Chapter

MiRNA-Based Therapeutics in Oncology, Realities, and Challenges

Ovidiu Balacescu, Simona Visan, Oana Baldasici, Loredana Balacescu, Catalin Vlad and Patriciu Achimas-Cadariu

Abstract

As master modulators of the human genome, miRNAs are involved in all cancer hallmarks, disrupting the normal function of their targets. By gaining or losing the function, miRNAs lead to the validation of tumor phenotype, its progression, and metastasis as well as to drug resistance. Increasing the evidence suggests that the modulation of miRNAs in cancer cells, by suppressing the oncogenic miRNAs (oncomiRs) and substituting the deficient tumor suppressive miRNAs (TS-miRNAs), could become a reliable tool for improving the cancer therapy. In this chapter, we will present an up-to-date overview of the role of miRNA-based therapeutics in oncology, highlighting their role in cancer management, how these therapies can be used, and which would be the future challenges related to miRNA-based therapies.

Keywords: cancer, invasions, metastasis, miRNA, therapy

1. Introduction

With an increasing incidence each year, cancer represents a major health problem worldwide, ranked second, after cardiovascular disease. According to the last estimation presented by the International Agency for Research on Cancer (IARC) in 2012, more than 14 million new cases of cancers were encountered, while the cancer-related deaths reached around 8.2 million people [1]. Unfortunately, the estimation for 2030 shows an increase in over 21 million new cancer cases and about 13 million cancer deaths [2]. Cancer is characterized by changing the phenotype of the cells in which it occurs, leading to an uncontrolled proliferation, invasion, and metastasis. Albeit the cancers in early stages are treatable, especially by surgery, the major challenge is to treat cancers in advanced stages. Currently, the therapeutic regimens for treating the advanced cancers depend on several factors such as localization, phenotype, and tumor size and are based on a combination of at least two of more approaches, represented by surgery, chemotherapy, radiotherapy, hormone therapy, and target therapy. Nevertheless, this anticancer armamentarium is not very efficient because about 90% of advanced cancers lead to metastasis and death ultimately [3]. Current anticancer therapies target either antiproliferative or pro-apoptotic pathways of tumor cells or activate immune response against tumors, but

none of the currently available antitumor therapies target the molecular pathways involved in invasion and metastasis.

Tumor invasion and metastasis, as they were pointed out by Hanahan and Weinberg [4], represent one of the most important hallmarks of cancer, and therefore, exploiting these features of tumor cells could bring new data to develop more powerful anticancer therapies. Tumor invasion and metastasis are very complex processes that involve a series of sequential and interrelated steps. In this line, epithelial-to-mesenchymal transition (EMT) represents the most important event underlying the tumor invasion [5]. During EMT, tumor cells lose their epithelial characteristics and adhesion and acquire increased motility by shifting toward a mesenchymal phenotype while also diminishing apoptosis and senescence and gaining stem cell properties. The EMT regulation includes a network of many regulators, inducers, and effector molecules, which sustains tumor cell dissemination to distant organs [6].

The "omics" revolution has brought us new data about the complexity of signaling pathways in cancer, the type of molecules that are involved in them, and which alterations are associated with cancer. Moreover, noncoding RNAs, including miRNAs, have proved their crucial role in the regulation of mRNA translation in both physiological and pathological status. Because of their high capacity to modulate mRNA expression, miRNAs are defined as master modulators of the human genome. Therefore, miRNAs are involved in all cancer hallmarks, disrupting the normal function of their targets. By gaining or losing the function, miRNAs lead to the validation of tumor phenotype, its progression, and metastasis as well as to drug resistance.

Increasing the evidence suggests that the modulation of miRNA expression in cancer cells, through the inhibition of oncogenic miRNAs (oncomiRs) and the substitution of deficient tumor suppressive miRNAs (TS-miRNAs), could represent a reliable tool for improving the cancer therapy. In this chapter, we will present an up-to-date overview about the role of miRNA-based therapeutics in oncology, highlighting their role in cancer management, how these therapies can be used, and which would be the future challenges related to miRNA-based therapies.

2. Short overview about biogenesis and function of miRNAs

If deciphering the whole human genome has represented a milestone of modern biology, the identification of its precise functionality is still a great challenge. However, by completing the ENCODE project, many data about how the human genome is functioning were revealed. Such as, it is estimated that about 1.5% of human genome includes coding DNA exons from protein-coding genes (PCGs), while the rest of 98% represents noncoding DNAs including regulatory sequences such as the ones defining noncoding RNAs (ncRNAs), as well as introns, and other DNA sequences with unknown functions [7].

About 80% of human genome is activated in cell physiology, and an important part of noncoding regulatory elements involved in the regulation of PCGs includes noncoding RNAs. Since their recognition as a distinct class of biological regulators [8], micro-RNAs (miRNAs) have become the most studied species of noncoding RNAs. miRNAs are coded by genes located in almost all regions of the genome, including both PCGs and noncoding transcripts. About a half of miRNA genes are located in both intronic (40%) and exonic (10%) regions of noncoding genes, while the majority of the other miRNA loci are located in intragenic regions of PCGs [9]. The first step of miRNA biogenesis includes the transcription of pri-miRNA, a primary long hairpin transcript with a length of hundreds or thousands of nucleotides (**Figure 1**). Furthermore, after its processing to a shorter

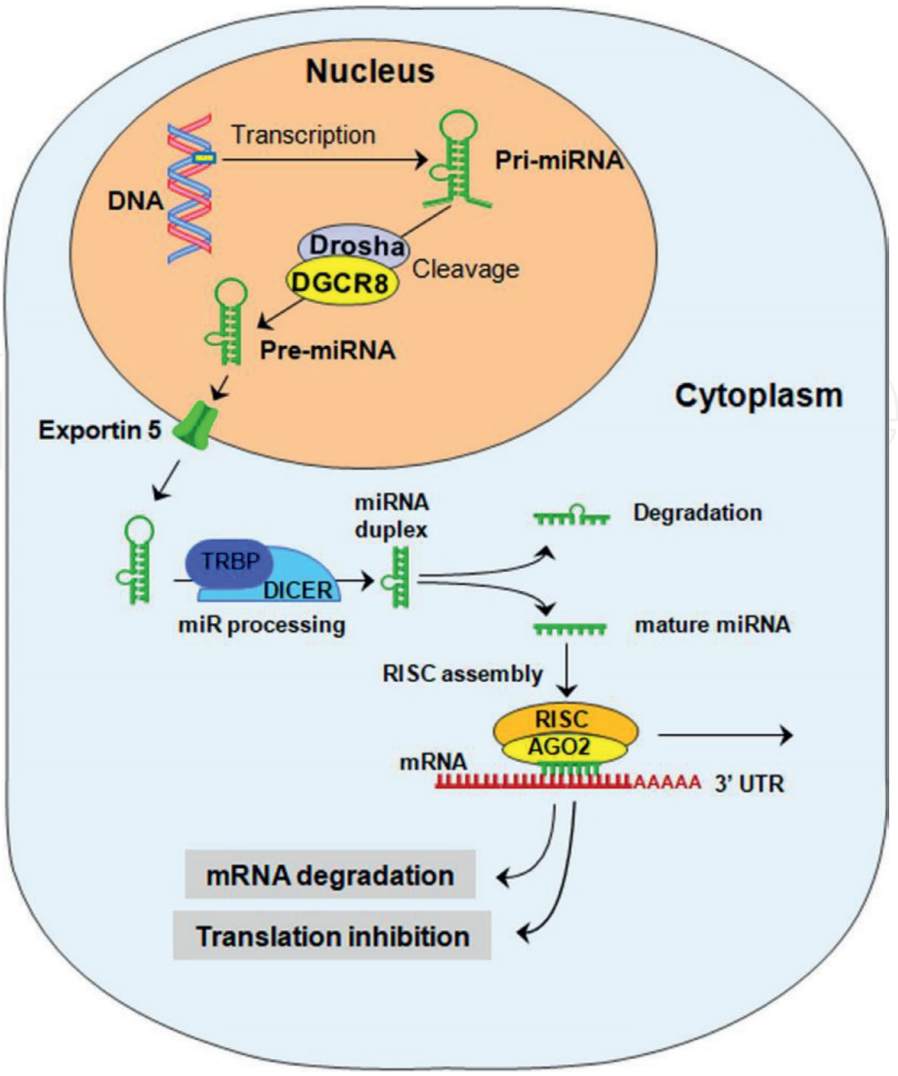

Figure 1.
miRNA biogenesis. The miRNA biogenesis starts in the nucleus, with a pri-miRNA transcription. Afterward, the pri-miRNA is processed to a shorter hairpin structure of about 70 nucleotides, by Drosha and DGCR8, and it is exported in the cytoplasm by Exportin 5. After enzymatic processing by DICER, double-stranded miRNAs are reduced to a single-stranded mature RNA (21–23 nucleotides in length). Furthermore, by incorporating into Argonaute 2 and RISC, mature miRNA will target mRNA transcripts, usually in the 3′ UTR, leading to mRNA degradation or repression.

hairpin structure of about 70 nucleotides, the pre-miRNA is exported into the cytoplasm, where under enzymatic processing, it is reduced to a single-stranded RNA (mature miRNA) of about 21–23 nucleotides in length. Afterward, by its incorporation into Argonaute 2 protein and then in the RNA-induced silencing complex (RISC), the mature miRNA will function as a guide molecule for silencing complex, targeting specific mRNA transcripts, usually by base-pairing specific mRNA transcripts, in the 3′ untranslated region (3′ UTR). Targeting the mRNA by a specific miRNA leads to the translational repression of mRNA and its exonucleolytic decay [9].

Because of their capacity to modulate up to 60% of PCGs, miRNAs are defined as "master modulators" of the human genome [10]. An important feature of miRNAs is that a single miRNA can target up to 200 mRNAs, while a single mRNA can be modulated by different miRNAs [11]. Nevertheless, to increase the accuracy of miRNA-mRNA binding, several combinatorial prediction tools based on thermodynamic modeling and machine learning techniques have been developed lately [12, 13], bringing new understanding about how miRNAs can exert their regulatory function through a combinatorial-cooperative activity.

At the moment, 48,885 mature miRNA products from 271 species, including 2654 mature human miRNAs, have been reported in the latest available miRNA database (miRBase release 22; http://www.mirbase.org/) [14]. In normal phenotype, by their modulatory effects, miRNAs maintain the cell physiology, while by their aberrant expression, miRNAs lead to the validation of many diseases including cancer.

