MicroRNAs as Therapeutic Targets for Cancer

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1 Introduction

MicroRNAs (miRNAs) are a recently discovered family of endogenous, non-coding RNA molecules approximately 22 nt in length [1]. They negatively modulate gene expression post-transcriptionally by binding to the complementary sequence in the 3′ untranslated region of target messenger RNAs (mRNAs) [1]. miRNAs are transcribed from genomic DNA by RNA polymerase II but not further translated into protein (non-coding RNA). Eventually, they are processed from primary transcripts known as pri-miRNAs to short stem-loop structures called pre-miRNA and finally to become functionally mature miRNA. Mature miRNA molecules are partially complimentary to target mRNA where they either repress translation or direct destructive cleavage [2]. The first miRNA was described in 1993 by Lee and colleagues, who found miRNA-*lin-4* is essential for the normal temporal control of diverse post-embryonic development in *Caenorhabditis elegans* by negatively regulating the level of LIN-14 protein via antisense RNA–RNA interaction [3]. miRNAs have a large-scale effect as a new layer of gene regulation mechanism. It has been estimated that the vertebrate genome encodes up to 1000 unique miRNAs, which can regulate expression of at least 30% of genes [4, 5].

Cancer is an intricate genetic disease attributed to the breakdown of gene regulatory networks governing the balance between oncogenes and tumor-suppressor genes. That cancer results from a deregulation of this highly regulated network suggests the use of gene modulation as a therapeutic approach for treating cancer. To date, several approaches such as antisense oligonucleotides (ODN), aptamers, ribozymes, and small interfering RNAs (siRNAs) have been explored as tools for modulating the production of aberrant proteins. Recent evidence indicates that miRNAs play an important role in cancer pathogenesis by functioning as novel oncogenes or tumor-suppressor genes and altered

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Y. Lu, R.I. Mahato (eds.), *Pharmaceutical Perspectives of Cancer Therapeutics*,
DOI 10.1007/978-1-4419-0131-6_14, © Springer Science+Business Media, LLC 2009

expressions of specific miRNA genes have been identified as signatures for certain cancer types [6– 8]. Since miRNAs are powerful regulators of gene expression and can regulate more than one target, they have the potential to serve as a new class of therapeutic targets for treating cancer by artificially manipulating their expression levels.

This chapter highlights the role of miRNAs in the initiation and progression of human cancer, their utility as diagnostics and prognostics and focuses on the potential of miRNAs as therapeutic targets for cancer treatment. We also describe the current strategies for modulating miRNAs to exploit them as therapeutics.

2 Gene Regulation

2.1 Type of Nucleic Acids Used for Gene Modulation

One of the major developments in modern biomedical research resulting from the fields of molecular biology and genetics is gene modulation-based gene therapy. During the past 30 years, several approaches of gene regulation have been developed to artificially alter the expression of a given gene (Fig. 1). These include antisense oligodeoxynucleotides (ODN), triplex-

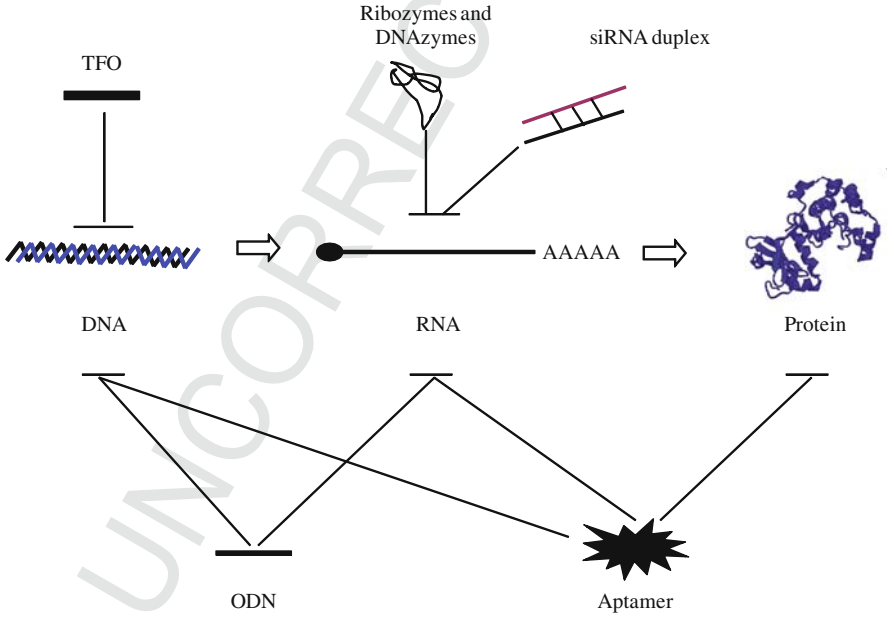

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Fig. 1 Schematic representation of gene modulation by TFO, ODN, Ribozymes, DNAzymes, siRNA duplex, and aptamer

forming oligonucleotides (TFOs), aptamers, ribozymes and DNAzymes, and small interfering RNA (siRNA). Among them, aptamers can regulate endogenous gene expression at both genomic and transcription levels [9]. TFOs and ODNs can exert their functions through binding the promoter region or open reading frame of target regions [10]. Ribozymes and DNAzymes can catalytically cleave the target gene [9]. All current approaches for gene regulation have been applied to selectively turn off specific genes in diseased tissue. This blockage of active target molecules by gene regulation has a potential to be developed into efficacious gene therapies for human diseases. Recently, RNA interference (RNAi), which is small interfering RNA (siRNA)-mediated specific gene silencing has become an important tool for analyzing gene function at the transcription level [11] and also provides a potentially therapeutic tool for treating genetic or acquired disease [12].

2.2 RNA Interference

RNAi was first described in *C. elegans* in 1998 by Andrew Fire and Craig C. Mello, who demonstrated substantially more effective gene silencing using dsRNA than either strand individually [11]. As per RNAi, introduction of dsRNA into organism results into the 21- or 22-nt dsRNA fragments that bear 2- or 3-nt 3′ overhangs upon cleavage by Dicer. These 21-nt dsRNAs, which are referred to as siRNAs, are then selectively incorporated into a RNA-induced silencing complex (RISC). Then a RISC undergoes an ATP-dependent activation step that involves unwinding of the double-stranded siRNA component to give a single-stranded guide RNA that targets RISC to homologous mRNAs. After mRNA binding, a RISC cleaves the target mRNA at the center of the region that is complementary to the guide RNA. dsRNAs can be produced by first chemical synthesis and the following in vitro annealing or are transcribed by plasmid or viral vectors encoding short hairpin RNA (shRNA). Due to its high specificity and efficiency, extensive efforts have led to the development of this technique as a potentially therapeutic strategy through gene regulation at the transcription level.

