA (MPM)	Expression in metastasis compared to benign tissue	Tissues	Target genes
Let-7 family	MSM [29, 101, 115, 131, 157, 162, 163, 165]	- [28, 29, 47, 48, 71, 101, 115, 157, 162, 163, 165]	Breast cancer [29], esophageal squamous cell carcinoma [163], Pancreatic cancer cells [72], gastric carcinoma [115, 162], Uveal melanoma [85], mouse melanoma [131], Lung cancer [71, 157], prostate cancer [47], hepatocellular carcinoma [28, 101], Colorectal cancer [165]	HMGA1 [72],HMGA2 [72], KRAS [72], Bsg [131], E2RAS and c-myc [49], MYH9 [162], COL1A2 [101], TRIM41, SOX13, SLC25A4, SEMA4F, RPUSD2, PLEKHG6, CCND2, and BTBD3 [165]
lin-28b	MPM [160]	+[160]	Colon cancer [160]	
miR-1		- [28]	Hepatocellular carcinoma [28]	
miR-9 family	MSM [36, 38]	+ [90], - [6, 35, 36, 38]	breast cancer [6, 90], lung[34], ovarian cancer [35], brain cancer [36]	CDH1 [90]
miR-10a	MPM [80]	+[80]	Pancreatic adencarcinoma [80]	
miR-10b	MPM [11, 92, 104, 118],MSM [107]	+[11, 44, 73, 74]	Breast carcinoma [11, 107], hepatocellular carcinoma [73], AML [74], pancreatic tumor cells [44], esophageal cancer [92], nasopharyngeal carcinoma [118]	HoxD10 [11], KLF4 [92], Tiam1 [107]
miR-15a		- [28]	Hepatocellular carcinoma [28]	
miR-16	MSM [70]	- [70]	Prostate cancer [70]	
miR-17-92 cluster	MPM [46,99]	+[99]	Neuroblastoma [46], Breast cancer [99]	
miR-19a		- [28]	Hepatocellular carcinoma [28]	
miR-20a	MSM [112]		Pancreatic cancer cell lines [112]	Stat3 [112]
miR-21	MPM [8, 21, 52, 59, 102, 103, 106, 108, 127, 148, 153, 161]	+ [3, 52, 21, 102, 103, 106, 127, 148, 153, 161]	Several types of cancer [3, 4, 5, 6, 8, 21, 52, 59, 102, 103, 106, 127, 148, 153, 161]	PTEN [5, 127], TPM1 [7], PDCD4 [8, 9, 56, 59, 106, 153], SPRY2 [10], MARCKS [53], RECK and TIMP3 [60, 103], Maspin [59], RHOB [108]
miR-22	MSM [122, 159]	- [122, 129, 159]	Ovarian cancer [122], Breast cancer [129, 159]	CDK6, SIRT1 and Sp1 [159]
miR-23b	MSM[39]	- [39,81]	Hepatocellular carcinoma[39], prostate cancer [81]	uPA e c-met[39]
Mir-24	MSM [26]	- [26]	Breast cancer [26]	DHFR [27]
miR-27a	MPM [75]	+[75]	Gastric cancer [75]	
miR-29a	MPM [67]	+[67]	Breast cancer [67]	
miR-29c		- [63]	nasopharyngeal carcinomas [63]	Laminin [63], collagen [63], tcl1 [64]
Mir-30a/b	MSM [109, 168]	- [28, 109, 168]	Hepatocellular carcinoma [28], breast tumor [109], non-small lung cancer cell [168]	
miR-30c		- [28]	Hepatocellular carcinoma [28]	
miR-30d	MPM[93]	+[93]	Hepatocellular carcinoma [93]	Galphai2 [93]
miR-30e		- [28]	Hepatocellular carcinoma [28]	
miR-31	MSM[33, 132, 152]	- [33, 132, 152]	breast cancer [33]	Fzd3, ITGA5, M-RIP MMP16, RDX, RhoA [33], WAVE3 [132]
miR-34a	MSM [77, 143]	- [77, 143]	Hepatocellular carcinoma [77], prostate cancer [143]	C-met [77], CDK6, cyclin D1 [78], MYCN [79], CD44 [143]