3. The role of miRNAs in cancer

Croce's group established for the first time an association of mRNAs with cancer, indicating an alteration of miR-15a/16-1 cluster, in chronic lymphocytic leukemia [15]. Further functional analyses have demonstrated that miR-15 and miR-16 can target and suppress the expression of BCL2 oncogene, inducing the apoptosis. [16]. Through exploring the role of miRNAs, Croce's group has demonstrated that miRNA profiling could be taken into consideration for characterizing the malignant phenotype [17], opening a new perspective for identifying new cancer-specific miRNAs. Interestingly, for poorly differentiated tumors, tissue miRNA profiling has revealed better diagnosis than mRNA profiling, highlighting their role as tumor biomarkers [18]. An important feature of miRNAs, given by their high stability in formalin-fixed paraffin embedded (FFPE) tissues, blood including serum and plasma, as well as other biological fluids such as urine, tears, breast milk, saliva, and seminal fluids, makes them important candidates for the discovery of new minimally invasive biomarkers [19, 20]

Therefore, a myriad of studies describing the role of miRNAs in cancer development have been provided, with more than 21,565 papers that are published in PubMed today, when "miRNA, miR, microRNA, and cancer" are used as a string search.

Alteration of miRNA expression in cancer is due to genetic and epigenetic events. Genetic alterations include: chromosomal rearrangements or loss of heterozygosis (LOH) (e.g. miR-15a/16-1), gene amplification (e.g. miR-17-92 cluster, miR-155), deletions (e.g. let-7 family member), or mutations (e.g. miR-16) [15]. Moreover, genetic alteration may occur in the PCGs involved in the synthesis of the protein components of the Drosha, DGCR8, Exportin 5, Dicer, and AGO2, the main enzymes that process the biogenesis and activation of miRNAs. Pre- and post-transcriptional controls of not only miRNA biogenesis but also epigenetic events, including methylation and acetylation, were also related to aberrant expression of tumor miRNAs [21, 22]. Not lastly, the presence of the single-nucleotide polymorphism (SNP) mutations in the miRNA-coding genes may lead to the alterations of mature miRNA structure, reducing its specificity to the mRNA target [23].

Functionality studies have demonstrated that the expression of oncogenes and tumor-suppressor genes in cancer is closely controlled by miRNAs (**Figure 2**). Such as, miRNAs that target and modulate the oncogenic expression are defined as tumor-suppressor miRNAs (TS-miRNAs), while the miRNAs that modulate the expression of tumor-suppressor genes are known as oncomiRs [24]. Genetic and epigenetic alterations occurring in cancer lead to "gain of function" of oncomiRs and inactivation or "loss of function" of TS-miR (**Figure 2**), which translate into regulating the expression of their targets through downregulation of tumor-suppressor genes and upregulation of oncogenes, respectively [25]. miRNAs are involved in all hallmarks of cancer, including self-sufficiency in growth signals (let-7 family, miR-21), insensitivity to antigrowth signals (e.g. miR-17-92 cluster, miR-195), evasion from apoptosis (e.g. miR-34a, miR-185, miR-15/miR-16), limitless replicative potential (e.g. miR-372/373 cluster, miR221/222), angiogenesis (e.g. miR-210, miR-26, miR-15b, miR-155), invasion and metastases (e.g. miR-10b, miR-31, miR-200 family, miR-21, miR-15b), reprogramming energy metabolism

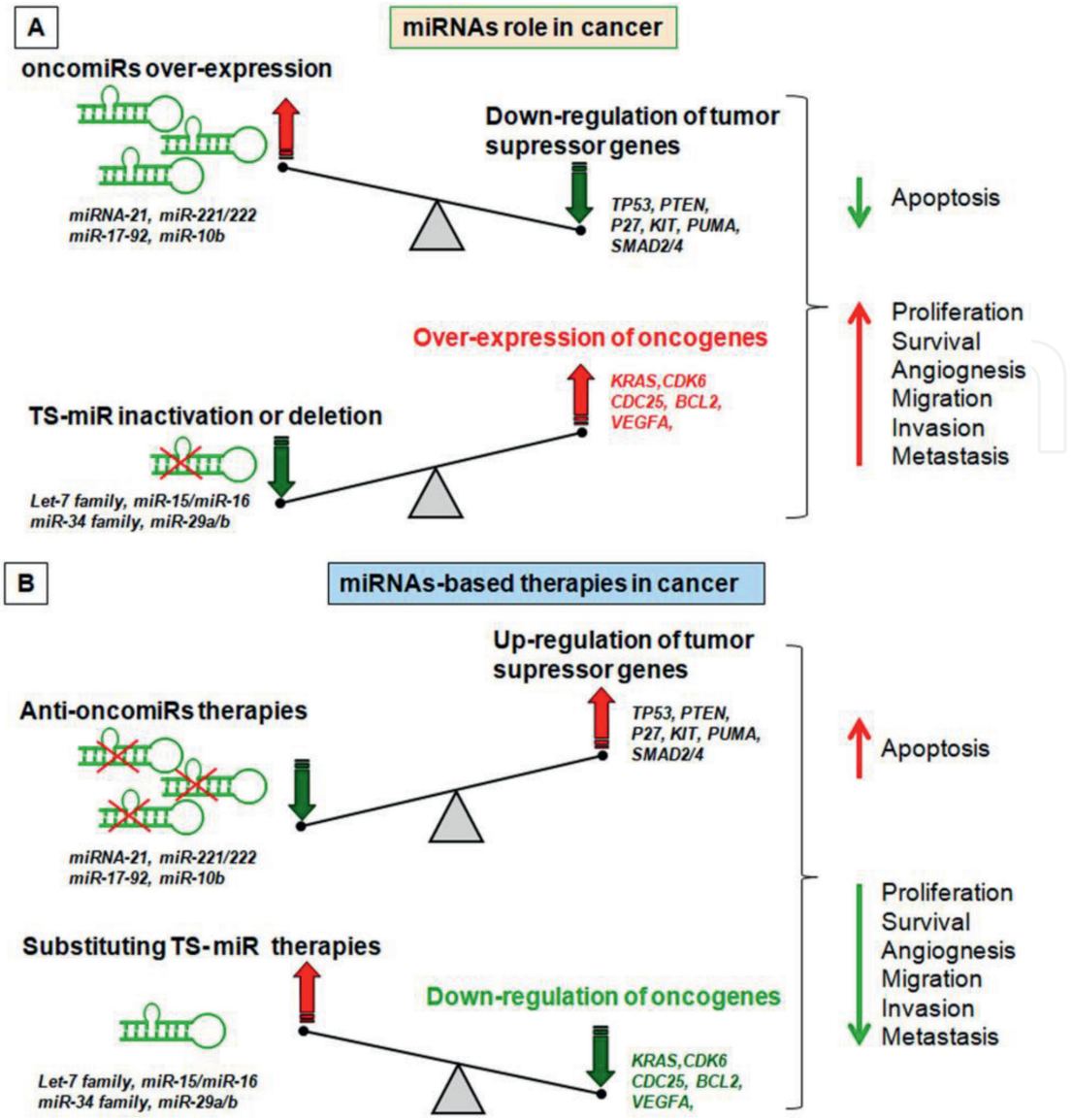

Figure 2.
The role of miRNAs in cancer and their use of miRNA-based therapy. **(A)** *miRNAs that function as oncomiRs and TS-miRs. Tumors are characterized by aberrant upregulation of oncomiRs that lead to downregulation of tumor-suppressor genes and inactivation of TS-miRs reflected in overexpression of oncogenes. All of these contribute to tumor development, invasion, and metastasis as well as decrease cell death.* **(B)** *The use of anti-miRNA therapies leads to block the oncomiR activities, resulting in the upregulation of tumor-suppressor genes, while substituting TS-miR therapies increases the cellular level of TS-miRs, leading to the inactivation of oncogenes. The effects of miRNA-based therapy indicate an increase in cell death concomitantly with inactivation of tumor development.*

(e.g. miR-23a/b, miR-378, miR-143, miR-15b), evading immune destruction (e.g. miR-124, miR-155, miR-17-92), tumor-promoting inflammation (miR-23b, miR-155, let-7d), and genomic instability (miR-21, miR-155, miR15b) [22, 26, 27].

4. Strategies used for miRNA-based therapies

4.1 miRNA inhibition therapies for oncomiRs

The choice to use mRNA-based therapies is based on the fact that the expression of mRNAs in tumor cells is altered, and tumor phenotype can be changed by the modulation of the miRNA expression [28].

MiRNA inhibition therapy is used to suppress the expression of oncomiRs that are frequently overexpressed in human cancers and reestablish the normal expression of tumor-suppressor genes that are targeting directly (**Figure 2**). The therapy for miRNA inhibition includes the following agents: antisense anti-miR oligonucleotides (AMOs), locked nucleic acid (LNA) anti-miRs, antagomiRs, miRNA sponges, and small molecule inhibitors of miRNAs (SMIRs) [27]. The principle of this therapy consists of an isolation of the endogenous miRNAs in an unrecognizable configuration, leading to inactivating and excluding the mature miRNAs from the RISC.

AMOs are single-stranded, chemically modified antisense oligonucleotides of about 17–22 nucleotides that are complementary to a miRNA of interest [28]. These antisense oligonucleotides anneal to the complementary mature miRNAs and inhibit their interaction with specific mRNA targets.

LNA anti-miRs represent an example of a modified antisense anti-miR oligonucleotide [29]. LNA-modified oligonucleotides present a higher thermal stability and affinity for their miRNA target molecules, as well as a higher aqueous solubility and increased metabolic stability for *in vivo* delivery [30].

The antagomiRs are single-stranded RNA molecules of about 23 nucleotides in length complementary to miRNA targets that are chemically modified to increase the stability of the RNA and protect it from degradation [31]. One of the most important aspects of using these agents is due to their lack of inducing any immune response.

miRNA sponges represent a class of RNAs that include multiple artificial binding sites similar to those found in the endogenous miRNA targets. The expression vectors represent the source of miRNA sponge transcription, thus reducing the miRNA's effects and increasing the expression of the miRNA's native targets [32].

SMIRs are small molecules that suppress the miRNA biogenesis or block the interaction between a miRNA and the target. The inhibition therapy using SMIRs is an encouraging one due to the reduced time of production, approval, and cost [33].

4.1.1 Discussion

Krützfeldt et al. [31] demonstrated that intravenous administration of several antagomiRs toward miR-16, miR-122, miR-192, and miR-194 leads to a significant reduction in the corresponding endogenous miRNAs.

Moreover, an important positive effect observed in this study was that after the administration of antagomiR-122, the cholesterol levels in plasma have decreased. Due to the fact that, so far, the therapy using antagomiRs did not induce a significant immune response, it is worth into consideration the development of a promising antisense therapy based on antagomiRs.