2.3 miRNA Versus siRNA

Both siRNA and miRNA are small RNAs of 18–25 nt in length that exert their functions by incorporating into related RISCs (Fig. 2). However, unlike siRNA, mature miRNAs exist as hairpin structures in the cytoplasm after undergoing two processing steps. In the nucleus, miRNAs are transcribed by RNA polymerase II into pri-miRNAs which are large precursor RNAs, often several kilobases long. These transcripts are then processed by an RNase III enzyme Drosha and its double-stranded binding domain protein Pasha into

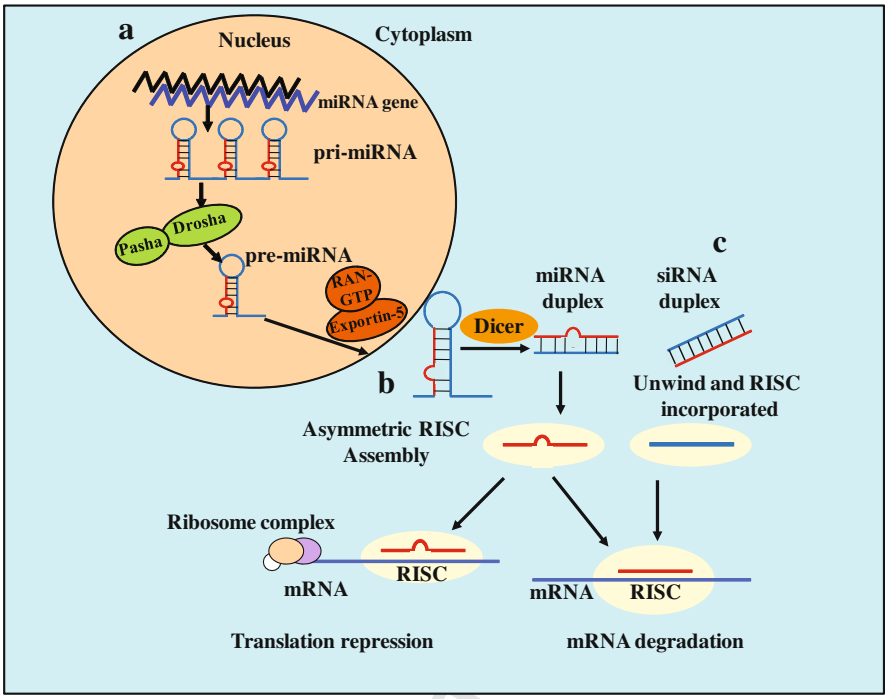

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Fig. 2 The biogenesis and function of miRNAs and siRNA pathway. (**a**) The primary miRNA (pri-miRNA) is transcribed from genome by RNA polymerase II, then is processed into precursor miRNA (pre-miRNA) by the Drosha/Pasha microprocessor complex and exported to cytoplasm by exportin-5. (**b**) pre-miRNA becomes a bioactive miRNA duplex upon removing the loop by Dicer. The miRNA duplex is unwinded into single-stranded mature miRNA, which is subsequently incorporated into related RISC. Asymmetric RISC including mature miRNA inhibits the translation of the target gene or cleaves mRNA of target gene. (**c**) Upon arrival in the cytoplasm, siRNA duplex is unwinded and the guide siRNA strand is incorporated into related RISC. The RISC specifically degrades the mRNA of target gene

~70 nt long stem-loop structures known as pre-miRNAs [13– 15]. The pre-miRNAs are then exported out of the nucleus by the GTP-driven exportin 5 transporter and further processed by the RNase III Dicer-TRBP microprocessor complex in the cytoplasm [16– 18]. This results in the release of a double-stranded RNA duplex composed of the mature miRNA bound to its complementary strand. The mature miRNA strand is separated from its complement due to differences in thermodynamic stability at the 5′ end and loaded into the RNA-induced silencing complex (RISC) where it has the capacity to regulate target genes; the unused strand is degraded [19]. The bound mRNAs either remain untranslated resulting in a decrease in the proteins they encode or are degraded by the RISC resulting in a decreased number of transcripts. miRNAs are involved in numerous cellular processes including development, differentiation, proliferation, apoptosis, and the stress response [20]. As to

siRNA-mediated RNAi, it is an evolutionary mechanism for protecting the genome against invasion by mobile genetic elements such as transposons and viruses. siRNAs directly trigger a sequence-specific post-transcriptional degradation of homologous genes by binding to its complementary mRNA, which is not limited to 3′ UTR. The siRNA sequence is required to strictly complement with target mRNA. It is recently noted that siRNA-mediated gene silencing could induce unwanted off-target effect and immune response.

3 Identification of miRNA Targets

As mentioned above, miRNAs function by binding to complementary sites on target mRNAs to induce cleavage or repression of productive translation. As such, identification of miRNA targets is a key step for analyzing miRNA function in organisms. Up to now, some miRNA targets have been identified and their functions assigned. For example, the *lin-4* and *let-7* miRNAs control developmental timing in *C. elegans* [21, 22]; *lsy-6* miRNA regulates left–right asymmetry in the nervous system [23]; *bantam* miRNA controls tissue growth [24]; *bantam* and *miR-14* control apoptosis [24]; *miR-181* is involved in hematopoietic differentiation [25]; *miR-375* regulates insulin secretion [26]; and *miR-373* and *miR-520c* stimulate cancer cell migration and invasion [27].

Computational prediction of miRNA targets provides an alternative approach to assigning biological functions of miRNAs. Several softwares with different governing algorithms have been developed and applied for prediction of a large number of targets genes for miRNA as listed in Table 1. In the following section, we introduce some of the softwares used for the prediction of miRNA targets. TargetScan is an algorithm developed by Lewis et al. for predicting miRNA targets in vertebrates [28]. Using this software, genes involved in transcriptional regulation were enriched even though the functions of the predicted target genes encompassed a broad range of activities. TargetScans criterion for target detection involves the existence of perfect complementarity to the "seed-region" of miRNA and the degree of complementarity of surrounding regions. Later, the authors improved the TargetScan algorithm and developed it into TargetScanS [5]. This updated software successfully predicated all of the known miRNA–target interaction and has been used in the prediction of over 5300 human genes as potential targets of miRNAs. The miRanda software was initially designed to predict miRNA target genes in *Drosophila melanogaster* [29] and utilizes dynamic programming to rapidly identify sites with a high-degree of miRNA complementarity. This software was also applied for prediction of human miRNA in targets [29, 30]. About 2000 putative human miRNA target genes were identified, suggesting that 10% or more of human genes are regulated by miRNAs. The RNAhybrid program presented by Rehmsmeier et al., which is the identification target miRNA based on energetically optimal binding sites for a small RNA within a large RNA sequences [31], can predict known and new

Table 1 List of some softwares used for predicting miRNA target

Name	Website	Applied proposes
TargetScan	http://www.targetscan.org/	Worm/fly/mammalian
miRanda	http://www.microrna.org/microrna/getGeneForm.do	Human/fly/zebrafish
PicTar	http://pictar.bio.nyu.edu/	Vertebrates/fly/nematode
RNAhybrid	http://bibiserv.techfak.uni-bielefeld.de/rnahybrid/	Worm
MicroTar	http://tiger.dbs.nus.edu.sg/microtar/	Worm/fly/mouse
ViTa	http://vita.mbc.nctu.edu.tw/	Virus
miRU	http://bioinfo3.noble.org/miRU.htm	Plants
RNAhybrid	http://bibiserv.techfak.uni-bielefeld.de/rnahybrid/	C. elegans
STarMir	http://sfold.wadsworth.org/starmir.pl	Worm/mammalian

miRNA targets. Because cross-species comparisons provide powerful criteria for identifying miRNA target genes, Kerk et al. developed Pic Tar algorithm to compare a group of orthologous 3′UTR from multiple species and retain the 3′UTRs with seeding match for miRNAs [32]. Then those candidate targets were further filtered according to their thermodynamic stability. They utilized this method to predict vertebrate miRNA targets and suggested that about 200 transcripts are regulated by a single miRNA. Yousef et al. also presented a machine learning approach for predicting miRNA target site based on the naïve Bayer (NB) classifiers [33]. They used the classifier as a filter for the output of the miRanda tool and demonstrated that the filtering step decreases the false-positive prediction by miRanda significantly. Calculation of mRNA secondary structures and favorable hybridization between miRNA and target mRNA can also be used to predict miRNA target such as RNAhybrid [31] and STarMir [34]. The microRNA.org site (http://www.microrna.org) is a resource for miRNA target predictions and miRNA expression that is widely used by the research community [35]. Other annotated miRNA databases such as miRBase [36], miRGen [37], Argonaute [38], miRNAMap [39], and smiRNAdb [40] could also provide some valuable information for predicting miRNA target.