miR-34b/c	MSM [38]	- [38]	colon cancer, head and neck cancer, melanoma [38]	MYC, CDK6, E2F3 [38]
miR-92a	MPM [135]	+[135]	Esophageal squamous cell carcinoma [135]	CDH1 [135]
miR-92b	MSM [36]	- [36]	brain tumor [36]	
miR-100		- [47, 81]	Prostate cancer [47, 81]	
miR-101	MSM [65]	-[65]	Prostate cancer [65]	EZH2 [65]
miR-103/107	MPM [113, 126]	+[113, 126]	Breast cancer [113], gastric cancer [126]	Dicer [113, 126]
miR-106b		- [114]	Renal cell carcinoma [114]	
Mir-122	MSM [55]	-[28, 54,55]	Hepatocellular carcinoma [28, 54,55]	ADAM17 [55]
miR-124a	MSM [174]	- [28, 174]	Hepatocellular carcinoma [28, 174]	ROCk2, EZH2 [174]
miR-125a-3p	MSM [111]	- [111]	Non-small cell lung cancer [111]	
miR-125a-5p	MPM [111], MSM [141]	+[111], - [141]	Non-small cell lung cancer [111], gastric cancer [141]	ERBB2 [141]
miR-125b	MSM [120]	- [28, 120]	Hepatocellular carcinoma [28], Liver cancer cell [120]	LIN28B [120]
miR-126	MSM [14, 62, 116]	- [14, 28, 62, 116]	Lung cancer [62], Breast cancer [14], hepatocellular carcinoma [28], gastric cancer [116]	Crk [62, 116]
miR-127	MPM [86]	+[86]	cervical carcinoma [86]	
miR-138	MSM[31,96]	-[31, 32,96]	head and neck squamous cell carcinoma[31], squamous cell carcinoma of the tong [96], thyroid carcinoma [32]	RhoC, ROCK2 [96], hTERT [32]
miR-81	MSM [124]	- [124]	Hepatocellular carcinoma [124]	ROCK2 [124]
miR-143	MPM [43, 156], MSM [154, 169]	+ [43, 156], - [154, 169]	Hepatocellular carcinoma[43], osteosarcoma [154], esophageal squamous cell carcinoma [156], prostate cancer [169]	FNDC3B[43]
miR-145	MSM [97, 169], MPM [156]	+[156], - [169]	esophageal squamous cell carcinoma [156], prostate cancer [169]	MUC1 [97]
miR-146a/b	MSM [12, 15, 30, 57, 58, 91, 167]	- [12, 15, 30, 57,58, 91, 167]	Glioblastoma [57], Breast cancer [12, 15, 30], prostate cancer [58], pancreatic cancer cells [91], gastric cancer [167]	MMP16 [57], ROCK1[58], TRAF6 [15, 16], IRAK1 [34, 16, 91, 167], EGFR [91, 167]
miR-148	MSM [38]	- [28, 38]	Hepatocellular carcinoma [28]	
Mir-151	MPM [94]	+[94]	Hepatocellular carcinoma [94]	RhoGDIA [94]
miR-155	MPM [61, 153]	+[61, 153]	colorectal cancer [153]	RHOA [61]
miR-181	MPM [144]	+[144]	Oral squamous cell carcinoma [144]	
miR-182	MPM [69]	+[69]	Melanoma [69]	MITF and FOXO3 [69]
miR-183	MSM [83,98, 121], MPM [164]	-[83, 121], +[164]	Lung cancer cells[83], breast cancer [121], medullary thyroid cancer [164]	ITGB1[98], KIF2A [98], Ezrin [83, 121]
miR-185		+[28]	Hepatocellular carcinoma [28]	
miR-194	MSM [125]	- [28, 125]	Hepatocellular carcinoma [28], liver cancer [125]	
MiR-196 family (miR-196a1, miR-196a2, and miR-196b)	MSM [119], MPM [45]	- [119], +[45]	Breast cancer [119], colorectal cancer [45]	HOXC8 [119], ANXA1 [87]
miR-198	MSM [170]	- [170]	Hepatocellular carcinomas [170]	c-MET [170]

