

**Review Article** 

# MicroRNAs as a Potential Target for Cancer Therapy

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#### Abstract

MicroRNAs (miRNAs) are evolutionary conserved small non-coding RNAs that negatively regulate gene expression by several mechanisms. Deregulation in expression of miRNAs has been reported in the pathogenesis of cancer. Accordingly, studies identified down regulation in the expression of miRNAs having tumor suppressor role and up-regulation in the expression of oncogenic miRNAs in different types of cancer. In response to these observations currently there are ongoing efforts to develop safe and effective miRNA-based therapeutics in the hope of fighting against cancer. This paper aimed at reviewing the role of miRNAs in tumorigenesis, and strategies for therapeutic targeting of miRNAs in cancer.

**Keywords:** MicroRNA; Tumor; Novel target; Cancer therapy; Tumorigenesis; Tumor suppressor

#### Introduction

Cancer is a complex group of diseases characterized by the presence of cells with uncontrolled growth, and high proliferation capacity [1]. Based on insights from decades of research eight changes in cell physiology has been ascribed as hallmarks of cancer, including: sustaining proliferative signaling; insensitivity to antigrowth signals; evasion of apoptosis; limitless replicative potential; sustained angiogenesis; activating invasion and metastasis; as well as reprogramming of energy metabolism and evading immune destruction [2,3]. During development and in response to cellular milieu there might be switch- on or -off of expression of genes encoding proteins that regulate the above process and altered expression of the genes (tumor-suppressor genes and proto-oncogenes) arise from mutations, or expression deregulation in a multistep process resulting in cancer [4,5]. Moreover, recent studies have revealed the involvement of class of small non-coding singlestranded RNAs including microRNAs (miRNAs), and long non-coding RNAs in the development and progression of cancer [6,7]. This review aimed at appraising the most current literatures regarding the link between miRNAs and molecular pathogenesis of cancer and also the current approach in targeting miRNAs as cancer therapy.

## Literature Review

#### **Overview of MicroRNAs**

MiRNAs, known to be a negative regulator of gene expression at posttranscriptional level, are small noncoding single-stranded RNAs of 18 to 24 nucleotides [6,8].

#### **Biogenesis and regulation**

As shown in Figure 1, biogenesis of miRNAs starts with formation of a primary transcript (pri-miRNA) by RNA polymerase II, or sometimes by RNA polymerase III [9]. Pri-miRNA has a stem-loop structure, is capped at the 5'-end and has a 3'-poly (A) tail [10]. Then, drosha complex (microprocessor complex), process pri-miRNAs into hairpin-structured precursor miRNAs (pre-miRNAs) inside the nucleus [11]. Furthermore, the pre-miRNAs are transported into the cytoplasm by Exportin-5 (XPO5) along with Ran-GTP [12]. Once it reaches in the cytoplasm, the pre-miRNA will be digested into a mature duplex miRNA by a Dicer (RNase III) [13]. In this regard, biogenesis of miRNAs has been known to be regulated at different levels, including at level of miRNA transcription [13,14], processing in to mature duple miRNA (Drosha, Dicer and their accessory proteins) [15-20].

On the other hand, so as to interfere in the expression of variety of genes, one strand of the miRNA duplex will be loaded onto the miRNAinduced silencing complex (RISC) [14], which then regulates the translation of complementary messenger RNA (mRNA) [15]. As studies identified, interaction of miRISC to mRNA achieved by recognizing its complementary sequences in the 3'untranslated region (UTR) of their target mRNAs through seed region, typically positions 2-7 in the miRNA [14,15]. As shown in Figure 2, miRNAs negatively regulate gene expression through different mechanisms including, inhibition of translation and decay [11,21-28]. Meanwhile strict complementarity is not obligatory for regulation, one miRNA may target a number of mRNAs and subsequent aberrant miRNA expression may affect a multitude of transcripts, which have remarkable effect on cancer-related signaling pathways [11].