Experimental validation of predicated miRNA targets is crucial for understanding miRNA functions as well as the biological significance of results from computational prediction. This is necessary since computational methods are not warranted due to the associated risk of false-positive prediction. Although experimental validation of miRNA target genes is challenging compared to computational validation, more and more miRNA target genes from various species have been identified using a combination of computational and biological approaches. Experimental validation is performed against two types of predicted miRNA targets that have different regulatory mechanisms: translational repression of target mRNAs and cleavage of target mRNAs. Methods such as reporter gene [41– 43], gene mutation [44– 46], rapid amplification of 5′ complementary DNA end (5′ RACE) [47], and proteomics [48] have been

applied for experimental validation of computationally predicated miRNA targets that are translationally regulated. In addition, microarray analysis, which provides a powerful and high-throughput method for observing cleaved target mRNAs, was also used to identify a large number of human miRNA targets that appear to be cleaved by miRNAs [49]. Recently, German et al. directly sequenced miRNA–mRNA pairs and identified miRNA targets from the mRNA cleaved site, which provided a novel method for predicting miRNA target [50]. However, there is no clear agreement as to which experimental procedures should be followed to demonstrate a predicated miRNA target is a target of specific miRNA. It is therefore necessary to experimentally confirm predicated targets by using a combination of techniques.

4 Roles of miRNAs in Cancer

4.1 miRNA Profile in Cancer

Several studies have shown that miRNAs are involved in cancers since they regulate the expression of genes responsible for cell growth and apoptosis [6, 7]. Calin and coworkers were the first to provide evidence regarding the involvement of miRNAs in cancers based on a study characterizing the frequently deleted and downregulated chromosome 13q14 in human chronic lymphocytic leukemia (CLL) [51]. They observed that this chromosome which is deleted in a majority of B-cell chronic lymphocytic leukemia contained two miRNAs: miR-15 and miR-16. Subsequently, numerous studies have been undertaken to identify the differentially expressed miRNAs between cancer and normal tissues to further understand their biological functions in tumor development. Because miRNA array technology allows hundreds of miRNAs to be studied simultaneously and enables the observation of altered profiles, investigation of miRNA profiles between cancer and normal tissues were initially carried out by miRNA array to identify miRNAs functions. To date, several microarray-based approaches have been used for miRNA profiling analysis. These include miChip which is a microarray platform for identifying miRNA expression profiles based on locked nucleic acid (LNA) oligonucleotide capture probes [52], an oligonucleotide microarray [53], and a novel bead-based flow cytometric technique designed by Lu et al. Using this novel technique, Lu and colleagues analyzed the expression of 217 miRNAs in 332 tissue samples, including many different types of tumors [54].

The miRNA expression profile seems to be tissue specific: different types of tumors have distinctive patterns of miRNA expression, and the miRNA profiles also reflect the developmental origin of tissues [54] as indicated in Table 2. In the following section, we highlight some representative miRNA profiles in cancer. Pallante et al. analyzed the genome-wide miRNA expression profile in human thyroid papillary carcinomas (PTCs) using a

Table 2 Loss of some miRNA expression profiles in cancer

Tumors/Cells	miRNA profiles	Identified methods	Comments	References
B-cell chronic lymphocytic leukemia (B-CLL)	*MiR-123,miR-220,*and *miR-192* down *MiR-190,miR-183-prec,miR-33,miR-19a,miR-140,miR-123,miR-10b,miR-15b-prec,miR-92-1,miR-188,miR-154,miR-227,miR-101,miR-141-prec,miR-153-prec,miR-196-2,miR-134,miR-141,miR-132,*and *miR-181b-prec* up	Microarray, confirmed by northern blot and real-time RT-PCR	miRNA profiles are associated with biological behavior and prognosis in B-CLL	146
Glioblastoma	*MiR-128,miR-181a,miR-181b,*and *miR-181c* down *MiR-221* up	Microarray, confirmed by northern blot	The alternation of miRNAs is associated with glioblastoma	147
Breast cancer	*miR-125b, miR-145, miR-21,* and *miR-155*down	Microarray, confirmed by northern blot	miRNAs expression correlates with specific breast cancer biopathologic features	115
Hepatocellular carcinoma	*MiR-92,miR-20,miR-18, miR-99a, and miR-18 prec* up	Microarray, confirmed by northern blot	miRNAs are potentially involved in the progress of liver tumor	148
Lung cancer	*MiR-21,miR-191,miR-210,miR-155,miR-205,miR-24-2,miR-212,miR-214,*and *miR-17-3 prec* up *MiR-126,miR-143,miR-192 prec,miR-224,miR-126,miR-30a-5 prec,miR-140,*and *miR-9*down	Microarray, confirmed by real-time RT-PCR	miRNA expression profiles could be diagnostic and prognostic markers of lung cancer	149
Colorectal cancer	*MiR-19a,miR-21,miR-29a,miR-92,miR-148a,*and *miR-200b* up *MiR-30c,miR-133a,*and *miR-145* down	Real-time PCR	miRNA profiles has relevance to the biological and clinical behavior of colorectal neoplasia	58

Table 2 (continued)

Tumors/Cells	miRNA profiles	Identified methods	Comments	References
Pituitary adenomas	*MiR-26a,miR-26b, miR-197,miR-103,miR-103-2,miR-192,miR-149, and miR-24* up *MiR-128a,miR-136,miR-132,miR-223,miR-7-3,let-7a-1,let-7f-1,miR-192-2/3,miR-9-3,miR-7-1,let-7e,miR-212,miR-164,miR-138-2,miR-7-3*,and *miR-100-1/2*down	Microarray, confirmed by real-time RT-PCR	Several differentially expressed miRNAs are involved in cell proliferation and apoptosis	150
Pancreatic cancer	*MiR-221,miR-424,miR-301,miR-100, miR-376a,miR-125b-1,miR-2, miR-16-1,miR-181, miR-92-1, miR-15b, miR-155,let-7f-1, miR-212,miR-107,miR-24*,and *let-7d* up *MiR-345,miR-142 prec*,and *miR-139* down	Real-time PCR, confirmed by northern blot	Differentially expressed miRNAs could be involved in tumorigenesis in pancreatic adenocarcinoma.	151
Thyroid anaplastic carcinomas	*MiR-30d, miR-125b,miR-26a*, and *miR-30a-5 prec* down	Microarray, confirmed by northern blot and real-time PCR	a miRNA signature is associated with ATC and suggest the miRNAs as an important event in thyroid cell transformation	152
Prostate cancer	*Let-7a, b,c,d,g; miR-16; miR-23a,b; miR-26a, miR-92, miR-143,miR-145,miR-195,miR-199, miR-221, miR-222,miR-497,miR-99a, miR-103*,and *miR-125a,b*; down *MiR-202,miR-210,miR-296,miR-320,miR-370,miR-373,miR-498*,and *miR-503* up	Oligonucleotide array, confirmed by northern blot	Differentially expressed miRNAs could become a novel diagnostic and prognostic tools for prostate cancer	57
Ovarian cancer	*MiR-200a,miR-141,miR-200c*,and *miR-200b* up *MiR-199a, miR-140, miR-145*, and *miR-125b1* down	Microarray, confirmed by northern blot and real-time PCR	miRNAs might play a role in the pathogenesis of human epithelial ovarian cancer.	56

Table 2 (continued)