One of the main advantages of using locked nucleic acid (LNA) anti-miRs is that they present a higher thermal stability, high-affinity Watson-Crick hybridization with their RNA target molecules, higher aqueous solubility, and increased metabolic stability for *in vivo* delivery. Overexpression of miR-21 is a common place in glioblastomas, and Griveau et al.'s [34] study was able to silence miR-21 in U87MG glioblastoma cell line, using a LNA conjugated to lipid nanocapsules (LNC). Another advantage of using LNA-LNC complexes in combination with external beam radiation is represented by the improvement of cell sensitivity to treatment.

4.2 miRNA replacement therapies for tumor-suppressor miRNAs

Also defined as miRNA restoration therapy, the replacement therapy with miRNAs includes the following agents: small molecules, synthetic miRNA mimics, and DNA plasmids encoding a miRNA gene that epigenetically alters endogenous expression of miRNAs [35].

Small molecules in miRNA replacement therapy are represented by hypomethylating agents (Decitabine or 5-azacytidine) and enoxacin, exerting a role in the nonspecific miRNA expression.

miRNA mimics are double-stranded synthetic RNAs, which are aimed to compensate the lack of tumor-suppressor miRNAs by replacing the lost miRNAs. These chemically structures are loaded into RISC to provide the downstream inhibition of the target mRNAs [27].

4.2.1 Discussion

One of the main challenges of miRNA replacement therapy is represented by finding the most suitable, efficient, and specific delivery system. The efficacy of this therapy is significantly decreased by an unsuitable size of the vector or by gene expression. Since miRNAs can be introduced into cells using a similar technique to small interference RNAs [36], it is recommended to improve those techniques based on the insertion of synthetic miRNA mimics, DNA plasmids, and small molecules, as well as to improve the quality of molecules used for this type of therapy.

4.3 miRNA delivery systems

An important aspect that is worth considering in miRNA cancer therapy aims to use miRNA delivery systems. One of these delivery systems including microvesicles and exosomes aim to block miRNA-entrapped exosomes released by tumors. It is already demonstrated that miRNA-entrapped exosomes secreted by tumor cells can regulate gene expression in the receiving cells by binding to their target mRNAs [37]. The use of some agents that block specific miRNAs (such as LNA anti-miR-21 and LNA anti-miR-29a) in tumor cells could lead to the reduction in miRNA-entrapped exosomes, released by cancer cells [38]. However, an ideal delivery system meets the following criteria: protects the miRNAs from early degradation in the bloodstream, efficient distribution to the target cells, facilitates cellular uptake, does not induce an immune response, and made of biocompatible and biodegradable materials [39].

The most commonly used vectors for miRNA delivery include viral and nonviral vectors. Previous data demonstrated that viral vectors mainly caused an immune response; therefore, the focus of the actual studies is on developing efficient nonviral vectors. Nonviral vectors are classified into three main groups, including polymeric vectors (polyethyleneimines, atelocollagen, polylactic-co-glycolic acid, polyamidoamine dendrimers), lipid-based carriers (positively, negatively or neutral charged), and inorganic materials (gold, diamond, silica, and ferric oxide) [40].

The delivery system based on viral vectors transfers the pri-miRNA or mature miRNAs, usually a TS-miR, into a plasmid, which contains a viral promoter, an antibiotic resistance gene, and a restriction enzyme gene, to the tumor cells. After nuclear integration of the miRNA and further transcription, the mature miRNA represses the translation and/or induces the degradation of the target mRNA [41].

4.3.1 Discussion

One of the most studied classes of polymeric vectors was represented by polyethylenimines (PEIs) but was removed from clinical studies due to their high toxicity, given by an excessive positive charge, low biological degradation, and inactivation in serum caused by a nonspecific protein. Ibrahim et al. [42] have demonstrated that by using low molecular weight, PEIs as system delivery for miR-145 and miR-33a would

decrease the toxicity and increase the antitumor effect, in a model of colon carcinoma. Recent studies [43, 44] have proved that codelivery of miR-200c with chitosan, a cationic polymer with a high specificity for nucleic acid binding, decreased the angiogenesis, invasion, EMT, and metastasis and increased the apoptosis, highlighting the role of miRNA concentration in treatment effectiveness. Hao et al. [45] used miRNA (MiR-15a, miR16-1)/ATE-APT complex formed by atelocollagen (ATE), a type I collagen positively charged polymer, in combination with a RNA aptamer (APT) used as a ligand to target PCa cells that express prostate-specific membrane antigen (PSMA). Their study concluded that miRNA/ATE-APT complex was more efficient than an ATE-miRNA complex and that by using a PSMA-targeted system, the chances for selective killing of prostate cancer cells significantly would increase.

Moreover, it is worth into consideration the administration methods used for synthetic miRNAs (miRNA mimics) delivery into cells. Previous studies of Trang et al. have shown that both intratumoral and intravenous administration of let-7a mimics lead to the diminishing of non-small-cell lung cancer (NSCLC) tumor size in mouse models [46, 47].

As a future improvement in miRNA delivery systems, it is recommended to be synthetized proteins or peptides in order to be used as vector polymeric due to their low cytotoxicity and immunogenicity. Finding a suitable delivery system for a specific miR according to tumor cell type and the development of systems to target specific cancer membrane antigens still represent major challenges.

5. MicroRNAs in cancer therapies

5.1 miRNA candidates used in preclinical trials

Increasing the evidence has demonstrated that miRNA expression is modified in cancer, and restoring the level of cellular miRNA could underpin the development of miRNA-based therapies. Below we briefly describe miRNAs that are currently used in preclinical and clinical trials and also represent examples that affect the emerging hallmarks of cancer such as evasion from apoptosis (miR-15/16, miR-34 cluster) [48], enabling replicative immortality (miR-34a) [48], activating invasion and metastasis (miR-10b) [49], tumor-promoting inflammation (miR-155), and genome instability and mutation (miR-155) [50].

5.1.1 miR-10b

Guessous et al. [51] observed that miR-10b is overexpressed in human glioblastoma and stem cell lines when compared to healthy tissues or astrocytes. After the modulation of miR-10b, they found out that the inhibition of miR-10b strongly reduced cell proliferation, invasion, and migration of glioblastoma and stem cell lines, whereas its overexpression caused cell migration and invasion. Moreover, in a previous study, Ma's group [52] has demonstrated that the use of miR-10b antagomiRs was correlated with reduced metastasis both in cell-culture lines and in animal model of breast tumor-bearing mice. Thus, miR-10b inhibition both *in vitro* and *in vivo* significantly decreased miR-10b levels and increased levels of Hoxd10 gene, an important miR-10b target. Curiously, the administration of miR-10b antagomiRs *in vivo* did not reduce primary mammary tumor growth but significantly suppressed the development of lung metastases, highlighting its antimetastatic role.

5.1.2 miR-221

Since miR-221 overexpression alters multiple cancer pathways, it becomes a potential target for miRNA-based therapy. In order to validate the role of miR-221 in tumorigenesis, Callegari et al.[53] showed that *in vivo* delivery of an AMO anti-miR-221 caused a significant decrease in the size and number of tumor nodules. Based on the results from their study, it was highlighted the promoter role of miR-221 in liver carcinogenesis, being also established a valuable animal model to investigate the anti-miRNA-based therapy for liver cancer.

Moreover, using a colorectal cancer model, Qin et al. [54] showed that miR-221 promotes cell migration and invasion *in vitro* and metastasis *in vivo,* identifying tumor-suppressor RECK gene as a direct target of miR-221.

With regard to the role of miR-221 in tumorigenesis combined with the need to limit its expression, Brognara's group demonstrated that a peptide nucleic acid conjugate targeted against miR-221 (Rpep-PNA-a221) caused a suppression of miR-221 expression and an upregulation of its target $p27^{Kip1}$ in two breast cancer cell lines (MCF-7 and MDA-MB-231), respectively [55]. On the other hand, in a recent study, Gallo et al. [56] evaluated the pharmacokinetic and pharmacodynamic properties of a locked nucleic acid anti-miR-221 (LNA-i-miR-221) in the models of mice and monkeys. Their data highlighted that LNA-anti-miR-221 has a short half-life, optimal tissue bioavailability and minimal urine excretion in both species. A very important aspect of their study was that no toxicity was present in the pilot monkey study. This finding defines the potential application of LNA-anti-miRNAs in clinical studies.

5.1.3 miR-222

Sometimes developing a miRNA-based therapy is difficult because the same miRNA can act both as an oncogene and as a tumor-suppressor gene, due to its multiple targets and mechanisms of action. Such an example in this way is represented by miR-222, which has a role of oncomiR in liver cancers, by targeting and suppressing the PTEN tumor-suppressor gene, or TS-miRNA, whose downregulation in erythroblastic leukemia leads to the overexpression of c-KIT oncogene [27].

5.1.4 miR-34

miR-34 is one of the most important TS-miRs, being positively controlled by TP53 [57], repressed by MYC [58], and silenced by aberrant CpG methylation [59].

Overexpression of miR-34 was related to apoptosis and cell cycle arrest [60], while its underexpression was linked to different tumor types, including nonsmall-cell lung cancer (NSCLC) [61], breast cancer [62], or ovarian cancer [63]. Several studies have proved that ionizing radiation upregulates the levels of expression from different miR-34 family members in a variety of human cell types: miR-34b in lymphocytes [64], miR-34c in prostate cancer cell lines [65], and miR-34a in thyroid cells [66]. Consequently, to increase the therapeutic efficiency, some of the future studies should focus on the combined use of DNA damage response related to miRNAs and radio- or chemotherapy. By performing a miR-34 modulation, Trang et al. [47] have demonstrated that synthetic miR-34 mimics incorporated in a lipid-based particle was able to block tumor growth in a mouse model of nonsmall cell lung. Likewise, Daige et al. [67] have proved that the use of encapsulating miR-34a mimics into liposomes (MRX34) leads to increase the level of miR34a in liver tumors, followed by significantly reducing several of its mRNA targets, and consequently tumor regression.

Based on these results, encapsulating miR-34a mimics into liposomes (MRX34, Mirna Therapeutics Inc.) was later proposed to be investigated in clinical trials [68].