#### Aberrant Expression in Cancer

The initial remark that provided a possible link between miRNA and carcinogenesis was the loss-of-function mutations phenotype of lin-4 and let-7 in C. elegans. Accordingly, mutants of lin-4 and let-7 resulted in extra cell divisions during the adult stage, beyond larval stages, linking in the control of cell differentiation and proliferation [21,22]. However, the most direct evidence linking miRNAs to cancer came from the discovery of deletions of the miR-15a/16-1 cluster in chromosome 13q14 in chronic lymphocytic leukemia cells by Calin [29]. According to this study, miR-15a/16-1 shown to regulate BCL2, signifying a mechanism by which CLL could be caused due to loss of mir-15a and mir-16-1. Since then, thousands of tumor miRNAs (Tables 1 and 2) [30-32]. In this

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Figure 2: miRNA function: potential mechanisms includes, (a) Repression of translation initiation. MicroRNA (miRNA)-mediated silencing complexes (miRISCs) inhibit the initiation of translation by affecting eukaryotic translation initiation factor 4F (eIF4F) cap recognition, 40S small ribosomal subunit recruitment and/or by inhibiting the incorporation of the 60S subunit and the formation of the 80S ribosomal complex. Some of the target mRNAs bound by the miRISC are transported into processing bodies (P-bodies) for storage and may re-enter the translation phase when induced by exogenous signals such as stress. (b) Post-initiation translational repression. miRISCs may inhibit the elongation of ribosomes, causing them to drop off the mRNAs and/or facilitate the degradation of newly synthesized peptides. (c) Destabilization of target mRNAs. Binding of miRISCs to target mRNAs may recruit RNA decapping and/or deadenylating enzymes that lead to mRNA destabilization. P-bodies are the key cellular organelles for the degradation and storage of targeted mRNAs. AGO2, Argonaute 2; DCP1, mRNA-decapping enzyme 1; PABP, poly(A)-binding protein [11].

MicroRNA	Cancer type	Function
miR-29b	AML	Represses Sp1 which resulted in c-KIT inhibition
miR-34b/c	Lung cancer	A positive feedback between p53 and miR-34 mediates tumor suppression in human lung cancer
miR-126	Breast, lung, colon cancers	Plays a critical tumor-suppressor role in tumor initiation and metastasis
miR-150		AML A critical tumor-suppressor gatekeeper in AML by targeting FLT3 and Myb
miR-155	Breast cancer	Downregulates RAD51 and sensitizes cancer cells to irradiation
miR-181a/b	AML	Their increased expression is associated with good prognosis and hinders tumor cell growth
miR-375	Breast cancer	Forced expression re-sensitizes cells to Tamoxifen treatment
miR-494	Lung cancer	Regulated by ERK1/2 it modulates proliferation and apoptosis response
miR-495	AML, gastric cancer	Specifically, down-regulated in MLL-rearranged, shown to block migration and invasion
miR-551a	Gastric cancer	Forced expression leads to a block in migration and invasion
Let-7	Lung cancer	Inhibits the expression of oncogenes involved in cell proliferation, such as Myc, RAS, and HMGA2
MiR-200	Lung cancer	Inducement of epithelial-mesenchymal transition

Table 1: Examples of tumor-suppressor miRNAs [30,31].

MicroRNA	Cancer type	Function	
miR-9	AML	Specifically overexpressed in MLL-rearranged AML and promotes leukemia progression	
miR-17-92	AML	Up-regulated in MLL-rearranged AML and targets p21 and RASSF2	
miR-21	Breast	Overexpression of miR-21 contributes to proliferation and metastasis	
miR-27a	Lung	Promotes proliferation in NSCLC cells	
miR-30a/c	RCC	Downregulation leads to increased expression of HIF2a	
miR-126	AML	Up-regulated in core-binding factor (CBF) leukemia	
miR-181a/b	Breast, liver, colon	Promote tumorigenesis and tumor progression	
miR-196a	Gastric	Promoted EMT, migration and invasion	
miR-196b	AML	Upregulated in MLL-rearranged AML and targets Fas	
miR-421	Gastric	Marker of circulating tumor cells	
miR-155	Lung	Promote the progression of NSCLCs, in part by downregulating SOCS1, SOCS6, and PTEN	
miR-221/222	Lung	Promotes cell migration and suppresses apoptosis by targeting TIMP3 and PTEN	
AML-Acute Myeloid Leuk	AL-Acute Myeloid Leukemia; FLT, FMS- like tyrosine kinase 3; NSCLC, Non-Small Cell Lung Cancer; RCC, Renal Cell Carcinomas		

Table 2: Examples of oncogenic miRNAs [30,32].

regard, miRNAs that has been up-regulated and support oncogenesis in some cancers are called "oncomiRs," while, miRNAs that has been known to be down-regulated that act as tumor suppressors are named as "tumor suppressor miRs".