Tumors/Cells	miRNA profiles	Identified methods	Comments	References
Kidney cancer	*MiR-28,miR-185,miR-27,* and *let-7f-2* up	Microarray, confirmed by northern blot and real-time PCR	Differently expressed miRNAs are involved in the development and progression of kidney cancer	153
Bladder cancer	*MiR-223, miR-26b, miR-221,miR-103-1,miR-185,miR-23b,miR-203,miR-17-5p,miR-23a,*and *miR-205* up *MiR-26b* down	Microarray, confirmed by northern blot and real-time PCR	Differently expressed miRNAs are involved in the development and progression of bladder cancer	153
Papillary thyroid carcinoma	*MiR-221,miR-222* ,and *miR-146* up	Microarray, confirmed by northern blot	Upregulated miRNAs are involved in papillary thyroid carcinoma pathogenesis	154

miRNACHIP microarray containing hundreds of human precursor and mature miRNA oligonucleotide probes [55]. Using this approach, they found an aberrant miRNA expression profile that clearly differentiates PTCs from normal thyroid tissues. In particular, a significant increase in *miR-221*, *miR-222*, and *miR-181b* was detected in PTCs in comparison with normal thyroid tissue. They also functionally studied the *miR-221*, and reported a miRNA signature associated with PTCs, and also suggested miRNA deregulation as an important event in thyroid cell transformation. Iorio et al. used microarray to identify miRNA profiles in human ovarian cancer and found that *miR-200a*, *miR-141*, *miR-200c*, and *miR-200b* were the most significantly overexpressed, whereas *miR-199a*, *miR-140*, *miR-145*, and *miR-125b1* were the most downmodulated miRNAs [56].

Unique miRNA signatures distinguishing between benign tumors and carcinoma tumors has also been established for prostate cancer. For example, Porkka et al. investigated differentially expressed miRNAs between prostate benign tumors and carcinomas tumors using an oligonucleotide array hybridization method. Fifty-one differentially expressed miRNAs were detected, 37 of them were downregulated while 14 miRNAs were upregulated in carcinoma tumors and indicated that those miRNAs could be significant in prostate cancer development and/or growth [57].

Bandrés et al. examined the expression of 156 mature miRNA in colorectal cancer by real-time PCR and the results suggested that miRNA expression profile could be relevant to the biological and clinical behavior of colorectal neoplasia [58]. Michael et al. identified 28 different miRNAs in a colonic adenocarcinoma and normal mucosa by using miRNA cloning and northern blotting. They also indicated that *miR-143* and *miR-145* are downregulated in cells derived from breast, prostate, cervical, and lymphoid cancers as well as colorectal tumors [59].

Because colorectal cancer develops through two differently pathological pathways, Lanza et al. used miRNA microarray chip to investigate different miRNA profiles of colorectal cancer between two differently pathological pathways. They reported the presence of 27 differently expressed gene, including 8 miRNAs and showed that their functions were most frequently associated with cell cycle, DNA replication, recombination, repair, gastrointestinal disease, and immune response [60].

Within a single developmental cell lineage such as acute lymphoblastic leukaemia, distinct patterns of miRNA expression can also be observed that represent different mechanisms of transformation [61]. Zanette et al. determined miRNA expression profile of chronic and acute lymphoblastic leukaemia (CCL and ALL) using TagMan MicroRNA Assay Human Panel [62]. The five most highly expressed miRNAs were *miR-128b*, *miR-204*, *miR-218*, *miR-331*, and *miR-181b-1* in ALL, and *miR-331*, *miR-29a*, *miR-195*, *miR-34a*, and *miR-29c* in CLL [62]. Using cloning and quantitative real-time PCR, *miR-21* and *miR-155* have been confirmed to be highly overexpressed in the patients with CLL [63].

Taken together, miRNA expression profiles in cancer provides important clues for further understanding tumor development/metastases as well as for

diagnosis and prognosis. The levels of some miRNAs are reduced in tumors with poor cell differentiation, reflecting the role of miRNAs in cell differentiation in cancer tissues. Conversely, the levels of some miRNAs are increased in tumors, potentially indicating the role of miRNAs as oncogenes. Consequently, investigation of miRNAs that are enriched in tumor but not normal tissues, or vice versa, may identify miRNA-regulated genes involved in human cancer. If the levels of miRNAs in cancer cells, relative to normal tissues, are different, it is important to identify those genes that are regulated by these miRNAs and to see how these altered expressions influence the development of malignancy. The study of tumor suppressor and oncogene miRNA targets using computational prediction softwares and carrying out experiments for validation should further our understanding of tumor development or metastases. Given the number of miRNAs and genes, it remains to be determined whether each miRNA can target the exact genes and genetic pathways under miRNA control in tumorigenesis.

4.2 miRNA Roles in Tumorigenesis

With extensive investigation of miRNA profiles between cancer and normal tissues, specific miRNAs have been suggested to be associated with tumor initiation, promotion, and progression as called tumorigenesis. Some miRNAs involved in tumorigenesis are summarized in Table 3. *lin-4* and *let-7* which control the timing of fate specification of neuronal and hypodermal cells in *C. elegans* during larval development was the first evidence of miRNAs involvement in cell proliferation [21]. Human *let-7* shows diminished expression in lung tumors, whereas Ras is overexpressed, which is an oncogene regulated by *let-7*miRNA. Moreover, overexpression of *let-7* in lung cancer cell lines decreases growth rates [64]. Recently, a published study by Schultz et al. indicated that the members of *let-7* miRNA family are downregulated in primary melanomas and demonstrated that *let-7b* represses expression of cyclins D1, D3, Cdk4, and CyclinA and consequently effects cell-cycle progression and anchorage-independent growth [65]. Additionally, Shell et al. demonstrated that the expression of *let-7* can define two differentiation stages of cancer using ovarian cancer as a model [66], suggesting that *let-7* is involved in tumor development at the specific manner. It is interesting to note that *let-7* miRNA family can suppress non-small cell development in lung tumor [67], suggesting that *let-7* acts as a tumor suppressor. These studies further provide clear evidences to support that miRNAs regulate tumor development. Sempere et al. observed that the mammalian ortholog of *C. elegans lin-28*, which is downregulated by *lin-4* in worms, was also repressed during neuronal differentiation of mammalian embryonal carcinoma cells and indicated that mammalian *lin-28* messenger RNAs contain conserved predicted binding sites in their 3′ untranslated regions for neuron-expressed *mir-125b*, *let-7a*,

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Table 3 Roles of some miRNAs in tumorigenesis

miRNA	Expressed patterns	Tumors	Target mRNAs	Functions/Observation	References
Let-7b	↓	Melanomas	Cyclin D1	*Let-7b* reduces cells cycle progress in primary melanomas	65
miR-378	↑	Numbers of tumors	SuFu and Fus-1	*miR-378* promotes cell survival, tumor growth and angiogenesis	155
miR-34b/34c	↓↑	Numbers of tumors	p53, E2F3 or MYCN	*miR-34b/ 34c* are targets of p53, E2F3, or MYCN and then inhibit cell proliferation, is also overexpressed in various type of human cancer	79,81,83,84
miR-15a /16-1	↓	Chronic lymphocytic leukemia	Bcl2	*miR-15a* and *miR-16-1* are natural antisense Bcl2 interactors	105
miR-372 /373	↑	Testicular germ cell tumors	LATS2	*miR-372 /373* are potential oncogenes in the development of human testicular germ cell tumors	98,156
miR-200 Family and *miR-205*	↓	MDCK cells with modification	ZEB1 and SIP1	*miR-200* family and *miR-205* downregulate tumor metastasis	157
miR-17-5p-92	↑	Neuroblastoma	p21 and BIM	The upmodulation of miR-*17-5p* mediates the oncogenic properties of *MYCN*, through a direct suppression of *p21* and *BIM* translation	131
miR-137	↓	Melanoma cell lines	MITF	*miR-137* downregulated MITF which is the master regulator of melanocyte development, survival, and functions	158

SuFu – suppressor of fused homolog; MYCN – MYC family of proto-oncogenes; Bcl2 – B-cell lymphoma 2 protein; MDCK – Madin Darby Canine Kidney; LAST2 – large tumor suppressor 2; ZEB1 – zinc finger E-box binding homeobox 1, SIP1 – survival of motor neuron protein interacting protein 1; BIM – Bcl 2 interacting mediator of cell death; MITF – micropthalmia-associated transcription factor.

mir-218 [68]. This result suggested miRNAs (*let-7*, *mir-125b*, *let-7a*, and *mir-218*) could be involved in tumor differentiation via binding to 3′ untranslated regions of target gene. Recent evidence strongly suggests *miR-373* and *miR-520c* are involved in stimulating cancer cell migration and invasion, indicating that those miRNAs can promote cancer metastasis [27].