5.1.5 miR-16

miR-16 represents another TS-miR whose decreased expression has been observed among different types of cancers, as well as in nonsmall cell lung cancer (NSCLC) [69], prostate cancer [70], or malignant pleural mesothelioma [71], making it a strong candidate for replacement therapy in future studies with potential use in clinical trials. The data presented by Takeshita et al. [72] revealed that systemic delivery of synthetic miR-16, conjugated to atelocollagen, significantly reduced bone metastases and tumor development in a prostate cancer animal model. Moreover, their *in vitro* data suggest that miR-16 suppresses prostate tumor growth by regulating the expression of genes associated with cell-cycle control and cellular proliferation such as CDK1 and CDK2. The Hao group has also revealed that miR-16-1/atelocollagen-aptamer complex used in a mice model of human prostate cancer with bone metastasis enhanced anticancer efficacy. They also demonstrated that the efficacy of this complex, including aptamers, was higher, both *in vitro* and *in vivo* models than the other atelocollagen complexes that do not include aptamers. Re-expression of miR-16 mimic in malignant pleural mesothelioma cell lines and nude mouse models has caused the inhibition of tumor growth, correlated with downregulation of target genes Bcl-2 and CCND1 [71].

5.1.6 miR-155

miR-155, one of the first described oncomiRs [73], was identified as highly expressed in a wide range of tumors including chronic lymphocytic leukemia [74], lung cancer [75], breast cancer [76], acute myeloid leukemia [77], solid tumor including stomach, prostate, colon, pancreas [78], and melanoma [79].

OncomiR-155 was discovered to target RAD51, an important gene in the homologous recombination DNA repair pathway, and the clinical study of Gasparini et al. [80] for triple negative breast cancer revealed that low miR-155 expression level correlated with worse progression-free survival. Moreover, Pouliot et al. [81] reported a reduced expression of miR-155 in human epidermoid carcinoma cisplatin-resistant cell lines. Dysregulated expression of this miRNA sensitizes the cells to cisplatin-induced apoptosis by targeting WEE1 and CHK1 kinases. Based on these results, future studies are encouraged with the focus on the use of exogenous agents, such as mimics or anti-miRs to sensitize cancer cells to chemo- or radiotherapy, thus overcoming resistance to therapy.

Alexander et al. [82] found that endogenous miR-155, an important microRNA that regulates inflammation, is released from dendritic cells within exosomes and transferred to recipient dendritic cells. Administration of miR-155 containing exosomes enhances inflammatory gene expression as a response to endotoxin-induced inflammation in mice. Their findings provide strong evidence that endogenous microRNAs follow a functional transfer between immune cells and represent a regulatory mechanism for inflammatory response.

More examples of tumor-suppressor miRNA mimics, which target multiple oncogenic transcripts, were recently presented by Hosseinahli et al. [41].

5.2 Clinical studies involving miRNA-based therapy

Given the results provided by *in vitro* and *in vivo* studies, several clinical trials including miRNA-based therapy in human cancers were subsequently initiated (**Table 1**).

Company	Drug	Targeted miRNA	Therapy type	Cancer type	Delivery system	Mechanism/effect	Trial status	Clinical trials. Gov identifier
Mirna Therapeutics. Inc	MRX34	miR-34	Mimic	Nonsmall-cell lung carcinoma, small cell lung cancer, primary liver cancer lymphoma, melanoma, multiple myeloma, renal cell carcinoma	LNPs (Smarticles)	Reduction in the expression of oncogenes, tumor regression, enhanced the survival, and inhibited the growth of other nonhepatic tumors	Multicenter phase I terminated	NCT01829971
EnGeneIC	MesomiR-1	miR-16	Mimic	Malignant pleural mesothelioma, nonsmall cell lung cancer	EnGeneIC delivery vehicle	Strong inhibition of tumor growth	Multicenter phase I completed	NCT02369198
miRagen Therapeutics	MRG-106	miR-155	Anti-miR	Cutaneous T cell lymphoma, mycosis fungoides, chronic lymphocytic leukemia, adult T-cell leukemia/lymphoma	LNA-modified antisense inhibitor	Reduce overexpression and of oncomiR, leading to decreasing aberrant cell proliferation	Multicenter phase I recruiting	NCT02580552

Table 1.
Clinical trials using miRNA therapy in human cancers (adapted after Christopher et al. [87] and Rupaimoole et al. [88]).

In April 2013, Mirna Therapeutics, Inc., a publicly traded company based in Carlsbad, California, which primarily focuses on anti-miRNAs technology, announced that their leading product candidate, MRX34, a mimic of miR-34 encapsulated in a liposomal nanoparticle formulation, called NOV40, was the first microRNA mimic to enter clinical development. The multicenter phase I trial of MRX34 included patients diagnosed with primary liver cancer, nonsmall cell lung cancer (NSCLC), lymphoma, melanoma, multiple myeloma, or renal cell carcinoma. The trial aimed to increase the number of intravenously doses with two times per week or five times per day schedule. In June 2016, a total of 99 patients suffering from HCC, NSCLC, or pancreatic cancer had been enrolled in the study [83]. The phase I clinical trial confirmed partial responses in a patient with metastasized hepatocellular carcinoma (HCC), a patient with advanced acral melanoma, and a patient with advanced renal cell carcinoma (RCC), evaluated through Response Evaluation Criteria in Solid Tumors (RECIST). Also, 14 patients were detected with stable disease (median duration 136 days; range 79–386 days). Results from white blood cell analysis indicated a significant reduction in two miR-34 target genes FOXP1 and BCL2. Nonetheless, because of the immune-related adverse responses involving patient deaths, the trial was finished. Since the cause of these immune reactions remains still unclear, preclinical trials will be need in order to better understand the immune-related toxicities. After passing successfully the phase I clinical trial, they intended to advance into two phase II clinical studies: one for patients diagnosed with advanced malignant melanoma and another for patients diagnosed with advanced renal cell carcinoma (RCC). In concordance with the advancements made by MRX34, their lead product candidate, through the clinical development, miR-34 mimics represent a new promising class of replacement therapy used in cancer. Additionally, Cortez et al. [84] reported that p53 regulated PDL1 expression via miR-34 in nonsmall-cell lung cancer. Administration of miR-34a mimics (MRX34), alone or in combination with radiotherapy (XRT), reduced PDL1 expression in the tumor and antagonized T-cell exhaustion.

In November 2014, EnGeneIC, a privately held Australian company in collaboration with Asbestos Diseases Research Institute, Sydney, Australia, announced the start of phase I clinical trial using miR-16 mimic charged in nanocells, a bacterial-derived transfection system EDV™. The trial included patients suffering from malignant pleural mesothelioma (MPM) and advanced nonsmall cell lung cancer (NSCLC), refractory to standard therapy. miR-16 mimic-based therapy were delivered intravenously, using EnGeneIC Delivery Vehicle (EDV)-Packaging, and were surface conjugated with an EGFR-targeting antibody in order to facilitate the target of tumor site [85]. Preliminary data presented by Van Zandwijk et al. [86] show manageable safety in response to infusion of 5 billion nanocells loaded with 1.5 μg miR-15/16 mimics as a first-dose level in the first five patients that had been enrolled. Because of the fact that this targomiR trial using miR-16 as a replacement therapy did not present adverse immune response and toxic effects, it is expected to continue to phase II study [86].

In March 2016, MiRagen Therapeutics, Inc., a privately held company based in Boulder, Colorado, announced the initiation of phase I clinical trial to investigate the anticancer product candidate: MRG-106, a synthetic microRNA antagonist of microRNA-155 (LNA anti-miR). The phase I clinical trial is currently tested in patients diagnosed with cutaneous T-cell lymphoma (CTCL) of the mycosis fungoides (MF) subtype [89].

Despite some promising preclinical results, the outcome of MRX34 translational clinical trial using miR-16 as a replacement therapy, designed to restore the expression of miR-34 in patients diagnosed with different types of cancer, was discouraging due to adverse toxic effects. At present, this clinical trial is finished, and its suitability to further development to phase II study remains under question.

However, this drawback can be addressed by optimizing therapeutic doses and applying organ-specific administration routes [41].

6. Future challenges: new reliable miRNAs for target therapies

Epithelial-to-mesenchymal transition (EMT) allows tumor cells to enter the metastatic cascade by changing their morphological and molecular characteristics, and it represents a wide spectrum that cancer cells keep transiting. The EMT program relies on an intricate network of signaling pathways that dictate a series of phenotypic changes in epithelial cells. One clearly visible aspect is the loss of apical-basal polarity and cell-to-cell interaction caused by the destabilization of tight junctions, decreased claudin, occludin, and E-cadherin repression by SNAIL, SLUG, ZEB, TWIST, and SMAD, and its interchange with N-cadherin, a process known as the "cadherin switch" [90, 91]. Moreover, the overexpression of vimentin, a cytoskeleton intermediate-filament protein of mesenchymal origin that connects the nucleus to the plasma membrane, enables filopodia formation and a fibroblastic spindle-like morphology. Vimentin was proved to be induced through Slug and Ras signaling, and it promotes cell movement and migration [92]. The next step is the degradation of extracellular matrix components under the activity of matrix metalloproteinases and the invasion of the surrounding stroma (**Figure 3**).

The EMT is coordinated by a series of signaling pathways triggered by either the tumor microenvironment or the intrinsic factors, and it falls under the incidence of regulatory noncoding RNAs, especially micro-RNAs [93].

This is the reason why finding potential microRNA targets for blocking EMT could support the efforts already made in blocking metastasis and improving cancer therapy strategies. Right below, we are succinctly reviewing the results of several studies that investigated the role of microRNAs in EMT and metastasis and their potential in becoming microRNA therapy targets (**Table 2**).