# Possible mechanisms of deregulated expression

A tumor cell has been known to have a deregulated expression of miRNAs [33,34]. The underling mechanisms include chromosomal abnormalities, changes in transcriptional and epigenetic regulation and also alteration in the miRNA biogenesis pathways (Figure 3) [29,34,35].

Alterations in genomic miRNA copy numbers and location changes: Abnormal miRNA expressions in tumor cells compared with normal cells are often attributed to alterations in genomic miRNA copy numbers and gene locations, i.e. amplification, deletion or translocation [5,34]. For instance, the loss of miR-15a/16-1 cluster gene at chromosome 13q14 was due to miRNA gene location change, which is frequently observed in B-cell chronic lymphocytic leukemia patients [29]. In addition, in lung cancer, the 5q33 region harboring miR-143 and miR-145 is often deleted, resulting in decreased expression of both miRNAs [36]. On the other hand, amplification of miR-17–92 group gene has been observed in B-cell lymphomas and lung cancers, and translocation of this gene was also observed in T-cell acute lymphoblastic leukemia, leading to overexpression of these miRNAs in these malignancies [37-39].

Moreover, Zhang [40] reported high frequency of DNA copy number alterations in miRNA loci based on high-resolution arraybased genomic analysis of 227 specimens obtained from human ovarian cancer, breast cancer and melanoma. Besides, other genome-wide investigations revealed that many miRNA genes are located in cancerassociated genomic regions [41]. Generally, these discoveries suggested that, abnormal miRNA expression in cancer cells could arise from amplification or deletion of specific genomic regions encompassing miRNA genes.

Alteration in transcriptional regulation: As mentioned above, expression of miRNA has been known to be controlled by transcription factors [14]. It is, therefore, alteration in the activity or expression of key transcription factors, including c-Myc and p53 will result in deregulated expression of miRNA in tumor [42-46]. For instance, a study done by O'Donnell [43] showed that over expression of c-Myc, known to be commonly up-regulated in different types of malignancies, promote expression of oncogenic miR-17-92 cluster via interaction with E-box elements in miR-17-92 promoter region so as to up-regulate tumor cell proliferation. In accordance with its oncogenic role, c-Myc also represses transcriptional activity of tumor suppressive miRNAs such as mir-15a, miR-26, miR-29, mir-30 and let-7 families [44]. Furthermore, a study done by Wang [42] showed the reciprocal regulation of c-Myc and tumor suppressor miR-122 in hepatocellular cancer. Accordingly, disruption of this feedback loop between miR-122 and c-Myc is essential for hepatocellular carcinoma (HCC) development.

Moreover, other studies also demonstrated that, p53 can induce the expression of miRNAs like miR-34a to trigger apoptosis through direct binding to the promoter of mir-34a gene. Consecutively, miR-34a promotes p53 expression by targeting SIRT1, a negative regulator of p53 via deacetylation [47,48]. Further studies also showed that p53 performs its function through regulating the expression of a range of miRNAs, such as miR-605, miR-1246, and miR-107 [49-51]. It is;



therefore, miRNA expression is finely tuned by transcription factors a that maintain its normal transcription, and dysregulation in this control might leads to tumorigenesis.

**Epigenetic alterations:** The epigenetic change is an eminent feature in cancer development and progression, including global genomic DNA hypomethylation, aberrant DNA hypermethylation of tumor suppressor genes and disruption of the histone modification patterns [52]. For example, a study done by Fazi [53] reported silencing of miR-223 expression by AML1/ETO, a most common AML-associated fusion protein. Furthermore, Lujambio [54] reported CpG methylation associated silencing of miR-148a and miR-34b/c group due to hypermethylation in tumor. In the same study, restoration of these miRNAs in tumor has been associated with inhibition of motility, tumor growth and metastasis *in vivo* [54]. By the same token, decreased expressions of miR-9-1, miR-124a and miR-145-5p were attributed to DNA hypermethylation in breast, lung and colon carcinomas, respectively [52]. To this end, these evidences highlighted the role of epigenetic regulation in miRNA expression during tumorigenesis.