miR-199a	MPM[128], MSM [150]	+[128], - [150]	Uveal melanoma [85], Gastric Cancer [128], testicular tumor [150]	MAP3K11 [128], PODXL [150]
Mir-199b-5p	MSM [51]	- [51]	Medulloblastoma [51]	HES1 [51]
miR-200 family (miR-200a, miR-200b, miR-200c, miR-141 and miR-429)	MSM[22, 23, 24, 25, 84,88,89, 117, 134, 139] MPM [37,100]	+[37, 100], - [22,23, 24, 25,84, 88, 89, 117, 134, 139]	Ductal adenocarcinomas of pancreas [37], breast carcinoma cells [24,25,100], primary serous papillary ovarian tumors[25], lung cancer [84, 117, 134], head and neck squamous cell carcinoma [139]	ZEB1 [22, 23, 24,25], ZEB2 [24,25], WAVE3 [89], Flt1 [134], BMI1 [139], SIP1 [22, 23], EP300 [37]
miR-203	MSM [137]	- [137]	Prostate cancer [137]	ZEB2, Bmi, Survivin, Runx2 [137]
miR-204	MSM [105, 151]	- [105, 151]	Squamous cell carcinoma of head and neck [105], endometrial cancer [151]	FOXC1 [151]
miR-205	MSM [22,68]	- [22, 68]	breast cancer [68], head and neck squamous cell carcinoma [66]	LRP1[1], ErbB3 [68], VEGF-A [68]
miR-206	MSM [14, 136]	- [14, 136]	Breast cancer [14], lung cancer [136]	
miR-207		+[28]	Hepatocellular carcinoma [28]	
miR-211	MPM [82], MSM [130, 133]	+ [82], - [130, 133]	Oral carcinoma [82], melanoma [130, 133]	KCNMA1 [130]
miR-214	MSM [140]	- [140]	Cervical cancer [140]	Plexin B1 [140]
miR-218	MSM[47,95]	- [47, 95]	Gastric cancer [100], prostate cancer [47]	LAMB3 [50], ROBO1 [95]
Mir-219		+[28]	Hepatocellular carcinoma [28]	
miR – 221	MSM [17]	- [17]	prostate cancer [17]	P27Kip1[18, 19, 20]
miR-222	MSM[40]	- [40]	Tongue squamous cell carcinoma[40]	MMP1 and SOD2[40]
miR-223	MPM [166]	+[166]	Gastric Cancer [166]	EPB41L3 [166]
miR-224	MPM [41]	+[41]	Pancreatic ductal adenocarcinomas [41]	
miR-328	MPM [155]	+[155]	Non-small cell lung cancer [155]	
miR- 335	MSM [14, 147]	- [14, 147]	Breast cancer [14, 147]	SOX4, TNC [14]
miR-338	MSM[42, 173]	- [42, 173], +[28]	Hepatocellular carcinoma[28, 42], liver cancer [173]	SMO [173]
miR-339-5p	MSM [123]	- [123]	Breast cancer [123]	
miR-340	MSM [142]	- [142]	Breast cancer [142]	C-met [142]
miR-345	MSM [171]	- [171]	Colorectal cancer [171]	BAG-3 [171]
miR-370	MPM [172]	+[172]	Gastric cancer [172]	TGFβ-RII [172]
miR-373	MPM [13]	+[13]	Breast cancer cells [13]	TXNIP e RABEP1 [146]
miR-375	MPM [164]	+[164]	Medullary thyroid cancer [164]	
MIR-452	MPM [76]	+[76]	Urothelial carcinoma [76]	
miR-486	MPM [41]	+[41]	Pancreatic ductal adenocarcinomas [41]	
miR-503	MSM [158]	- [158]	Hepatocellular cancer [158]	
miR-516a-3p	MSM [138]	- [138]	Gastric cancer [138]	Sulfatase1 [138]
miR-520b	MSM [149]	- [149]	Breast cancer [149]	HBXIP e IL-8 [149]
miR-520c	MPM [13]	+[13]	Breast cancer cells [13]	
miR-661	MPM [110]	+[110]	Breast cancer [110]	
miR-1258	MSM [145]	- [145]	breast cancer [145]	HPSE [145]