The influence of cancer-associated fibroblasts (CAFs) secreted exosomes over endometrial cancer progression was questioned in a study by Li et al. [94]. CAFs secreted exosomes contained significantly lower levels of miR-148b than normal fibroblasts, and miR-148b expression was lower in endometrial cancer specimens than in normal adjacent tissues. miR-148b was correlated with improved prognosis, *in vitro* and *in vivo* studies suggesting its role as an EMT inhibitor. Downregulation of DNMT1 oncogene was the mechanism proposed for miR-148b-mediated suppression of endometrial cancer progression. Also, relating to the tumor microenvironment, emerging evidence shows the prometastatic effect of a hypoxic

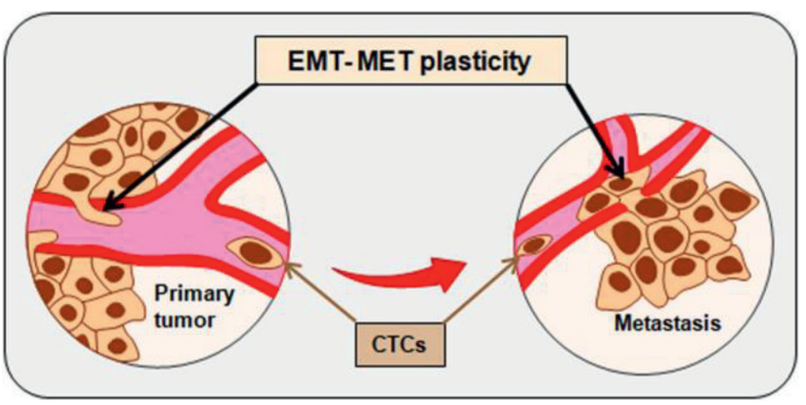

Figure 3.
The EMT-MET plasticity of tumor cells, their migration, and invasion as tumor circulating cells (CTCs).

microenvironment over tumor cells. Acidic conditions were shown to promote miR-210 overexpression by activating HIF1 [95, 96]. In prostate cancer, high levels of miR-210 were detected in bone metastases, and they were correlated with poor prognosis of prostate cancer patients. Exogenously overexpression of miR-210-3p in cancer cell lines enhanced cell motility and migration as well as bone metastasis in mouse model by inducing NF-κB signaling and EMT. Moreover, miR-210-3p inhibition reversed EMT and impaired the metastatic potential of cancer cells [95]. Another study confirmed the hypoxia-induced EMT activation and metastasis by HIF1-miR-210 axis in breast cancer. Tang et al. [96] identified high expression of miR-210 in hypoxia grown breast cancer stem cells and in isolated human breast cancer stem cells. The overexpression of MiR-210 in poorly metastatic MCF7 cell lines leads to their invasiveness and migration *in vitro* as well as increasing metastatic potential *in vivo*. One suggested mechanism is related to the direct binding of miR-210 to the Open Reading Frame (ORF) of the E-cadherin mRNA and its post-transcriptional inhibition in breast cancer cells.

miR-652 downregulation in the acidic microenvironment of pancreatic cancer promoted EMT by ZEB1 activation, and it was correlated with a progressive stage, lymphatic invasion, vascular infiltration, and distant metastasis. *In vitro* experiments showed that miR-652 overexpression has an antimetastatic effect by inducing MET in PANC-1-A miR-652-mimic transfected cells, and it reduced their migration and invasion. Also, *in vivo* studies confirmed the *in vitro* results with lower tumor mass, fewer metastases, and overexpression of E-cadherin over vimentin/N-cadherin in mouse xenografts PANC-1-A miR-652-mimic transfected cells vs. miR-652 inhibitor [97].

Partially due to cancer tumors heterogeneity in construction and behavior and the relative novelty of noncoding RNAs as potential targets for cancer therapy, there are still missing bricks in understanding the mechanism that triggers the regulation of cancer-related microRNAs. However, as shown above, tumor microenvironment has a great impact over cancer progression, and probably understanding its role in modulating microRNA expression in tumor cells might bring light to new potential targets for improved therapy.

From a different perspective, there are many well-known pathways underlying cancer development and progression. Wnt signaling pathway represents one of the fundamental pathways involved in cell proliferation and specialization, as well as cell movement during both embryonic development and tissue homeostasis. The canonical Wnt signaling functions by regulating the amount of the transcriptional coactivator β-catenin, a molecule that controls key developmental gene expression programs [98]. Wnt signaling aberrations have been shown to regulate various processes that are important for cancer progression, including tumor initiation, tumor growth, cell senescence, cell death, differentiation, and metastasis. Wnt signaling molecules and downstream effectors can promote transcriptional changes in order to induce EMT in cancer cells while also being further activated by EMT in a continuous feedback loop [99].

Several microRNAs were proven to modulate Wnt signaling in EMT and cancer metastasis. In endometrial cancer, Wnt signaling can be activated by miR-652, which targets and inhibits retinoid orphan nuclear receptor alpha (RORA) gene. RORA represents a tumor-suppressor gene that represses the Wnt/β-catenin pathway through attenuating β-catenin transcriptional activity. Expression of miR-652 is frequently increased in human endometrial cancer tissues, its high expression being correlated with poor tumor differentiation, shorter overall survival, and recurrence. Overexpression of miR-652 in endometrial cancer cell promotes their proliferation and migration *in vitro* and *in vivo* [100].

Another metastasis enhancer, miR-374a, acts by activating the Wnt/β-catenin cascade and promoting EMT. miR-374a maintains constitutively activated

Wnt/β-catenin signaling by suppressing multiple negative regulators including WIF1, PTEN, and WNT5A. miR-374a was upregulated in primary tumor samples from breast cancer patients with distant metastases, and it was associated with poor metastasis-free survival. miR-374a transfection into poorly metastatic MCF7 cell line promoted its motility and invasiveness *in vitro* and lung metastasis forming abilities in BALB/c mice, while miR-374a knockdown in highly invasive MDA-MB-231 cells decreased their motility and metastatic potential [101].

On the other hand, microRNA-590-5p was found to function as a tumor-suppressor in breast cancer, inhibiting EMT, cell migration, and invasion by downregulating the Wnt/β-catenin pathway. Quantitative RT-PCR analysis on breast tumor tissues and paired adjacent normal tissues showed that miR-590-5p was downregulated in breast cancer together with E-cadherin, while its target PITX2, b-catenin, Wnt-1, N-cadherin, and vimentin were upregulated. *In vitro* experiments and mouse xenografts showed that miR-590-5p upregulation or PITX2 silencing inhibits the activation of Wnt/β-catenin signaling pathway and suppresses the EMT of breast cancer [102].

Another metastasis inhibitor, miR-625, was characterized in different pathologies, and surprisingly, it maintained its function, even though it was involved in different pathways. miR-625 was reported as downregulated in hepatocellular carcinoma, gastric cancer, and colorectal cancer, and its low expression was associated with local invasion, lymph node, and distant metastasis. Ectopic expression of miR-625 induces suppression of migration and invasion of hepatocellular carcinoma cells by post-transcriptionally inhibiting IGF2BP1. Loss of IGF2BP1 suppressed F-actin polymerization, inhibiting the formation of cell protrusions, required for cell migration. The same effect was observed *in vivo*, where miR-625 overexpression decreased intrahepatic and lung metastasis [103]. Moreover, when it was ectopically induced, miR-625 suppressed the migration and invasion of gastric cancer cells as well as metastasis in nude mice by inhibiting ILK protein synthesis [98]. While the exact signaling pathway was not fully elucidated in colorectal cancer, ectopic miR-625 expression inhibited cell migration and invasion and suppressed colorectal cancer cell metastasis in nude mice [99]. The aforementioned findings highlight miR-625 as an interesting candidate for further *in vivo* studies in order to test its potential for developing a therapeutic microRNA for blocking invasion and metastasis.

A dual and somewhat contradictory behavior can be observed in the case of miR-409. While low miR-409 expression in breast cancer and nonsmall cell lung carcinoma (NSCLC) was associated with poorer prognosis and its ectopic uptake decreased the invasiveness of cancer cells [104, 105], it also seems to exert the negative effect in the case of prostate cancer, where it promotes tumorigenesis and EMT [106, 107]. Qi Song et al. [104] showed that miR-409 inhibits NSCLC cell migration, growth, and proliferation abilities by inhibiting SPIN1 translation. miR-409 downregulates PI3K/AKT pathway in NSCLC and inhibits its downstream targets such as CREB1, BCL2, and Cyclin D. Overexpression of miR-409 led to fewer lung metastases in nude mice, confirming its antimetastatic potential. Interestingly, miR-409 targeted the same pathway and suppressed cell growth and invasion in breast cancer. miR-409-3p inhibits the proliferation, migration, and invasion of breast cancer by targeting and suppressing the AKT expression. miR-409-3p was downregulated in several human tumors compared to their corresponding nontumor tissues [105]. On the other hand, miR-409-entrapped exosomes secreted by cancer-associated fibroblasts (CAFs) and promoted EMT and prostate tumorigenesis. In their study, Josson et al. demonstrated that miR-409-3p was highly expressed in CAFs derived from human patients, and it was correlated with higher Gleason score in prostatic tissues. Moreover, the ectopic expression of miR-409 in normal prostate stromal fibroblasts conferred them a CAF phenotype *in vitro*. Exosome-mediated transport of miR-409 into normal prostate stromal cells induced cell growth and

miRNA	Pathology	Effect	Target	Mechanism	Effect *in vitro* and *in vivo*	Clinical associations	Ref.
miR-148b	Endometrial cancer	Metastasis inhibitor	Inhibits DNMT1	Anti-EMT, increased E-cadherin over vimentin, fibronectin, N-cadherin	Decreased motility and invasion *in vitro*, decreased metastasis *in vivo*	—	[94]
miR-210-3p	Prostate cancer	Metastasis promoter	Inhibits TNIP1 and SOCS1	HIF1-miR-210-3p- > enhances NF-κB signaling inducing EMT	Promoted EMT, invasion, and migration of Pca cells and bone metastasis of Pca cells in mouse	Overexpressed in bone metastatic tissues, correlates with high PSA levels, Gleason grade and bone metastasis status in prostate cancer patients	[95]
miR-210	Breast cancer	Metastasis promoter	Inhibits E-cadherin synthesis	Blocks E-cadherin mRNA by binding to the ORF region	Upregulated in mammosphere cells, induced by hypoxia, promotes invasion, migration, proliferation, and self-renewal, induces EMT by loss of E-cadherin *in vitro* and leads to poorly differentiated tumors, high proliferation, more metastases and higher tumor mass *in vivo*	Upregulated in BCSC CD44+/CD24-sorted from breast cancer tissue samples	[96]
miR-652	Pancreatic cancer	Metastasis inhibitor	Inhibits ZEB1	Acidic microenvironment-miR-652 downregulation-ZEB1 upregulation > EMT	Anti-EMT, reduced migration and invasion, promotes MET *in vitro*, lower tumor mass, fewer metastases, increased expression of E-cadherin vs. vimentin, N-cadherin *in vivo*	Downregulated in pancreatic cancer, correlated with progressive stage, lymphatic invasion, vascular infiltration, distant metastasis	[97]
miR-652	Endometrial cancer	Metastasis promoter	Inhibits RORA	Enhanced b-catenin expression, Wnt-b-catenin signaling pathway	Increased proliferation, and increased metastasis potential *in vitro* and *in vivo*	Upregulated in EC, associated with poor differentiation, poor prognosis, shorter overall survival, and recurrence; not associated with cancer stage, localization, tumor size	[100]