Alteration in the biogenesis pathway: Several studies reported abnormal expression and subcellular localization of miRNA processing enzymes/proteins in to mature duple miRNA including Drosha, Dicer and TARBP1, exportin5, TARBP2, correlation with tumor progression and poor prognosis [55-62].

# **Role in Tumor Development and Progression**

It is believed that alteration in the expression of miRNAs could

affect one or several of the cancer hallmarks for tumor initiation and progression. Depending on their target genes, miRNA could act as either oncogene or tumor suppressor under certain circumstances [33,35].

# **Unlimited proliferation**

The E2F proteins are known to be critical regulators of cell proliferation in a cell-cycle-dependent manner [62]. The E2F member E2F1 induces target gene transcription during the G1 to S transition and is defined as a tumor suppressor because the E2F1-deficient mice developed a wide variety of cancers [63]. Among several studies that revealed E2F as target of miRNAs, a study done by O'Donnell [43] was considered as a pioneering study. This study revealed that miR-17-92 inhibits E2F1 translation after being activated by the transcription factor c-Myc. In support with this notion, forced expression of miR-1792 cluster shown to promote increased proliferation and undifferentiated phenotype of normal lung cells [64]. Moreover, miR-17–92 cluster was also found to regulate E2F2 and E2F3 translation, and the E2F transcription factors can in turn induce the expression of the miR-17–92 cluster [63,65]. These studies signify the important role of miR-1792 in proliferation and survival.

Furthermore, cell-cycle progression depends on different cyclins, Cdks and their inhibitors, which are widely regulated by miRNAs [66-71]. In this regard, Gillies and Lorimer [66] reported that Cdk inhibitor p27Kip1 as a target for miR-221/222 and revealed the role of these mRNAs in promoting the aggressive growth of human glioblastoma.

Other studies done by Galardi [67] and Visone [68] also confirmed the above-mentioned role of miR-221/222 using human prostate and thyroid papillary carcinoma cell lines, respectively. Similarly, other G1/S transition regulators like p21CIP1 and p16INK4a are also regulated by miRNAs such as miR-663, miR-302 family and miR-24 [69,70]. To this end, miRNAs have a hand in in cell proliferation, not only through targeting cell-cycle components but also by far regulating multiple signaling pathways. Such as, miR-486, significantly down-regulated in non-small-cell lung cancer, was found to affect cell proliferation and migration through insulin like growth receptor (IGFR) and PI3K pathways by targeting IGF1, IGF1R and p85α [71,72].

#### **Evasion of apoptosis**

Resisting apoptosis is another significant hallmark of tumor progression, which is also controlled by miRNAs [73,74]. Tumor cells develop a variety of strategies so as to circumvent apoptosis; however, the loss of p53 tumor suppressor function is most important. Other ways to escape apoptosis include upregulation of anti-apoptotic regulators, down-regulation of pro-apoptotic factors and inhibition of death pathway induced by extrinsic ligands. The components involved in pro-apoptotic and anti-apoptosis pathways were known to be broadly inhibited or activated by miRNAs [75].

For instance, studies done by Fornari [76] and Burns [77] showed that, miR-122 enhances p53 activity through targeting cyclin G1 and cytoplasmic poly (A) element-binding protein that improves tumor sensitivity to the drug doxorubicin, founding a root headed for the development of combined chemo and miRNA-based therapy for HCC. Similarly, alteration of other p53-regulated miRNAs also confers cancer cells resistant to apoptosis. Such as, miR-17–92 cluster is a novel target for p53-mediated transcriptional repression under hypoxia. In support of this, studies identified that down-regulation of miR-17–92 expression sensitizes cells to hypoxia-induced apoptosis, whereas its overexpression inhibits apoptosis [78,79]. It is therefore, p53 and its regulated miRNAs form a network to elaborately determine cell fate under normal conditions.