Apart from downregulated miRNAs, upregulated miRNAs are also associated with tumorigenesis. It is well established that *miR-17-92* polycistron is located in chromosome 13q31 that is amplified in human B-cell lymphomas. He et al. demonstrated that *miRNA-17-92* cluster can cooperate with oncogene gene c-Myc, which is a oncogenic transcription factor, to promote tumor development [69]. To further reveal mechanism behind this phenomenon, O'Donnell et al. also investigated the interaction between *miR-17-92* cluster with Myc [70]. They found that Myc directly binds to *miR-17-92* locus on chromosome 13 and activates expression of the miRNA cluster and also shows that two miRNAs from this cluster (*miR-17-5p* and *miR-20a*) negatively regulate expression of E2F1 transcription factor, which is an additional target of Myc that promotes cell-cycle progression. Further evidence provided by Chang and coworkers revealed that c-Myc can regulate a much broader set of miRNAs than previously anticipated and indicated that much of this repression is likely to be a direct result of Myc binding to miRNA promoters [71]. In addition, Hayashita et al. observed that the overexpression of *miR-17-92* is associated with the enhancement of cell proliferation in human lung cancers [72]. Wang et al. indicated that *miR-17-92* cluster can accelerate adipocyte differentiation by negatively regulating tumor-suppressor gene Rb2/p130 [73]. Recently published results indicated that *miR-17* polycistron (*miR-17-18-19-20-92*) with transcript factor Myc can synergistically contribute to tumor development, probably by repressing tumor-suppressor genes [74]. Using transgenic mice to overexpress *mir-17-92* cluster in embryonic lung epithelium, it has been observed that *mir-17-92* promotes the high proliferation and undifferentiated phenotype of lung epithelial progenitor cells [75]. Another well-characterized miRNA-*miR-21*, which is overexpressed in a wide variety of cancers, will be introduced in the section of miRNAs as oncogenes.

miR-34a has been demonstrated to target MYCN, which is commonly found to be amplified in neuroblastoma as well as brain tumor [76], breast tumor [77], and cervix cancer [78]. Wei and coworkers demonstrated that *miR-34a* causes significant decrease of cell growth through increased apoptosis and inhibited DNA synthesis [79], suggesting that *miR-34a* is a tumor-suppressor miRNA. Further studies indicated *miR-34*, *miR-34b*, and *miR-34c* can cooperate with p53 tumor suppressor in control of tumor growth and development [80, 81]. The expression of *miR-34a* could lead to cell apoptosis by p53-dependent pathway. Conversely, perturbation of *miR-34a* expression, as occurs in some human cancers, could contribute to tumorigenesis [82]. In addition, Welch et al. also demonstrated that *miR-34a* functions as a potential tumor suppressor by inducing apoptosis in neuroblastoma cell though targeting E2F3 protein, which is a potent transcriptional inducer of cell-cycle progression [83]. However, Dutta

et al. indicated that the overexpression of *miR-34a* can also be observed in various types of human cancers such as mucinous adenocarcinoma and infiltrating papillary carcinoma, which is associated with cell proliferation [84]. Those results suggest that even one miRNA probably has different roles in different type of cancer.

So far, the main epigenetic alternations in cancer are aberrant DNA hypermethylation of tumor-suppressor genes, global genomic DNA hypomethylation, and disruption of the histone modification patterns, which result in inappropriate cellular proliferation and survival. The epigenetic modification could attribute to miRNA-mediated tumorigenesis due to the downregulated expression of miRNAs that are considered as tumor-suppressor gene [56, 85]. For example, *miR-127* and *miR-124a* are transcriptionally inactivated by CpG island hypermethylatin in colorectal cancer [86], whereas in lung cancer, the overexpression of miRNA with oncogenic function, *let-7a-3*, seems to be due to DNA hypomethylation [87].

Overall, the upregulated miRNAs with oncogenic function in caner could be considered oncogenic miRNAs, named as oncomirs. The downregulated miRNAs with tumor-suppressor function could be considered as tumor-suppressor miRNAs. We will discuss the most well-understood oncomirs and tumor-suppressor miRNAs in the following sections.

4.3 miRNAs as Oncogenes

miRNAs act as oncogenes by inhibiting the expression of tumor suppressors or by downregulating genes that inhibit the activity of known oncogenes [88]. Table 4 summarizes some well-characterized oncogenic miRNAs. *miR-155*, which is a product of the *BIC* transcript, was the first oncomir discovered and it has been shown to be upregulated in many solid tumors as well as in leukemias and lymphomas [89]. In an avian model, oncogenic cooperation between the *BIC* and the *MYC* genes in lymphomagenesis and erythroleukemogenesis has been observed, indicating that MYC and BIC might cooperate in human tumors [90]. Costinean and coworkers developed the first transgenic mouse that specifically overexpresses *miR-155* in B cells, thus modeling the human B-cell leukemia where the upregulation of *miR-155* is observed. The transgenic mice developed polyclonal pre-leukemia B-cell type followed by B-cell malignancy. However, the death of these mice is not an early event, suggesting that *miR-155* deregulation needs additional genetic alterations for the development of the fully malignant phenotype [91].

The best characterized oncogenic miRNA is *miR-17-92* cluster. This miRNA cluster is encoded by a polycistronic transcript from the chromosome 13 open reading frame 25. *miR-17-92* cluster interacts with c-myc, which is pathologically activated in many human cancers, to accelerate tumor development [69]. *miR-10b* has also been shown to positively regulate cells migration and invasion

Table 4 List of some miRNAs as oncogenes

miRNA	Tumors	Effect factors/proposed targets	Functions/Observation	References
miR-17-92 clusters	Human B-cell lymphomas	c-myc	miRNAs can modulate tumor formation	69
miR-373 /520c	Testicular germ cell tumors	CD44	miRNAs stimulate cancer cell migration and invasion	27
miR-10b	Breast cancer	Twist	*miR-10b*positively regulates cell migration and invasion	92
miR-221/222	Prostate carcinoma	Kip1	The overexpression of these miRNAs probably contribute to the oncogenesis and progression of prostate carcinoma	97
miR-372/373	Testicular Germ Cell Tumors	LATS2	These miRNAs neutralize p53-mediated CDK inhibition	98
miR-21	Numbers of tumors	PTEN or TPM1	*miR-21*suppresses the expression of tumor-suppressor gene	93,95

LAST2 – large tumor suppressor 2; PTEN – phosphatase and tensin homolog; TPM1 – tumor-suppressor tropomyosin-1.

in breast cancer upon inducing by transcription factor twist [92]. Both the overexpression of *miR-17-92* and *miR-10b* results from the oncogenic transcript factors binding with genome and initiating mRNA transcript. *miR-21*, which is another well-characterized oncogenic miRNA, is overexpressed in a wide variety of cancer and has been demonstrated to be linked to cell proliferation, apoptosis, and cell migration. Knockdown of *miR-21* in cultured glioblastoma cells triggers activation of caspase activity, leading to increased apoptotic cell death. In addition, the disruption of *miR-21* affects the glioma growth in vivo. Si et al. demonstrated that *miR-21* can promote growth of the breast cancer cell line MCF-7 both in vitro and in vivo, which may be due to the ability of *miR-21* to suppress the expression of the tumor-suppressor PTEN and tropomyosin 1 (TPM1) [93– 95]. *miR-21* can also target and downregulate the expression of sprouty 2 and inhibit the cell of outgrowth [96]. Galardi et al. indicated that *miR-221*/*222* can be a new family of oncogenes, directly targeting the tumor-suppressor p27(Kip1), and that their overexpression might be one of the factors contributing to the oncogenesis and progression of prostate carcinoma through p27(Kip1) downregulation [97].