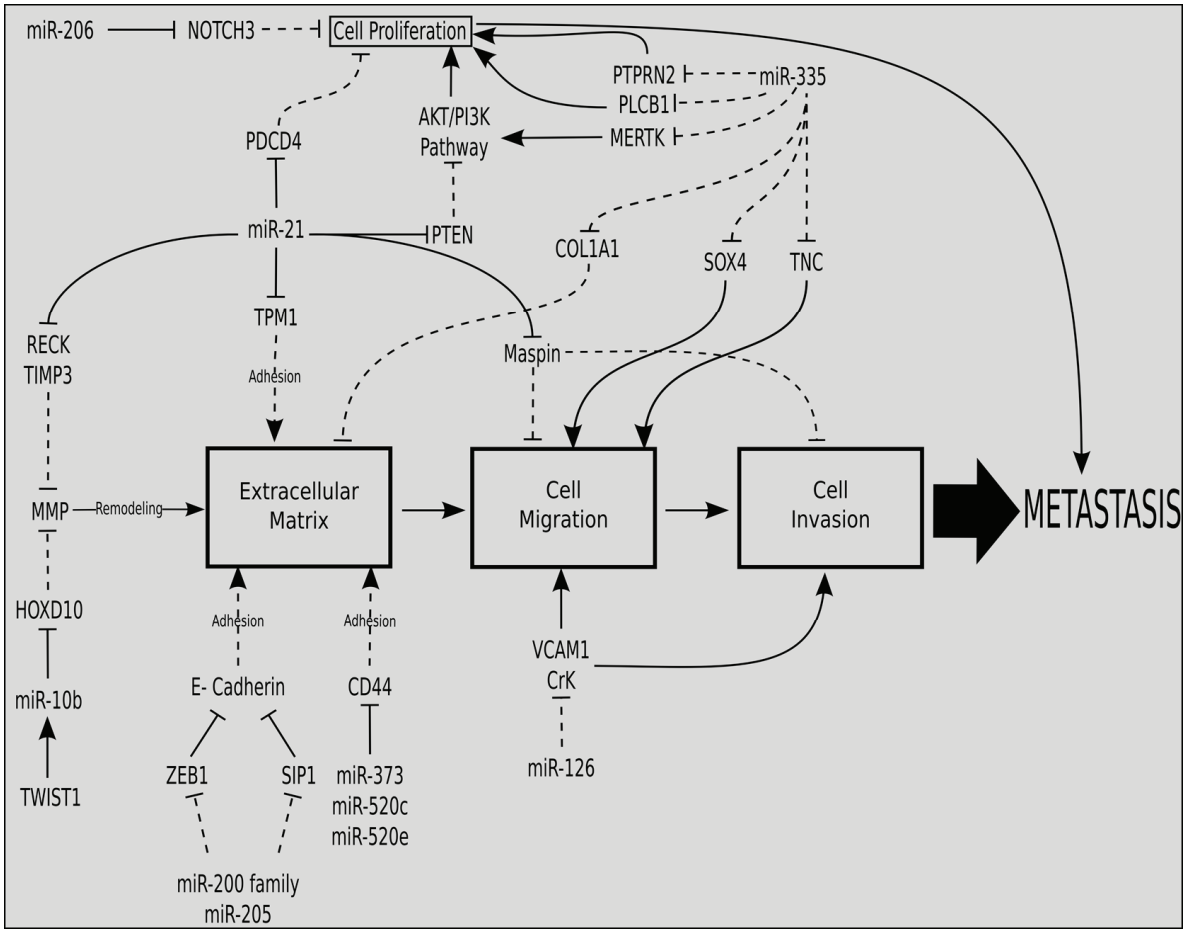

Figure 3. Overview of miRNAs involved in metastasis progression. Dashed lines denote the original function in normal cells that is lost or altered in tumor cells.