miRNA	Pathology	Effect	Target	Mechanism	Effect *in vitro* and *in vivo*	Clinical associations	Ref.
miR-374a	Breast cancer	Metastasis promoter	Inhibits PTEN, WIF1, WNT5A	Wnt-b-catenin signaling, promoting the transcriptional activity of TCF/LEF	Induces EMT and enhanced motility in MCF7 transfected cells, decreases MDA cells motility in knockout, promotes lung metastases with MCF7 miR-374a transfected cells, impaired MDA-435 metastases by the administration of antagomiR	Upregulated in patients that presented metastases within the 51 months follow up; associated with low WIF1, PTEN, and WNT5A expression in tumor samples; high b-catenin low E-cadherin in miR-374a overexpressing samples	[101]
miR-590-5p	Breast cancer	Metastasis inhibitor	Inhibits PITX2	Blocks Wnt signaling induced by PITX2	Upregulation of miR-590 inhibits EMT genes, inhibits proliferation after 48-72 h, impairs migration and invasion of cancer cells, and promotes smaller tumor size for miR-590 mimic or PITX2 inhibition and less lung and lymphatic metastases in nude mice	Downregulated in breast cancer tissues vs. normal tissue, together with E-cadherin, while PITX2, b-catenin, Wnt-1, N-cadherin, and vimentin are upregulated	[102]
miR-625	Hepatocellular carcinoma	Metastasis inhibitor	Inhibits IGF2BP1	PTEN and Akt signaling downregulation, inhibiting F-actin polymerization	Suppressed migration and invasion, proliferation was not influenced in cell lines, decreased intrahepatic and lung metastasis in mice xenografts	Downregulated in HCC, correlated with aggressiveness of cancer and tumor metastasis	[103]
miR-625	Gastric cancer	Metastasis inhibitor	Inhibits ILK	Suppressed LIMS1-ILK-parvin axis signaling	Reduced migration and invasion rate *in vitro*, suppressed motility and extravasation from vessels, fewer lung metastatic nodes	Downregulated in gastric cancer, associated with lymph-node metastasis; no relation with tumor localization, differentiation, and local invasion	[98]
miR-625	Colorectal cancer	Metastasis inhibitor	—	—	Reduced migration in cancer cell lines; fewer and smaller liver metastases in mouse xenografts	Downregulated in cancer tissues, associated with lymph node and liver metastases and lower overall survival rate in 5 years	[99]

miRNA	Pathology	Effect	Target	Mechanism	Effect *in vitro* and *in vivo*	Clinical associations	Ref.
miR-409	NSCLC	Metastasis inhibitor	Inhibits SPIN1	Downregulation of SPIN1-AKT signaling	Inhibits cell growth, proliferation, and migration *in vitro*; fewer lung metastatic foci *in vivo*	Downregulated in NSLC tissues, correlated with tumor size, stage, pleural invasion, and metastasis; worse overall survival and disease-free survival	[104]
miR-409-3p	Breast cancer	Metastasis inhibitor	Inhibits AKT1 p	Downregulation of PI3K-AKT pathway	Inhibited breast cancer cell proliferation, migration, and invasion suppressed tumor growth *in vivo*	Downregulated to a significant extent in tumor samples, relative to the corresponding nontumor tissues	[105]
miR-409-3p/5p	Prostate cancer	Metastasis promoter	Inhibits RSU1 and STAG2	Upregulation of Ras and Akt signaling	Induces EMT in normal prostate epithelia, promotes tumorigenicity and stemness *in vivo*	Upregulated in human prostatic tissues with higher Gleason score and prostate cancer bone metastasis	[106]
miR-409-3p/5p	Prostate cancer	Metastasis promoter	Inhibits STAG2 and RSU1	Upregulation of Ras-ILK signaling	Induction of an EMT phenotype, decreased E-cadherin, increased vimentin, b-actin and downregulation of tumor suppressing genes	Higher miR-409 expression in the stromal fibroblasts, correlated with higher Gleason score in prostatic tissues	[107]
miR-409-5p	Breast cancer	Metastasis promoter	Inhibits RSU1	Upregulation of Ras signaling	Induces proliferation and migration in cancer cells and tumor growth in mice xenografts	Upregulation in breast can carcinoma tissues, correlated with shorter survival	[108]

Table 2.
In vitro and in vivo validated miRNAs related to EMT, invasion, and metastasis that could represent future therapy targets.

EMT *in vitro* and *in vivo.* In this case, the predicted targets of miR-409 were the tumor-suppressor proteins STAG2 and RSU1, which appeared downregulated [108]. Considering these, it would be worth exploring if miR-409 has a positive or a negative impact over EMT and metastasis in more human cancers, and if it could be taken into consideration as a future miRNA therapy target.

Table 2 comprises a set of microRNAs that were evaluated both *in vitro* and *in vivo*, while also being analyzed in tumor samples, in correlation with the clinical outcome of the patients. As it can be easily observed, the effect of microRNAs in modulating EMT and metastasis-related pathways varies in different cancer types. In certain situations, the same microRNA can induce completely opposite outcomes by targeting multiple signaling pathways. This would be one of the biggest challenges that need to be overcome when designing new microRNA-based therapeutic compounds, and it is, at the same time, an interesting research niche worth exploring, especially for miR-625, that maintained its anti-EMT function in three different cancer types (hepatocellular carcinoma, gastric cancer, and colorectal cancer) and miR-409 that is able to target multiple pathways with opposing effects in NSCLC, breast cancer, and prostate cancer.

7. Conclusions

miRNAs represent key modulators of the human genome because of their capacity to affect up to 60% of protein-coding genes. In cancer, genetic and epigenetic events lead to the alteration of miRNA expression and consequently their mRNA target genes. Functional studies have demonstrated that miRNA modulation in tumor cells causes changes in the phenotype, leading to increased apoptosis and cell death, suppression of tumor development, invasion, and metastasis by inhibiting the oncogenic miRNAs (oncomiRs) and/or substituting the deficient tumor suppressive miRNAs (TS-miRNAs). Considering the encouraging preclinical and clinical data, miRNA-based therapy could become a reliable tool in cancer management.

Acknowledgements

The work for this chapter was supported by the UEFISCDI Program-PN-III-P2-2.1-PED-2016-1750.

Conflict of interest

The authors declare no conflict of interest.

Author details

Ovidiu Balacescu[1,2*], Simona Visan[1], Oana Baldasici[1,3], Loredana Balacescu[1,2], Catalin Vlad[4,5] and Patriciu Achimas-Cadariu[4,5]

1 Department of Functional Genomics, Proteomics and Experimental Pathology, The Oncology Institute "Prof. Dr. Ion Chiricuta", Cluj-Napoca, Romania

2 Department of Medical Oncology, University of Medicine and Pharmacy "Iuliu Hatieganu", Cluj-Napoca, Romania

3 Faculty of Pharmacy, University of Medicine and Pharmacy "Iuliu Hatieganu", Cluj-Napoca, Romania

4 Department of Surgical and Gynecological Oncology, University of Medicine and Pharmacy "Iuliu Hatieganu", Cluj-Napoca, Romania

5 Department of Surgery, The Oncology Institute "Prof. Dr. Ion Chiricuta", Cluj-Napoca, Romania

*Address all correspondence to: obalacescu@yahoo.com

IntechOpen

References

[1] Society AC. Global Cancer Facts & Figures. 3rd ed. Atlanta: American Cancer Society; 2015

[2] Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. International Journal of Cancer. 2015;**136**(5):E359-E386

[3] Chaffer CL, Weinberg RA. A perspective on cancer cell metastasis. Science. 2011;**331**(6024):1559-1564

[4] Hanahan D, Weinberg RA. Hallmarks of cancer: The next generation. Cell. 2011;**144**(5):646-674

[5] Polyak K, Weinberg RA. Transitions between epithelial and mesenchymal states: Acquisition of malignant and stem cell traits. Nature Reviews. Cancer. 2009;**9**(4):265-273

[6] Jolly MK, Tripathi SC, Somarelli JA, Hanash SM, Levine H. Epithelial/mesenchymal plasticity: How have quantitative mathematical models helped improve our understanding? Molecular Oncology. 2017;**11**(7):739-754

[7] Pennisi E. Genomics. ENCODE project writes eulogy for junk DNA. Science 2012;**337**(6099):1159-1161

[8] Ambros V. The evolution of our thinking about microRNAs. Nature Medicine. 2008;**14**(10):1036-1040

[9] Kim VN, Han J, Siomi MC. Biogenesis of small RNAs in animals. Nature Reviews. Molecular Cell Biology. 2009;**10**(2):126-139

[10] Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. Cell. 2005;**120**(1):15-20

[11] Krek A, Grun D, Poy MN, Wolf R, Rosenberg L, Epstein EJ, et al. Combinatorial microRNA target predictions. Nature Genetics. 2005;**37**(5):495-500

[12] Coronnello C, Benos PV. ComiR: Combinatorial microRNA target prediction tool. Nucleic Acids Research. 2013;**41**(Web Server issue):W159-W164

[13] Friedman Y, Karsenty S, Linial M. miRror-suite: Decoding coordinated regulation by microRNAs. Database (Oxford). 6 Jun 2014;**2014**:pii:bau043. DOI: 10.1093/database/bau043

[14] Kozomara A, Griffiths-Jones S. miRBase: Annotating high confidence microRNAs using deep sequencing data. Nucleic Acids Research. 2014;**42**(Database issue):D68-D73

[15] Calin GA, Dumitru CD, Shimizu M, Bichi R, Zupo S, Noch E, et al. Frequent deletions and down-regulation of micro-RNA genes miR15 and miR16 at 13q14 in chronic lymphocytic leukemia. Proceedings of the National Academy of Sciences of the United States of America. 2002;**99**(24):15524-15529

[16] Cimmino A, Calin GA, Fabbri M, Iorio MV, Ferracin M, Shimizu M, et al. miR-15 and miR-16 induce apoptosis by targeting BCL2. Proceedings of the National Academy of Sciences of the United States of America. 2005;**102**(39):13944-13949

[17] Calin GA, Liu CG, Sevignani C, Ferracin M, Felli N, Dumitru CD, et al. MicroRNA profiling reveals distinct signatures in B cell chronic lymphocytic leukemias. Proceedings of the National Academy of Sciences of the United States of America. 2004;**101**(32):11755-11760

[18] Lu J, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, et al.