Some oncogenic miRNAs control tumor development by mediating the expression of tumor-suppressor genes such as p53 and TPM1. For example, Voorhoeve et al. demonstrated that *miR-372*/*373* neutralize p53-mediated CDK inhibition and then potentially become novel oncogenes participating in the development of human testicular germ cell tumors [98]. On the other side, some miRNAs can also regulate tumor development by adjusting the activities of some kinases. For example, *miR-106-363* cluster potentially regulates homeodomain-interacting protein kinase 3 and then promotes cancer metastasis [99].

4.4 miRNAs as Tumor Suppressors

miRNAs function as tumor suppressors by inhibiting the expression of oncogenes and then blocking tumorigenesis as summarized in Table 5. miRNA- *let-7* has been shown to act as a tumor suppressor in extensive tumors including lung cancers [64], colorectal [100] and breast cancers [101], and leiomyoma [102]. Now it has been demonstrated that, *let-7* can regulate cell differentiation, proliferation, apoptosis, and transformation by suppressing the expression of Ras and high-mobility group A2 (HMGA2), which are kind of oncogenes [64, 102]. Disruption of the pairing between *let-7* and HMGA2 can enhance oncogenic transformation [103]. Furthermore, the introduction of *let-7* miRNA by intranasal administration can suppress the growth and formation of lung tumor in xenografts mice [104]. These observations suggest *let-7* miRNA may be useful as a therapeutic agent in cancer.

miRNAs can also downregulate the expression of antiapoptotic proteins or kinases in cancer by serving as tumor suppressors. For example, Cimmino et al. demonstrated that *miR-15* and *miR-16* can induce apoptosis by targeting

Table 5 List of some miRNAs as tumor suppressors

miRNA	Tumors	Target mRNA	Functions	References
miR-221/222	Glioblastomas	$p27^{Kip1}$	*miR-221/222* inhibit tumor proliferation	159
miR-29	Cholangiocarcinoma cell line and lung cancer	Mcl-1, DM3a, and DM3b	*miR-29* induces tumor cell apoptosis	106,109
Let-7	Numbers of cancers	Ras or HMGA	*Let-7* inhibits tumor cell growths and proliferation	64,102,104
miR-15/16	Chronic lymphocytic leukemia	Bcl2	*miR-15/16* induce apoptosis by targeting BCL2	105
miR-119a	Fibroblasts tumor	Met or ERK2	*miR-199a* may be effective in inhibiting cell proliferation and motility and invasive capabilities of tumor cells	107
miR-7	Glioblastoma	EGFR, Ras 1, or Ras2	*miR-7* is a regulator of major cancer pathway	108

DM3a – DNA methyltransferase 3A; DM3b – DNA methyltransferase 3b; HMGA – HMAG, high mobility group A; BCL2 – B-cell lymphoma 2 protein; ERK2 – extracellular signal-regulated kinase 2; EGRF – epidermal growth factor receptor.

antiapoptotic B-cell lymphoma 2 protein (BCL2), which is a central player in the genetic program of eukaryotic cells favoring survival by inhibiting cell death in B-cell chronic lymphocytic leukemia [105]. *miR-29* can induce tumor cell apoptosis by negatively regulating Mcl-1 protein expression, which belongs to BCL2 protein family [105, 106]. Kim et al. documented that *miR-199a* regulates not only the Met-proto-oncogene but also the downstream extracellular signal-regulated kinase 2 (ERK2), leading to tumor cell apoptosis [107]. Recently, *miR-7* has been identified a new tumor suppressor by suppressing epidermal growth factor receptor expression (EGFR) and inhibiting the Akt pathway via targeting upstream regulators such as Ras 1 or Ras 2 [108].

miRNAs can also regulate the expression of enzymes involved DNA methylation and then control tumor development. For example, Fabbri et al. demonstrated that *miR-29* family including *miR-29a*, *b*, and *c*, which is downregulated in tumor cells line, target DNA methyltransferase 3A and 3B. The enforced expression of *miR-29* in lung tumor induces reexpression of methylation-silenced tumor-suppressor gene and inhibits tumorigensis both in vitro and in vivo [109].

5 miRNA for Diagnosis and Prognosis in Cancer Patients

miRNA expression profiling using bead-based flow cytometry, northern blot analyses, RT-PCR, and miRNA microarrays has been used to demonstrate distinct expressions of specific miRNAs in certain tumor tissues [49, 54, 110–113]. These characteristic miRNA signatures suggest that miRNAs have the potential to be used as diagnostic and prognostic tools, especially since they are more informative in distinguishing between cancer types and for determining cancer metastases when compared with traditional biomarkers. For example, Lu *et al*. used a novel bead-based flow cytometry miRNA expression profiling method to analyze the systematic expression of 217 mammalian miRNAs in normal and human cancer samples. Overall, they observed a downregulation of the miRNAs in tumors compared with normal tissues. Additionally, Lu and coworkers were able to classify poorly differentiated tumors using expression profiles of relatively few miRNA [54]. Furthermore, Chen et al. showed that miR-181, miR-223, and miR-142 s were overexpressed in hematopoietic tissues using northern blot analysis [25]. Using real-time PCR to analyze the expression of miR-21 in 37 gastric patients, Chan et al. found a 92% overexpression of miR-21 in tumor tissue, demonstrating the potential of miR-21 as diagnostic marker for gastric cancer [114]. With the application of oligonucleotide microchips, Croce's group observed unique miRNA signatures for human breast cancer, human megakaryocytopoiesis, and B-cell CLL [115, 116].

miRNA signatures can also provide useful information for determining prognosis in patients with cancer. For example, in ovarian cancer the expression of *let-7* and HMGA2 is a better predictor of disease progression than classical

markers such as E-cadherin, vimentin, and snail [66]. Also, the high expression of *miR-21* has been linked with poor survival and therapeutic response in patients with colon adenocarcinoma [117]; while high expression of *miR-191* and *miR-199a* in acute myeloid leukemia patients correlated with poor prognosis compared with those with low expression [118]. Guo and coworkers have also shown that a high expression of *miR-103/107* leads to low survival in patients with esophageal cancer [119]. Another example of miRNA expression and cancer prognosis is the high *miR-155* expression and low *let-7a-2* expression resulting in poor survival in the patients with lung cancer.

It is important to note, that some miRNA signatures are efficient diagnostic markers but poor indicators of clinical prognosis. For example, Chan et al. used quantitative PCR to examine the expression level of *miR-21* in 37 patients with gastric cancer and found *miR-21* to be an efficient diagnostic marker, but a poor prognostic tool for gastric cancer [114]. Taken together, distinct miRNA signatures can be used as cancer diagnostics and to foretell prognosis. However, there is still a long way to adopt this technology clinically since the function of unique miRNAs differently expressed in different tumors should first be well understood.

6 Therapeutic Implication of miRNAs

Many cancer types are characterized by an aberrant expression of miRNAs and as such represent a potential therapeutic target for cancer therapy. Presently, two technologies adapted from gene therapy and RNAi technology may be used to alter the levels of expression of miRNAs. In the first approach, antisense oligonucleotides (ASOs) can be used to inhibit the effects of miRNA by specifically binding with target miRNA and then blocking its normal function, while in the second approach, cancer causing miRNAs can be replaced by vectors overexpressing a specific miRNA or by transiently transfected double-stranded miRNAs.