and miR-335 had poor metastasis-free survival rates, and the inhibition of miR-335 in human non-metastatic breast cancer cells increased the colonization ability of these cells in the lung. Indeed, miR-126 is associated with the silencing of genes related to cell growth, adhesion, migration and invasion, such as Crk, an adhesion adaptor protein [89], vascular cell adhesion molecule 1 (VCAM-1) [90], insulin receptor substrate 1 (IRS-1) [91], and PIK3R2, which regulates the activity of PI3 kinase [92]. The re-expression of miR-335 in metastatic human cells also affects the expression of several genes, including those associated with cytoskeleton control and extracellular matrix (type 1 collagen, *COL1A1*), signal transduction (receptor-type tyrosine protein phosphatase, *PTPRN2*; c-Mer tyrosine kinase, *MERTK*; and phospholipase 1, *PLCB1*) and cell migration (tenascin C, *TNC*; and SRY-box-containing transcription factor 4, *SOX4*) [88]. Additionally, miR-206 blocks the anti-apoptotic activity of NOTCH3 at the transcriptional and post-transcriptional levels [93].

Another miRNA with great importance in metastatic tumors is miR-21, whose role in cancer is well known. This miRNA represses several tumor suppressor or metastasis suppressor genes, such as PTEN [94], a phosphatase that regulates the cell cycle by suppressing Akt/PKB signaling; tropomiosin1 (TPM1) [95], an actin-binding protein that regulates microfilament organization and anchorage-independent growth; programmed cell death 4 (PDCD4) [95, 96]; maspin [95, 97], an epithelial-specific member of the serine protease inhibitor (serpin) superfamily that inhibits the invasion and motility of tumor cells; TIMP3 [98], a metalloproteinase inhibitor; and RECK [98], a membrane-

anchored glycoprotein. In a human breast cancer model, the inhibition of miR-21 with an antisense oligonucleotide reduced the tumor invasion capacity *in vitro* and *in vivo* by up to 60% and induced a lower number of metastases in the lung [95]. MiR-21 is also upregulated in esophageal cancer cells compared with normal esophageal epithelial cells, as determined by microdissection [99].

In another study, Huang et al. [100] conducted a migration assay using a breast cancer cell line, MCF-7, that does not exhibit migration or metastatic properties. MCF-7 cells were transduced with approximately 450 different miRNA-expressing vectors. In the migratory population prone to a metastatic phenotype, the authors found an enrichment of cells transduced with miR-373, miR-520c and miR-520e compared with the total population. When exogenous miR-373 or miR-520c was introduced into MCF-7 cells, they showed a more invasive and migratory phenotype compared with the control cells. In addition, cells that endogenously express miR -373, such as human breast MDA-MB-435 and human colon HCT-15 cancer cell lines, show a high capacity for migration, which was reduced in more than 70% of cells when a miR-373 antagomiR was introduced [100]. In an *in vivo* assay, mice that received MCF-7 cells expressing exogenous miR-373 or miR-520c developed metastases to the skull, brain and lung, but this was not observed in mice that were injected with control MCF-7 cells [100]. The seed sequences of both miRNAs are similar, indicating that they might have overlapping functions by controlling the same targets. Among the targets predicted for miR-373 and miR-520c, CD44, which is a glycoprotein that modulates matrix degradation, cell growth, adhesion and motility [101], was confirmed as a direct target of both. In agreement with these findings, miR-373 was overexpressed in metastatic clinical samples compared with primary breast cancer samples from the same patients [100].