Furthermore, anti-apoptotic regulators {B-cell CLL/lymphoma 2 (Bcl-2) and Bcl2-like 1 L isoform (Bcl-xL)} and proapoptotic factors (Bax, Bim and Puma) are known to be potential targets of some miRNAs having key role in cell death [80-84]. For instance, miR-15a and miR-16-1 are significantly down-regulated in chronic lymphocytic leukemia and their expression inversely correlates with Bcl-2 expression. Subsequent study demonstrated that, these two miRNAs repress Bcl-2 expression and induce apoptosis. Moreover, Bcl-2 has been identified to be regulated by other miRNAs, such as miR-204, miR-148a and miR-365 [80-82]. On the other hand, a study done using ovarian cancer cell lines reported that efficient induction of apoptosis when miR-491-5p expression was restored through inhibition of Bcl-xL expression and inducing Bim accumulation in its activated form [83]. According to the same study, dephosphorylated form of Bim accumulation was through targeting of epidermal growth factor receptor (EGFR) by miR-491-5p and ensuing inhibition of downstream AKT and MAPK signaling cascade [83].

On the other hand, miRNAs have been also involved in controlling apoptosis via regulation of extrinsic apoptotic pathway [84]. For instance, a study done by Mo [85] showed that miR-196B directly repressed one of the element of the extrinsic apoptotic cascade first apoptosis signal (FAS) expression in colorectal cancer cells, however, FAS expression was constitutively elevated in normal human colorectal tissues. In the study, anti-miR-196B resulted in up-regulation of FAS expression and improved apoptosis in colorectal cancer cell lines. The authors concluded that over-expression of miR-196B modifies apoptosis in colorectal cancer cells in part by repressing FAS expression.

# **Invasion and Metastasis**

Even if the mechanisms are not entirely understood, some miRNAs have been identified as important effectors in cell migration and metastatic pathways [86-89]. For instance, miR-10b that is transcriptionally controlled by an epithelial-mesenchymal transition (EMT) facilitator, Twist 1 has been identified as a potential promoter of metastasis. Accordingly, elevated expression of miR-10b in metastatic breast tumor brings cell migration and metastasis through targeting of HOXD9 and subsequent upregulation of a pro-metastatic gene RHOC, which is suppressed by HOXD9 [87]. Conversely, miR-221, being one of the significant miRNAs in tumor progression and metastasis, is downregulated in metastatic tissue compared with their primary carcinomas. Moreover, it has been suggested as a prognostic marker for high-risk prostate cancer [88]. In addition, in a recent study, it has been shown that miR-34a, which is transcriptionally controlled by p53, directly targets CD44 and represses its expression. It has been reported that miR-34a is down-regulated in CD44+ cells isolated from xenografts and primary tumors, leading to an increased metastatic potential [90]. Similarly, miR-373 and miR-520c, members of the same miRNA family, have been shown to suppress CD44 through preventing its translation [91]. Moreover, miR-708 has been found to be down-regulated in CD44+ cells extracted from xenografts, and CD44 has been validated as a direct target for miR-708 [89]. Furthermore, recent reports revealed that several miRNAs including let7c, miR-16, miR-21, miR-34a, miR-100, miR-126, miR-145, miR-200, miR-205, miR-218 and miR-335 have been identified as potential markers for the metastatic status of distinct cancers [86,92].

## **Sustained Angiogenesis**

According to studies, miRNAs have been found to regulate various stages of angiogenesis [93-100]. For instance, miR-210 is the most consistently and significantly induced miRNA during hypoxia [94]. Accordingly, some studies demonstrated that miR-210 overexpression in normoxic human umbilical vein endothelial cells stimulates the formation of capillary-like structures and Vascular endothelial growth factor (VEGF)-dependent cell migration [95,96]. However, miR-210 inhibition using anti-miRNA transfection prevented these processes [95]. In this regard, miR-210 induced promotion of angiogenesis known to be mediated through down regulation of the receptor tyrosine kinase ligand ephrin-A3 (an antiangiogenic factor) expression [95], and also down regulation of VEGF and VEGF receptor-2 expression [97]. Besides, miR-424 has been shown to promote angiogenesis in vitro and *in vivo* during hypoxia in endothelial cells via cullin 2, a scaffold protein critical to the assembly of the ubiquitin ligase system that resulted in stabilized hypoxia-inducible factor (HIF)-1a and enhanced expression of VEGF [98]. On the other hand, miR-20b and miR-519c have been recognized as a negative regulator of angiogenesis by targeting VEGF and/or HIF1a [99,100].