6.1 miRNA Inhibition

Inhibition of miRNA can be achieved by introducing antisense molecules targeting mature miRNA or introducing siRNA/shRNA to silence the various components involved in miRNA processing.

6.1.1 miRNA Inhibition Using Modified Antisense Oligonucleotide

ASO-based gene therapy has been clinically applied for human disease [120]. Now it also was extended for silencing miRNA. To the best of our knowledge, the first reported study on miRNAs silencing by using ASO was presented by

Boutla et al. in 2003 [121]. In this study, the authors synthesized 11 DNA oligonucleotides complementary to 11 miRNAs and then injected them into *Drosophila* embryos, then a variety of developmental defects were observed, suggesting that these oligonucleotides inhibited miRNA activity and could become a potential target for treating cancer by inhibiting oncogenic miRNA.

To improve potency for the target nucleic acid, resistance to endogenous nuclease, or improved activation of RNase H or other protein involved in the terminating mechanism, several strategies have been applied to modify ASO for silencing a given gene [12]. The first generation of ASO was designed to resist nuclease attack by replacing one of the non-bridging oxygen atoms in the phosphate group of ASO with either sulfur or a methyl group [122]. However, this modification leads to several side effects due to unspecific interaction and poor solubility in water [123]. Then, sugars were also considered to be modified by adding *O*-methyl, fluro, *O*-propyl, *O*-allyl or other groups at the 2′ position to increase affinity for RNA and impart some nuclease stability, named as the second generation of ASO [124]. Additionally, 3′-hydroxyl group of the 2′-deoxyribose has been replaced with a 3′-amino group for ASO, named as N3′→P5′ phosphoramidates, and shown a very high-binding affinity to complementary DNA or RNA [125].

To date, phosphorothioate and/or 2′ sugar modified ASO has also been used to silence miRNA (Fig. 3). To the best of our knowledge, the first report to use modified ASO to silence miRNA was presented by Hutvagner et al. and coworkers in 2004 [126]. They injected the 2′-*O*-methyl modified ASO, which is complementary to the miRNA *let-7*, leading to a *let-7* loss-of-function phenocopy in *C. elegans* [126]. Davis et al. systematically evaluated the correlation between ASO with 2′-sugar and 2′-F modified backbone and its potency

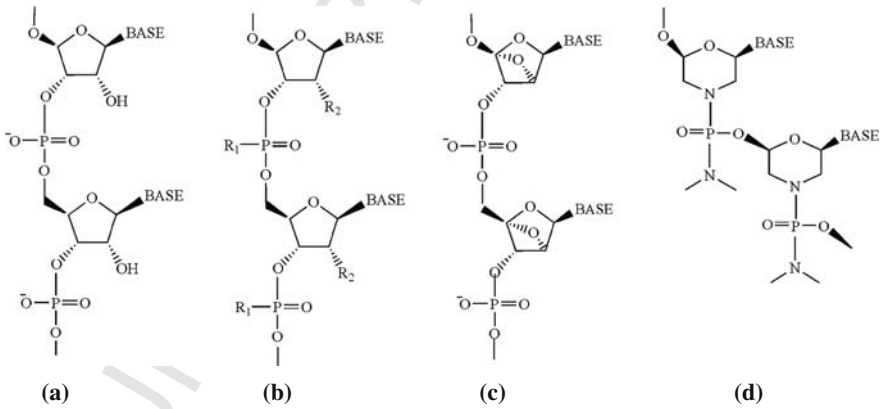

Fig. 3 Chemically modified ASOs that have been used for anti-miRNA. (**a**) Unmodified ASO. (**b**) Chemically modified ASO with phosphorothioate backbone (R1 position) and 2′ sugar modification such as 2′-*O*-methyl, 2′-*O*-methyoxyethyl, and 2′-flouro (R2 position). (**c**) Locked nucleic acid. (**d**) Morpholino

targeting miRNA and demonstrated that ASO modification improve miRNA ASO activity by improving target affinity [127]. They also noted that the positioning of high-affinity modifications had dramatically different effects on miRNA activity. Locked nucleic acid (LNA) modified oligonucleotides have been used as sensitive and specific miRNA detection probes in northern blots due to its high efficiency for binding targeting sequence [128]. Then LNA modified nucleotide has been shown to have specific and long-lasting silence effect on miRNA in cell line [129, 130]. Fontana et al. used 2′-*O*-methyl modified antagomir targeting *miR-17-5p-92* and demonstrated that both in vitro and in vivo treatments with antagomir-*17-5p* abolishe the growth of *MYCN*-amplified and therapy-resistant neuroblastoma through p21 and BIM upmodulation, leading to cell-cycling blockade and activation of apoptosis, respectively [131]. As we mentioned above, *miR-21* has been found to be upregulated in variety of solid tumors. Transfection of breast cancer MCF-7 cells with anti-miR-21 oligonucleotide with 2′-*O*-methyl modification suppressed cell growth in vitro and tumor growth in the xenograft mouse model by increasing cell apoptosis and increasing cell proliferation [93]. All these data suggest that downregulation of *miR-21* in solid tumors might be a way for tumor regression. Apart from modified ASOs, which are targeting mature miRNAs, Morpholino has been shown to affect the processing of the pri-miRNA and pre-miRNA and then inhibit the activity of mature miRNA as presented by Kloosterman and coworkers [132]. In this study, they also observed that knockdown of *miR-375* causes morphological defect in pancreatic islets by using Morpholino [132].

To further improve potency for targeting miRNA in vivo, modified ASOs could be conjugated with delivery agents such as cholesterols or be formulated with lipid, polymer or peptide-based delivery system (Fig. 4). Krützfeldt et al. [133] used cholesterol to conjugate with a series of modified miRNA antisenses, named as 'antagomirs' and demonstrated that antagomirs are efficient and specific silencers of endogenous miRNAs in mice. Intravenous administration of antagomirs against *miR-16*, *miR-122*, *miR-192,* and *miR-194* resulted in a marked reduction of corresponding miRNA levels in most organ, suggesting that the silencing of endogenous miRNAs by this novel method is specific, efficient and long-lasting, and potentially toward therapeutic propose. They also noted that injection of unmodified single-stranded RNA targeting *miR-122* had no effect on level of *miR-122* in liver, whereas injection of unconjugated, but chemically modified, single-stranded RNAs with a partially modified or fully modified phosphorothioate backbone and 2′-OMe sugar modification led to an incomplete effect, suggesting that modified miRNA antisenses can significantly improve potency in vivo. Fontana et al. also used 2′-OMe modified, phosphorothioate backbone, and cholesterol-conjugated ASO to target *miR-17-5p-92* cluster and demonstrated that the abrogation of *miR-17-5p-92* leads to cell-cycling blockade and activation of apoptosis [131]. Overall, modifications of different positions of ASO including 2′-hydroxyl group and backbone