An important event seen in metastasis is the transition of cells from an epithelial to a mesenchymal phenotype (EMT). This phenomenon is important for the initiation of embryonic development, as well as in adults, and is involved in tissue regeneration, fibrosis and healing [102]. Cells that undergo this transition show altered expression of several genes, such as loss of E-cadherin expression and, therefore, loss of cell-to-cell adhesion and increased motility [103]. In metastatic cells, EMT confers the ability to exit the primary site and to colonize distant sites. The miR-200 family has been implicated in EMT [104]. This family consists of five members grouped in two different regions of the genome: miR-200a, miR-200b and miR-429 on chromosome 1p36.33 and miR-141 and miR-200c on chromosome 12p13.31 [104]. These miRNAs, as well as miR-205, target and regulate *ZEB1* and *SIP1* (*ZEB2*), which are factors that repress the transcription of E-cadherin [104].

Interestingly, several miRNAs involved in cancer metastasis, including miR, are downregulated by cigarette smoke [23]. In particular, lung cancer metastasis is linked to the downregulation of anti-angiogenic miRNAs, allowing blood vessel penetration into the neoplastic mass. This event is linked to the hypoxic condition characterizing the cancer tissue but promoted by hypoxic compounds contained in cigarette smoke, such as carbon monoxide and hydrogen cyanide, which render CS a pro-angiogenic factor due to its ability to induce the expression of hypoxia-related factors, triggering angiogenesis [23]. Furthermore, *FOXO1* upregulation characterizes cigarette smoke-induced lung cancer and is correlated with the silencing of specific miRNAs that target this gene, including miR-182 [105]. These molecular events contribute to the establishment of poor prognosis in cancer patients and explain the poor cancer prognosis of smoke-induced lung cancer.

MiRNAs and chemoresistance

Chemotherapy is a treatment widely used in cancer, but in many cases, tumors can became refractory to this treatment by mutations, alterations in gene expression caused by epigenetic deregulation, or drug-induced karyotypic alterations (reviewed in [106]). Tumor chemoresistance to certain types of drugs may be influenced by miRNA regulation. Meng et al. [107] showed that the inhibition of miR-21 and miR-200b increases the cytotoxicity induced by gemcitabine in cholangiocarcinomas, while the transfection of non-malignant cholangiocytes with pre-miR-21 and pre-miR-200b increases cell viability. Indeed, Si et al. [108] have shown an association between silencing miR-21 and the sensitization of MCF-7 cells to the chemotherapeutic agent topotecan. In corroboration with these findings, miR-21 targets PTEN, a

negative regulator of the PI3 kinase pathway, which is involved in cell survival through the activation of AKT/mTOR [107].

On the other hand, the overexpression of the tumor suppressor miRNAs miR-15 and miR-16, which are negative regulators of BCL2 [20], increases the sensitivity to several cancer chemotherapeutic drugs, while its inhibition has the opposite effect. These miRNAs are downregulated in a multidrug-resistant (MDR) gastric cancer cell line compared with their parental cell line [109]. The inhibition of miR-30 and miR-138 before cancer onset and of mir-378 in full-blown cancer has been associated with the induction of MDR protein expression in lungs of rodents exposed to cigarette smoke [105].

Mishra and colleagues [110] have demonstrated that polymorphisms can also promote drug resistance. The SNP 829C->T in the 3'UTR of the dihydrofolate reductase (*DHFR*) gene is frequently found in the Japanese population, resulting in an increase in its expression [111]. Mishra et al. [110] reported that this SNP is located next to the target site of miR-24, preventing its binding. When *DHFR* is overexpressed, cells become resistant to treatment with the chemotherapeutic agent methotrexate. Thus, the authors proposed that SNPs in miRNAs or around their binding sites in target genes involved in the response to chemotherapeutic agents may contribute to the increasing resistance or sensitivity of cells to chemotherapeutics.