MicroRNA expression profiles classify human cancers. Nature. 2005;**435**(7043):834-838

[19] Weber JA, Baxter DH, Zhang S, Huang DY, Huang KH, Lee MJ, et al. The microRNA spectrum in 12 body fluids. Clinical Chemistry. 2010;**56**(11):1733-1741

[20] Lan H, Lu H, Wang X, Jin H. MicroRNAs as potential biomarkers in cancer: Opportunities and challenges. BioMed Research International. 2015;**2015**:125094

[21] Hata A, Kashima R. Dysregulation of microRNA biogenesis machinery in cancer. Critical Reviews in Biochemistry and Molecular Biology. 2016;**51**(3):121-134

[22] Di Leva G, Garofalo M, Croce CM. MicroRNAs in cancer. Annual Review of Pathology. 2014;**9**:287-314

[23] Croce CM. Causes and consequences of microRNA dysregulation in cancer. Nature Reviews. Genetics. 2009;**10**(10):704-714

[24] Zhang B, Pan X, Cobb GP, Anderson TA. microRNAs as oncogenes and tumor suppressors. Developmental Biology. 2007;**302**(1):1-12

[25] Romero-Cordoba SL, Salido-Guadarrama I, Rodriguez-Dorantes M, Hidalgo-Miranda A. miRNA biogenesis: Biological impact in the development of cancer. Cancer Biology & Therapy. 2014;**15**(11):1444-1455

[26] Berindan-Neagoe I, Monroig Pdel C, Pasculli B, Calin GA. MicroRNAome genome: A treasure for cancer diagnosis and therapy. CA: A Cancer Journal for Clinicians. 2014;**64**(5):311-336

[27] Shah MY, Ferrajoli A, Sood AK, Lopez-Berestein G, Calin GA. microRNA therapeutics in cancer—An emerging concept. eBioMedicine. 2016;**12**:34-42

[28] Garzon R, Marcucci G, Croce CM. Targeting microRNAs in cancer: Rationale, strategies and challenges. Nature Reviews. Drug Discovery. 2010;**9**(10):775-789

[29] Vester B, Wengel J. LNA (locked nucleic acid): High-affinity targeting of complementary RNA and DNA. Biochemistry. 2004;**43**(42):13233-13241

[30] Elmen J, Lindow M, Schutz S, Lawrence M, Petri A, Obad S, et al. LNA-mediated microRNA silencing in non-human primates. Nature. 2008;**452**(7189):896-899

[31] Krützfeldt J, Rajewsky N, Braich R, Rajeev KG, Tuschl T, Manoharan M, et al. Silencing of microRNAs in vivo with 'antagomirs'. Nature. 2005;**438**(7068):685-689

[32] Ebert MS, Neilson JR, Sharp PA. MicroRNA sponges: Competitive inhibitors of small RNAs in mammalian cells. Nature Methods. 2007;**4**(9):721-726

[33] Monroig Pdel C, Chen L, Zhang S, Calin GA. Small molecule compounds targeting miRNAs for cancer therapy. Advanced Drug Delivery Reviews. 2015;**81**:104-116

[34] Griveau A, Bejaud J, Anthiya S, Avril S, Autret D, Garcion E. Silencing of miR-21 by locked nucleic acid-lipid nanocapsule complexes sensitize human glioblastoma cells to radiation-induced cell death. International Journal of Pharmaceutics. 2013;**454**(2):765-774

[35] Bader AG, Brown D, Winkler M. The promise of microRNA replacement therapy. Cancer Research. 2010;**70**(18):7027-7030

[36] Kota J, Chivukula RR, O'Donnell KA, Wentzel EA, Montgomery CL, Hwang HW, et al. Therapeutic microRNA delivery

suppresses tumorigenesis in a murine liver cancer model. Cell. 2009;**137**(6):1005-1017

[37] Zhang J, Li S, Li L, Li M, Guo C, Yao J, et al. Exosome and exosomal microRNA: Trafficking, sorting, and function. Genomics, Proteomics & Bioinformatics. 2015;**13**(1):17-24

[38] Fabbri M, Paone A, Calore F, Galli R, Gaudio E, Santhanam R, et al. MicroRNAs bind to toll-like receptors to induce prometastatic inflammatory response. Proceedings of the National Academy of Sciences of the United States of America. 2012;**109**(31):E2110-E2116

[39] Aagaard L, Rossi JJ. RNAi therapeutics: Principles, prospects and challenges. Advanced Drug Delivery Reviews. 2007;**59**(2-3):75-86

[40] Bakhshandeh B, Soleimani M, Hafizi M, Ghaemi N. A comparative study on nonviral genetic modifications in cord blood and bone marrow mesenchymal stem cells. Cytotechnology. 2012;**64**(5):523-540

[41] Hosseinahli N, Aghapour M, Duijf PHG, Baradaran B. Treating cancer with microRNA replacement therapy: A literature review. Journal of Cellular Physiology. 2018;**233**(8):5574-5588

[42] Ibrahim AF, Weirauch U, Thomas M, Grunweller A, Hartmann RK, Aigner A. MicroRNA replacement therapy for miR-145 and miR-33a is efficacious in a model of colon carcinoma. Cancer Research. 2011;**71**(15):5214-5224

[43] Kaban K, Salva E, Akbuga J. The effects of chitosan/miR-200c nanoplexes on different stages of cancers in breast cancer cell lines. European Journal of Pharmaceutical Sciences. 2016;**95**:103-110

[44] Kaban K, Salva E, Akbuga J. In vitro dose studies on chitosan nanoplexes for microRNA delivery in breast cancer cells. Nucleic Acid Therapeutics. 2017;**27**(1):45-55

[45] Hao Z, Fan W, Hao J, Wu X, Zeng GQ, Zhang LJ, et al. Efficient delivery of micro RNA to bone-metastatic prostate tumors by using aptamer-conjugated atelocollagen in vitro and in vivo. Drug Delivery. 2016;**23**(3):874-881

[46] Trang P, Medina PP, Wiggins JF, Ruffino L, Kelnar K, Omotola M, et al. Regression of murine lung tumors by the let-7 microRNA. Oncogene. 2010;**29**(11):1580-1587

[47] Trang P, Wiggins JF, Daige CL, Cho C, Omotola M, Brown D, et al. Systemic delivery of tumor suppressor microRNA mimics using a neutral lipid emulsion inhibits lung tumors in mice. Molecular Therapy. 2011;**19**(6):1116-1122

[48] Dalmay T, Edwards DR. MicroRNAs and the hallmarks of cancer. Oncogene. 2006;**25**(46):6170-6175

[49] Nicoloso MS, Spizzo R, Shimizu M, Rossi S, Calin GA. MicroRNAs—The micro steering wheel of tumour metastases. Nature Reviews. Cancer. 2009;**9**(4):293-302

[50] Valeri N, Gasparini P, Fabbri M, Braconi C, Veronese A, Lovat F, et al. Modulation of mismatch repair and genomic stability by miR-155. Proceedings of the National Academy of Sciences of the United States of America. 2010;**107**(15):6982-6987

[51] Guessous F, Alvarado-Velez M, Marcinkiewicz L, Zhang Y, Kim J, Heister S, et al. Oncogenic effects of miR-10b in glioblastoma stem cells. Journal of Neuro-Oncology. 2013;**112**(2):153-163

[52] Ma L, Reinhardt F, Pan E, Soutschek J, Bhat B, Marcusson EG, et al. Therapeutic silencing of miR-10b inhibits metastasis in a mouse mammary tumor model. Nature Biotechnology. 2010;**28**(4):341-347

[53] Callegari E, Elamin BK, Giannone F, Milazzo M, Altavilla G, Fornari F, et al. Liver tumorigenicity promoted by microRNA-221 in a mouse transgenic model. Hepatology. 2012;**56**(3):1025-1033

[54] Qin J, Luo M. MicroRNA-221 promotes colorectal cancer cell invasion and metastasis by targeting RECK. FEBS Letters. 2014;**588**(1):99-104

[55] Brognara E, Fabbri E, Aimi F, Manicardi A, Bianchi N, Finotti A, et al. Peptide nucleic acids targeting miR-221 modulate p27Kip1 expression in breast cancer MDA-MB-231 cells. International Journal of Oncology. 2012;**41**(6):2119-2127

[56] Gallo Cantafio ME, Nielsen BS, Mignogna C, Arbitrio M, Botta C, Frandsen NM, Rolfo C, Tagliaferri P, Tassone P, Di Martino MT. Pharmacokinetics and pharmacodynamics of a 13-mer LNA-inhibitor-miR-221 in mice and non-human primates. Molecular Therapy Nucleic Acids. 21 Jun 2016;**5**(6). DOI: 10.1038/mtna.2016.36

[57] Chang TC, Wentzel EA, Kent OA, Ramachandran K, Mullendore M, Lee KH, et al. Transactivation of miR-34a by p53 broadly influences gene expression and promotes apoptosis. Molecular Cell. 2007;**26**(5):745-752

[58] Chang TC, Yu D, Lee YS, Wentzel EA, Arking DE, West KM, et al. Widespread microRNA repression by Myc contributes to tumorigenesis. Nature Genetics. 2008;**40**(1):43-50

[59] Lodygin D, Tarasov V, Epanchintsev A, Berking C, Knyazeva T, Korner H, et al. Inactivation of miR-34a by aberrant CpG methylation in multiple types of cancer. Cell Cycle. 2008;**7**(16):2591-2600

[60] He L, He X, Lim LP, de Stanchina E, Xuan Z, Liang Y, et al. A microRNA component of the p53 tumour suppressor network. Nature. 2007;**447**(7148):1130-1134

[61] Gallardo E, Navarro A, Vinolas N, Marrades RM, Diaz T, Gel B, et al. miR-34a as a prognostic marker of relapse in surgically resected non-small-cell lung cancer. Carcinogenesis. 2009;**30**(11):1903-1909

[62] O'Day E, Lal A. MicroRNAs and their target gene networks in breast cancer. Breast Cancer Research. 2010;**12**(2):201

[63] Corney DC, Hwang CI, Matoso A, Vogt M, Flesken-Nikitin A, Godwin AK, et al. Frequent downregulation of miR-34 family in human ovarian cancers. Clinical Cancer Research. 2010;**16**(4):1119-1128

[64] Girardi C, De Pitta C, Casara S, Sales G, Lanfranchi G, Celotti L, et al. Analysis of miRNA and mRNA expression profiles highlights alterations in ionizing radiation response of human lymphocytes under modeled microgravity. PLoS One. 2012;**7**(2):e31293

[65] Josson S, Sung SY, Lao K, Chung LW, Johnstone PA. Radiation modulation of microRNA in prostate cancer cell lines. The Prostate. 2008;**68**(15):1599-1606

[66] Nikiforova MN, Gandhi M, Kelly L, Nikiforov YE. MicroRNA dysregulation in human thyroid cells following exposure to ionizing radiation. Thyroid. 2011;**21**(3):261-266

[67] Daige CL, Wiggins JF, Priddy L, Nelligan-Davis T, Zhao J, Brown D. Systemic delivery of a miR34a mimic

as a potential therapeutic for liver cancer. Molecular Cancer Therapeutics. 2014;**13**(10):2352-2360

[68] Bader AG. miR-34—A microRNA replacement therapy is headed to the clinic. Frontiers in Genetics. 2012;**3**:120