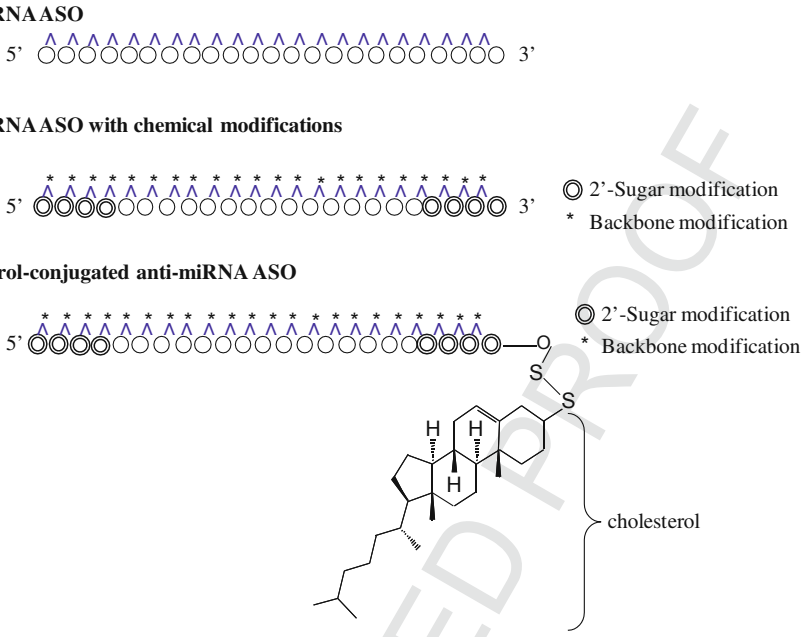

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Fig. 4 Scheme for improving ASO stability and silencing effect for miRNA. (**a**) Unmodified ASO. (**b**) 2′ Sugar and backbone modified ASO. (**c**) Cholesterol-conjugated ASO at 3′ end

as well as conjugation with delivery agent could at most facilitate miRNA silencing effect.

Esau and coworkers also inhibited miR-122 in normal mice by intraperitoneally injecting an unconjugated 2′-MOE modified AMO [134]. This led to an increase in mRNA levels of miR-122 target genes and a decrease in plasma cholesterol. While the mechanism governing the reduction of miRNA by the AMOs is not clear, it is known that the AMO acts on the mature miRNAs since the levels of pre-miR-122 remain unchanged.

In spite of the potential therapeutic benefits of using AMOs for treating cancer, clinical application is presently inhibited due to a lack of effective delivery of AMOs into target sites. Possible approaches to delivering AMOs include complexing them with lipids or proteins; using cationic liposomes [135, 136] or conjugating AMOs with homing signals for site-specific delivery. Recently, Akinc et al. developed a new class of lipid-like delivery molecules, termed lipidoids, as delivery agent for RNAi therapeutics and reported that lipidoids facilitate high levels of specific silencing of miRNAs when formulated with single-strand antisense 2′-*O*-methyl oligoribonucleotides [137]. With progress in the development of better delivery vehicles, miRNA targeting will ultimately become commercially available as a therapeutic agent.

6.1.2 Targeting miRNAs Processing Using Antisense Oligonucleotide

Another approach for therapeutically inhibiting miRNA expression involves downregulating the various components in the miRNA biogenesis pathway to prevent the production of mature miRNA [138]. Small molecule drugs or RNAi interference can be used to achieve this by inhibiting *Drosha*, *Dicer*, and other miRNA pathway components [15, 18]. However, this therapeutic approach is often accompanied by pleiotropic effects which can be minimized by tightly controlling the expression of miRNA pathway components using a tetracycline-inducible shRNA for downregulation. Nevertheless, recent studies indicate that the inhibition of components involved in the miRNA biogenesis pathway has very little effect on miRNA levels possibly due to the slow turnover of mature miRNA [15, 16].

The application of RNAi interference for silencing various component of the miRNA pathway has historically been inhibited by the fact that the miRNA/RISC complex and the hairpin pre-miRNA are not easily accessible by siRNA. Furthermore, since siRNA is active in the cytoplasm, it cannot be used to target pri-miRNA which is located in the nucleus. In one study, Lee and coworkers used siRNA to target the loop region of a pre-miRNA in the cytoplasm and found it to be highly inefficient requiring a very high dose compared to other mRNA targets. Since then, developing ways to obtain long-term suppression of miRNA has been the subject of intense research.

One approach which may be used to target pri-miRNAs involves using ASOs utilizing the RNase H mechanism since their primary site of action is in the nucleus [139, 140]. This strategy may be valuable for inhibiting polycistronic pri-miRNAs where one ASO can be used to target the transcripts of pri-miRNAs and hence inhibit the processing of the mature miRNA; whereas multiple AMOs would be needed to target each miRNA individually.

6.2 miRNA Replacement

As mentioned above, the replacement of cancer causing miRNAs may be used as a therapy for various cancers. This may be done by transiently transfecting double-stranded miRNA mimetics or by using vectors overexpressing a particular miRNA. In both approaches, the goal is to re-establish the levels of miRNA expression occurring prior to the inception of cancer. The double-stranded miRNA mimetic introduced is equivalent to the *Dicer* product and analogous in its function on target mRNAs. Since double-stranded miRNA mimetics are analogous in structure to siRNAs they are subject to all the limitations associated with siRNA therapy, including the difficulty in systemic delivery to tissues and the need for modifications to enhance their stability and cellular uptake [141– 143]. An additional issue with this method is the transient nature of miRNA expression.

One way of addressing this limitation is to use a gene therapy approach that results in longer lasting miRNA expression. In this approach, a plasmid or viral vector driven by a pol II or III promoter upstream of short hairpin RNA (shRNA) is used to express a specific miRNA which is then loaded into RISC after being processed into mature miRNA by *Dicer*. The benefits associated with these constructs are their long-lasting silencing compared to double-stranded miRNA mimetics and their capacity to express multiple miRNAs from one transcript. Examples include the use of adeno-associated virus (AAV) and lentivirus vectors as therapeutics for miRNA delivery to the liver and brain [144, 145]. However, the eliciting of immune response and the possibility of insertional mutagenesis has limited clinical use of AAVs and lentiviruses, respectively.

7 Concluding Remarks

miRNAs are small non-coding RNAs of about 22 nt, which regulate gene expression in a sequence-specific manner via binding to 3′-UTR of target mRNAs to suppress translation or cleave mRNAs. It has been established that miRNAs play an important role in cell proliferation, differentiation, and apoptosis. Because cancer is a class of disease in which a group of cells display the trait of uncontrolled growth, invasion, and metastasis, accumulated evidence indicated that miRNAs act as an important regulator in tumorigenesis such as oncogenes, tumor-suppressor factor, or other effective factors. Unique miRNA signatures have provided useful information for diagnosis and prognosis in patients with cancer. Blockage of miRNAs with oncogenic function by ASO has been shown to inhibit tumor cell growth and proliferation. Several types of modified ASOs as well as conjugation with cholesterols or lipid-based delivery have been demonstrated to improve potency both in vitro and in vivo. Overexpression of tumor-suppressor miRNA by introducing plasmid encoding miRNA or infecting lentivirus encoding miRNAs can suppress tumor development.

Although miRNA therapeutic modulation is potentially attractive for treating cancer as well as diagnosis and prognosis, there is still a great need to deeply understand the roles of miRNA in tumorigenesis. It is also necessary to develop an effective and efficient method for delivering antagomirs in vivo. Finally, the inhibition of miRNAs in conjunction with other chemotherapy strategies could significantly improve the outcome of cancer patients.

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AQ10

MicroRNAs as Therapeutic Targets for Cancer

Query No.	Line No.	Query
AQ1	590	"Recent evidence. . .mastassis" has been changed to "Recent evidence. . .metastasis". Is this OK?
AQ2	916	The sentence "Then, sugar. . .O-methy. . .ASO" has been changed to "Then, sugar. . .O-methyl. . .ASO". Is this OK?
AQ3	953	Please check the edit made to the sentence "Then LNA. . .. . ." is OK.
AQ4	1019	"Esau and coworkers...2′-MOE modifies AMO" has been changed to "Esau and coworkers...2 modified AMO". Is this OK?
AQ5	1234	Please provide volume and page numbers.
AQ6	1274	Please provide volume and page numbers.
AQ7	1313	Please provide volume and page numbers.
AQ8	1354	Please provide volume and page numbers.
AQ9	1381	Please provide volume and page numbers.
AQ10	1517	Please provide volume and page numbers.