With regard to the use of selective estrogen receptor modulators for breast cancer therapy, miRNAs have major roles in inducing drug resistance in cancer cells. Indeed, miR-221 and miR-222 are elevated in estrogen receptor alpha (ERα)-negative breast cancer cells compared with ERα-positive cells [112] and in tamoxifen-resistant cells, whereas miR-21, miR-342 and miR-489 show decreased expression. Another study suggested that miR-342 regulates the tamoxifen response in breast cancer cells *in vitro*, and clinical data indicate a link between reduced miR-342 expression and tamoxifen resistance [113]. Mir-221 and miR-222 play roles in resistance to fulvestrant, which is used in hormone-sensitive breast cancers following the failure of previous tamoxifen or aromatase inhibitor therapies [114].

MiRNAs and radiotherapy

Ionizing radiation (IR) induces breaks and other types of DNA damage, but the correct reparation of these breaks in tumor cells can make them resistant to treatment [115]. Based on the fact that the oncogene *RAS* participates in cell protection against IR [116] and is regulated by the let-7 miRNA family [50], Weidhaas et al. [117] irradiated A549 lung cancer cells and CLR2741 normal lung epithelial cells and examined their miRNA expression profiles. A significant change in response to radiation was seen in both cell lines after irradiation. Significant downregulation of the let-7 family members was seen, with the exception of let-7g, which was significantly upregulated. A549 cells transfected with pre-let-7a or pre-let-7b showed an increase in radiosensitivity compared with control cells, while transfection with anti-let-7b led to radioprotection [117]. The opposite occurred with let-7g, whose overexpression was radioprotective and whose inhibition led to radio-sensitization. Experiments with *C. elegans* have shown the same effects *in vivo* [117]. These results suggest that miRNA expression can be modulated to act synergistically with radiotherapy, causing normal cells to become more resistant or sensitizing tumor cells.

Additionally, Yan and colleagues [115] have studied the role of miRNAs in the sensitization of tumor cells to radiotherapeutic treatment by targeting several genes involved in different DNA repair pathways. They identified *in silico* and validated miR-101 as a regulator of the DNA-PKcs and ATM genes. Next, they observed that the overexpression of this miRNA increased the sensitivity of tumor cells to radiation both *in vitro* and *in vivo*, indicating an important role of miR-101 in the regulation of genes involved in the repair of radiation-induced breaks in DNA.

Despite the fact that radiation is widely used in the treatment of cancer, its undesirable effects and their causes are not fully understood. A possible effect of radiotherapy is the bystander effect, which consists of a destabilization of non-irradiated cells next to the irradiated cells, leading to the carcinogenesis of normal cells (reviewed in [118]). This destabilization may be due to genomic instability, which could be caused by epigenetic alterations (reviewed in [119]). Koturbash and colleagues [120] exposed rats to localized cranial irradiation and

monitored its effects in the spleen 24 hours and 7 months after exposure. A decrease in global methylation in the spleen was observed, as well as in the expression of proteins that are part of the epigenetic machinery, such as DNMT3a and MeCP2. The consequent reactivation of retrotransposons in that tissue was also seen. In addition, miR-194 was over-expressed after irradiation, both in plasma and in the spleen, and an *in silico* analysis revealed that the mRNAs of *DNMT3a* and *MeCP2* were predicted targets of this miRNA. All of these alterations remained even 7 months post-irradiation, indicating a possible contribution of epigenetic alterations to the destabilization of distant tissues, achieved by miRNA regulation and possibly contributing to carcinogenesis.

Therapeutic modulation of miRNAs in cancer

The modulation of cancer-related miRNAs, either to study their functions or for future applications in therapy, has been receiving a great amount of attention due to several promising results.

The two strategies used in miRNA modulation are (a) the introduction of molecules mimicking the expression of protective miRNAs downregulated in cancer and (b) the introduction of antagomiRs, which are synthetic miRNAs complementary to the miRNAs of interest, to inhibit oncomiRs overexpressed in tumor cells (reviewed in [121]).