# **Targeting Micro-RNAs for Cancer Therapy**

## Inactivating oncogenic miRNAs

This strategy aimed at inactivating oncogenic miRNAs that are highly expressed in tumor cells. They are targeted for inhibition with complementary sequences that impair the processing of endogenous miRNA by RISC. Potential therapeutic candidates include antisense

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anti-miR oligonucleotides (AMOs), miRNA sponges, and small molecule inhibitors [101,102].

## Anti-miRNA oligonucleotides

AMOs are chemically modified anti-sense oligonucleotides (ASOs) that are designed as complementary to a selected oncogenic miRNA to be inhibited [103]. In this regard, they could be considered as competitive inhibitors of oncogenic miRNAs by forming duplex with mature miRNA and preventing its interaction with its target mRNAs (Figure 4) [104]. Moreover, RNAse-H-mediated degradation of target miRNA gene will be resulted once ASO-miRNA duplex formed via Watson Crick binding [105]. To improve affinity to target RNA, cellular uptake and resistance to nuclease numerous modification has been done to AMOs [106]. For instance, resistance to nuclease and affinity to target RNA has been improved by sugar modifications, including 2'-O-methyl, 2'-methoxyethyl, 2'-fluoro, or bicyclic locked nucleic acid (LNA) [107]. Whereas, substitution of phosphodiester

backbone linkages with phosphorothioate, as well as, using peptide nucleic acid or morpholino oligomers, has been shown to enhance their pharmacokinetic properties and stability [108-110]. According to studies, miR-21 has been shown to be overexpressed in glioblastomas, and their knockdown has been associated with improved apoptotic activity [111]. In support of this, a study done by Griveau [112] showed that silencing of miR-21 by LNA-lipid nanocapsule complexes sensitize human glioblastoma cells to radiation-induced cell death.

# MicroRNA sponges

As a DNA construct(s), they contain artificially designed miRNAbinding sites in the 3'-UTR of a nontoxic gene and they serve as competitive inhibitors by binding to complementary oncogenic miRNAs, thereby 'soaking up' existing oncogenic miRNAs pool (Figure 4) [113]. According to few studies, they have shown to inhibit target miRNAs *in vitro* and *in vivo* [114]. However, their therapeutic value has been limited due to issues related to their delivery target cancer cells [102].



Figure 4: miRNA inhibition strategies. (a) MicroRNA (miRNA) sponges. Multiple miRNA-binding sites are inserted downstream of a reporter gene. When delivered into cells, the binding sites serve as decoys for the targeted miRNA, thereby reversing the suppression of endogenous target genes. (b) Chemically modified miRNA-targeting antisense oligonucleotides (anti-miRs) are designed to be fully complementary to the target miRNA and bind with high affinity (high melting temperature; T<sub>m</sub>). When delivered into cells, the anti-miRs bind to the target miRNA, relieving inhibition of the endogenous target genes. Many anti-miRs also induce degradation of targeted miRNAs. (c) Small-molecule inhibitors can target at least three steps of miRNA assembly and function. First, small molecules can interfere with the transcription of primary miRNAs (pri-miRNAs). This inhibition could be at multiple steps, including transcription initiation, elongation and intron splicing. Second, small molecules can inhibit pri-miRNA processing by Dicer and loading into Argonaute 2 (AGO2) to form an active RNA-induced silencing complex (RISC). Third, interactions between RISC and target mRNA can be perturbed by small molecules. All of these mechanisms would lead to the loss of repression of a target mRNA by miRs. miRISC, miRNA-induced silencing complex [11].