[69] Bandi N, Zbinden S, Gugger M, Arnold M, Kocher V, Hasan L, et al. miR-15a and miR-16 are implicated in cell cycle regulation in a Rb-dependent manner and are frequently deleted or down-regulated in non-small cell lung cancer. Cancer Research. 2009;**69**(13):5553-5559

[70] Bonci D, Coppola V, Musumeci M, Addario A, Giuffrida R, Memeo L, et al. The miR-15a-miR-16-1 cluster controls prostate cancer by targeting multiple oncogenic activities. Nature Medicine. 2008;**14**(11):1271-1277

[71] Reid G, Pel ME, Kirschner MB, Cheng YY, Mugridge N, Weiss J, et al. Restoring expression of miR-16: A novel approach to therapy for malignant pleural mesothelioma. Annals of Oncology. 2013;**24**(12):3128-3135

[72] Takeshita F, Patrawala L, Osaki M, Takahashi RU, Yamamoto Y, Kosaka N, et al. Systemic delivery of synthetic microRNA-16 inhibits the growth of metastatic prostate tumors via downregulation of multiple cell-cycle genes. Molecular Therapy. 2010;**18**(1):181-187

[73] Kluiver J, Poppema S, de Jong D, Blokzijl T, Harms G, Jacobs S, et al. BIC and miR-155 are highly expressed in Hodgkin, primary mediastinal and diffuse large B cell lymphomas. The Journal of Pathology. 2005;**207**(2):243-249

[74] Calin GA, Ferracin M, Cimmino A, Di Leva G, Shimizu M, Wojcik SE, et al. A MicroRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. The New England Journal of Medicine. 2005;**353**(17):1793-1801

[75] Yanaihara N, Caplen N, Bowman E, Seike M, Kumamoto K, Yi M, et al. Unique microRNA molecular profiles in lung cancer diagnosis and prognosis. Cancer Cell. 2006;**9**(3):189-198

[76] Iorio MV, Ferracin M, Liu CG, Veronese A, Spizzo R, Sabbioni S, et al. MicroRNA gene expression deregulation in human breast cancer. Cancer Research. 2005;**65**(16):7065-7070

[77] Garzon R, Volinia S, Liu CG, Fernandez-Cymering C, Palumbo T, Pichiorri F, et al. MicroRNA signatures associated with cytogenetics and prognosis in acute myeloid leukemia. Blood. 2008;**111**(6):3183-3189

[78] Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, et al. A microRNA expression signature of human solid tumors defines cancer gene targets. Proceedings of the National Academy of Sciences of the United States of America. 2006;**103**(7):2257-2261

[79] Philippidou D, Schmitt M, Moser D, Margue C, Nazarov PV, Muller A, et al. Signatures of microRNAs and selected microRNA target genes in human melanoma. Cancer Research. 2010;**70**(10):4163-4173

[80] Gasparini P, Lovat F, Fassan M, Casadei L, Cascione L, Jacob NK, et al. Protective role of miR-155 in breast cancer through RAD51 targeting impairs homologous recombination after irradiation. Proceedings of the National Academy of Sciences of the United States of America. 2014;**111**(12):4536-4541

[81] Pouliot LM, Chen YC, Bai J, Guha R, Martin SE, Gottesman MM, et al.

Cisplatin sensitivity mediated by WEE1 and CHK1 is mediated by miR-155 and the miR-15 family. Cancer Research. 2012;**72**(22):5945-5955

[82] Hoshino A, Costa-Silva B, Shen TL, Rodrigues G, Hashimoto A, Tesic Mark M, et al. Tumour exosome integrins determine organotropic metastasis. Nature. 2015;**527**(7578):329-335

[83] David S, Hong Y-KK, Brenner AJ, Sachdev JC, Ejadi S, Borad MJ, et al. MRX34, a liposomal miR-34 mimic, in patients with advanced solid tumors: Final dose-escalation results from a first-in-human phase I trial of microRNA therapy. Journal of Clinical Oncology. 2016;**34**(15_suppl):2508

[84] Cortez MA, Ivan C, Valdecanas D, Wang X, Peltier HJ, Ye Y, et al. PDL1 regulation by p53 via miR-34. Journal of the National Cancer Institute. 17 Nov 2015;**108**(1):pii:djv303. DOI: 10.1093/jnci/djv303

[85] Reid GW M, Kirschner MB, Mugridge N, Weiss J, Brahmbhatt H, et al. Targeted delivery of a synthetic microRNA-based mimic as an approach to cancer therapy. Cancer Research. AACR; 2015;**75**(15 Suppl):Abstract nr 3976. DOI: 10.1158/1538-7445.AM2015-3976

[86] Van Zandwijk N, Pavlakis N, Kao S, Clarke S, Lee A, Brahmbhatt H, et al. MesomiR 1: A phase I study of TargomiRs in patients with refractory malignant pleural mesothelioma (MPM) and lung cancer (NSCLC). Annals of Oncology 2015;**26**(suppl_2):ii16

[87] Christopher AF, Kaur RP, Kaur G, Kaur A, Gupta V, Bansal P. MicroRNA therapeutics: Discovering novel targets and developing specific therapy. Perspectives in Clinical Research. 2016;**7**(2):68-74

[88] Rupaimoole R, Slack FJ. MicroRNA therapeutics: Towards a new era for the management of cancer and other diseases. Nature Reviews. Drug Discovery. 2017;**16**(3):203-222

[89] Rupaimoole R, Calin GA, Lopez-Berestein G, Sood AK. miRNA deregulation in cancer cells and the tumor microenvironment. Cancer Discovery. 2016;**6**(3):235-246

[90] Peinado H, Zhang H, Matei IR, Costa-Silva B, Hoshino A, Rodrigues G, et al. Pre-metastatic niches: Organ-specific homes for metastases. Nature Reviews. Cancer. 2017;**17**(5):302-317

[91] Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. Nature Reviews. Molecular Cell Biology. 2014;**15**(3):178-196

[92] Vuoriluoto K, Haugen H, Kiviluoto S, Mpindi JP, Nevo J, Gjerdrum C, et al. Vimentin regulates EMT induction by Slug and oncogenic H-Ras and migration by governing Axl expression in breast cancer. Oncogene. 2011;**30**(12):1436-1448

[93] Zheng H, Kang Y. Multilayer control of the EMT master regulators. Oncogene. 2014;**33**(14):1755-1763

[94] Li BL, Lu W, Qu JJ, Ye L, Du GQ, Wan XP. Loss of exosomal miR-148b from cancer-associated fibroblasts promotes endometrial cancer cell invasion and cancer metastasis. Journal of Cellular Physiology. 26 Aug 2018. DOI: 10.1002/jcp.27111. [Epub ahead of print]

[95] Ren D, Yang Q, Dai Y, Guo W, Du H, Song L, et al. Oncogenic miR-210-3p promotes prostate cancer cell EMT and bone metastasis via NF-kappaB signaling pathway. Molecular Cancer. 2017;**16**(1):117

[96] Tang T, Yang Z, Zhu Q, Wu Y, Sun K, Alahdal M, et al. Up-regulation of miR-210 induced by a hypoxic microenvironment promotes breast cancer stem cells metastasis, proliferation, and self-renewal by targeting E-cadherin. FASEB Journal. 6 Sep 2018:fj201801013R. DOI: 10.1096/fj.201801013R. [Epub ahead of print]

[97] Deng S, Li X, Niu Y, Zhu S, Jin Y, Deng S, et al. MiR-652 inhibits acidic microenvironment-induced epithelial-mesenchymal transition of pancreatic cancer cells by targeting ZEB1. Oncotarget. 2015;**6**(37):39661-39675

[98] Wang M, Li C, Nie H, Lv X, Qu Y, Yu B, et al. Down-regulated miR-625 suppresses invasion and metastasis of gastric cancer by targeting ILK. FEBS Letters. 2012;**586**(16):2382-2238

[99] Lou X, Qi X, Zhang Y, Long H, Yang J. Decreased expression of microRNA-625 is associated with tumor metastasis and poor prognosis in patients with colorectal cancer. Journal of Surgical Oncology. 2013;**108**(4):230-235

[100] Sun X, Dongol S, Qiu C, Xu Y, Sun C, Zhang Z, et al. miR-652 promotes tumor proliferation and metastasis by targeting RORA in endometrial cancer. Molecular Cancer Research. 9 Aug 2018. DOI: 10.1158/1541-7786.MCR-18-0267. [Epub ahead of print]

[101] Cai J, Guan H, Fang L, Yang Y, Zhu X, Yuan J, et al. MicroRNA-374a activates Wnt/beta-catenin signaling to promote breast cancer metastasis. The Journal of Clinical Investigation. 2013;**123**(2):566-579

[102] Gao J, Yu SR, Yuan Y, Zhang LL, Lu JW, Feng JF, Hu SN. MicroRNA-590-5p functions as a tumor suppressor in breast cancer conferring inhibitory effects on cell migration, invasion, and epithelial-mesenchymal transition by downregulating the Wnt-β-catenin signaling pathway. Journal of Cellular Physiology. 7 Sep 2018. DOI: 10.1002/jcp.27056. [Epub ahead of print]

[103] Zhou X, Zhang CZ, Lu SX, Chen GG, Li LZ, Liu LL, et al. miR-625 suppresses tumour migration and invasion by targeting IGF2BP1 in hepatocellular carcinoma. Oncogene. 2015;**34**(8):965-977

[104] Song Q, Ji Q, Xiao J, Li F, Wang L, Chen Y, Xu Y, Jiao S. miR-409 inhibits human non-small-cell lung cancer progression by directly targeting SPIN1. Molecular Therapy Nucleic Acids. 1 Sep 2018;**13**:154-163. DOI: 10.1016/j.omtn.2018.08.020. [Epub ahead of print]

[105] Zhang G, Liu Z, Xu H, Yang Q. miR-409-3p suppresses breast cancer cell growth and invasion by targeting Akt1. Biochemical and Biophysical Research Communications. 2016;**469**(2):189-195

[106] Josson S, Gururajan M, Hu P, Shao C, Chu GY, Zhau HE, et al. miR-409-3p/−5p promotes tumorigenesis, epithelial-to-mesenchymal transition, and bone metastasis of human prostate cancer. Clinical Cancer Research. 2014;**20**(17):4636-4646

[107] Josson S, Gururajan M, Sung SY, Hu P, Shao C, Zhau HE, et al. Stromal fibroblast-derived miR-409 promotes epithelial-to-mesenchymal transition and prostate tumorigenesis. Oncogene. 2015;**34**(21):2690-2699

[108] Yu H, Xing H, Han W, Wang Y, Qi T, Song C, et al. MicroRNA-409-5p is upregulated in breast cancer and its downregulation inhibits cancer development through downstream target of RSU1. Tumour Biology. 2017;**39**(5):1010428317701647