An important characteristic of miRNA expression modulation is that one miRNA is capable of silencing several genes, in contrast to RNA interference technology, which is capable of silencing only one gene or a few genes belonging to the same gene family. This turns the therapeutic modulation of aberrantly expressed miRNAs into a powerful tool for the treatment of cancer, as well as for the understanding of carcinogenesis.

The antisense technology based on antagomiRs is the major method used to reduce unwanted miRNA expression in tumor cells. AntagomiRs have been progressively improved by chemically modified oligonucleotides, providing more stability and affinity to the target miRNA and, consequently, more efficiency than their natural counterparts (reviewed in [122]). The main modifications used are 2'-*O*-methyl and 2'-*O*-methoxyethyl groups [123], 2'-*O*-methyl-modified RNAs conjugated with cholesterol bound to phosphorothioate (known as antagomiRs) [124], and "locked nucleic acid" (LNA) constructs [125]. Other widely used constructs are "miRNA sponges", which have multiple binding sites to one or several miRNAs, thus competing with the targets of the miRNA(s) of interest [126]; and miR-masking antisense oligonucleotides (miR-masks), which are oligonucleotides that are complementary to the binding site of the miRNA in the 3'UTR of the target gene and that prevent the binding of the miRNA to its target site [127]. On the other hand, the introduction of tumor suppressor miRNAs that are down-regulated in tumor cells may be achieved by delivering synthetic double-stranded RNAs or vectors expressing the pre-miRNAs (reviewed in [121]).

However, delivery to the cell is still a great obstacle for miRNA introduction *in vivo*. Despite the fact that the molecules are very small and can receive chemical modifications to increase their delivery efficiency, the nucleic acids are negatively charged and do not easily cross the cell membrane. Many strategies have been described to overcome this issue, such as the encapsulation of the oligonucleotides in liposomes and the introduction of viral vectors (reviewed in [121]).

Another major problem is nucleotide sequencing of artificial miRNA probes. Indeed, an exceedingly high GC content activates endogenous TLR activation, triggering side effects ranging from fever to the activation of autoimmune diseases. The fact that miRNA overload may have adverse consequences in humans has been recently highlighted for a rare genetic disease, Aicardi-Goutieres syndrome, targeting RNase H and thus resulting in endogenous miRNA accumulation in the central nervous system, thereby impeding brain development [128].

In 2008, the first clinical trial using miRNA-based therapy began. A phase I trial was initiated to treat hepatitis C using a LNA oligonucleotide complementary to miR-122 to inhibit the expression of this oncogenic miRNA, which normally facilitates virus replication. In 2010, the success of this clinical trial and the beginning of phase II was announced [129]. Despite the fact that this is the only study addressing the manipulation of a specific miRNA expression, several other clinical trials are currently

being performed, aiming at the treatment of several diseases, such as asthma, leukemia, and other cancer types (see www.clinicaltrials.gov).

Conclusion

MiRNAs are small molecules that have important roles in regulating gene expression, maintaining the differentiation status and controlling the cell cycle. It has been estimated that half of them are epigenetically regulated, while epigenetic machinery is also targeted by miRNAs, demonstrating how these gene regulatory pathways are interrelated and involved in tumorigenesis. MiRNA expression deregulation triggers cancer development, as well as cancer cell aggressiveness, chemoresistance, radioresistance, migration and metastasis. The identification of miRNAs that are associated with the most varied types of cancer, the resistance to drugs and radiation, and the invasion and metastasis of tumors will lead to more individualized and efficient treatments for cancer. Additionally, miRNA expression profiles may provide a powerful tool for the diagnosis of metastasis of tumors from unknown primary sites.

In conclusion, a better knowledge of miRNA functions, their interrelationships with other cellular processes and the already available treatments against unwanted proliferation can generate new approaches, such as combined therapies, in which the manipulation of miRNA expression can play a pivotal role.

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Supplementary Data

Table 1 References

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