#### Small molecule inhibitors

As shown in Figure 4, they have been designed to target oncogenic miRNA. They can interfere with primary RNA transcription, or premiRNA processes by DICER and RISC, or RISC and target mRNA interaction [101]. One limitation of using such inhibitors could be non-specific targeting of miRNA and off target consequences [115]. The first specific small molecule inhibitor of specific miRNA (SMIR) to be discovered was an azobenzene that shown to inhibit miR-21 by inhibiting miR-21 precursor [116]. Further studies have focused on SMIRs to inhibit miR-122, which plays an important role in HCV and have identified several compounds which inhibit miR-122 [117] for further testing.

# **Restoring Tumor Suppressor miRNA Expression**

Micro-RNA replacement therapy can be made by two different strategies: using viral vector-based gene restoration or by miRNA mimics [118].

## **MicroRNA Mimics**

Based on studies, ectopic expression of synthetic miRNAs mimics with tumor suppressor role in have been shown to induce apoptosis and block proliferation in cancer [119]. For example, restoration of miR-15-a and miR-29 induced cell death in acute myeloid leukemia (AML) and prostate cell lines, respectively [120,121]. Further studies reported that, intra-tumoral injection of miR-29 mimics shown to decrease tumorigenicity in human liver and AML xenograft murine models [122,123]. Moreover, intranasal administration of let-7 in a K-ras mutant mouse effectively restrained the growth of the tumors by repression of proliferation and cell cycle pathways [124,125].

Furthermore, a study done by Trang [126] using Kras-activated autochthonous mouse model of non-small cell lung cancer (NSCLC), systemic delivery of miR-34a in complex with novel neutral lipid emulsion has been shown rise the level of this tumor suppressor mi-RNA and has been associated with a significant decrease in tumor burden. Besides in other studies, miR-34a has shown to inhibit tumor growth in different preclinical studies using mouse model of cancer, including HCC and prostate cancer [127,128].

Owing to its promising anti-tumor activities in preclinical studies, phase 1 clinical trial was initiated by the biopharmaceutical company Mirna Therapeutics in 2013. The study has been started using MRX34 (a lipid-formulated miR-34 mimic) in order to evaluate its safety in patients with HCC or other selected solid tumors or hematologic malignancies but unfortunately, the clinical study was halted in 2016 because of multiple immune related severe adverse events (SAEs) [NCT01829971].

# Adenovirus Associated Vectors (AAV)

Another strategy to enhance the expression of a tumor suppressor miRNA in cancer utilizes adenovirus associated vectors (AAV) [129]. These vectors do not integrate into the genome and are eliminated efficiently with minimal toxicity, as shown in phase I and phase II clinical trials in about 200 patients [101]. Another advantage of AAV vectors is the efficient transduction of target cells [130]. Moreover, Kota [131] revealed that, miR-26 role as tumor suppressor miRs in human HCC. Similarly, in a study done by Chen [132] tumor-specific expression of microRNA-26a has shown to suppress human HCC growth through cyclin-dependent and -independent mechanisms. In addition, AAVmediated systematic administration of miR-26a has shown to inhibit tumor growth in a spontaneous murine liver cancer model, in a study conducted by Kota [131]. This way of systemic administration could be worthwhile for HCC treatment, since it is readily targeted by both viral and non-viral gene and small molecule delivery systems. But, the efficacy of this system for other types of tumors and in different locations is not yet clearly known [133].

# **Discussion and Conclusion**

Over the past two decades several studies have proven the significance of miRNAs in normal cellular homeostasis and in the initiation and progression of cancer. In this regard, the novel approach of cancer treatment using miRNAs is particularly inspiring in showing the importance of basic research, which in this case, has proven to be relevant in the development of new treatments for cancer. However, miRNA-based therapeutics are still in their infancy stage and there are many important challenges that the scientific community still needs to address. Efficient delivery of miRNAs faces various barriers, such as delivery-associated toxicity, poor transfection, systemic clearance and bio-distribution, degradation in circulation, immune response, and endosomal sequestration. Even though, remarkable strategies for miRNA delivery are being developed to overcome these obstacles and facilitate miRNA transport, in the future the rational design of safe and efficient new generation carriers would promote clinical translation of miRNAs. Safety of miRNA-based therapeutics is another challenge that would be clarified in the future.

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