EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

MicroRNA silencing and the development of novel therapies for liver disease

Gyongyi Szabo[1,*], Peter Sarnow[2], Shashi Bala[1]

[1]*Department of Medicine, University of Massachusetts Medical School, MA 01605, United States;* [2]*Department of Microbiology and Immunology, Stanford University Medical School, Stanford, CA 94305, United States*

Summary

In recent years microRNAs have emerged as crucial small non-coding RNA molecules with diverse roles in various diseases including diseases of the liver. In this review, we highlight the latest advances in the field of microRNA biology and their potential as emerging therapeutic targets in liver disease.

© 2012 European Association for the Study of the Liver. Published by Elsevier B.V. All rights reserved.

Introduction

Since the discovery of small interfering RNAs (siRNA) by Mello and Fire in 1998, the biological importance and potential therapeutic target of various forms of small RNAs have rapidly emerged. MicroRNAs (miRs) are small (20–22 nucleotide), non-coding RNA molecules that are encoded by genomic DNA and were discovered in 1993 by Ambros and colleagues [1]. miRNAs affect a broad range of biological functions including cell proliferation, differentiation, immunity, tissue remodeling, and cancer development [2,3].

The biogenesis of miRNAs is more complex than earlier thought and has been reviewed extensively. Briefly, miRNAs are transcribed in the nucleus as mono or polycistronic mRNAs, known as pri-miRNAs (Fig. 1). The pri-MiRs are cleaved by the ribonuclease Drosha to yield pre-miRNAs (60–90 bp). The pre-Mirs are then exported from the nucleus to the cytoplasm via the exportin 5 complex (reviewed in [4]), where they are further processed by ribonuclease Dicer to yield miRNA duplexes (20–22 bp). miRNA duplexes are then transferred to the RNA-induced silencing complex (RISC) where the mature guide strand becomes associated with Argonaute proteins and the passenger strand gets expelled from RISC and degraded (Fig. 1). It is known that a single miRNA can have hundreds of target mRNAs and that a given mRNA can be targeted by numerous miRNAs. Usually, miRNAs bind to the 5′ and 3′ untranslated (UTR) regions of mRNAs and the outcome of this interaction depends on the degree of complementarity between the miRNA and mRNAs [5]. Imperfect base pairing of miRNA with target mRNA UTR results in the inhibition of mRNA translation or mRNA degradation, and perfect base complementarity almost always results in mRNA cleavage by Argonaute 2 [5]. Recent studies suggest that miRNAs can also interact with the DNA methylation machinery and modulate the chromatin state [6]. Curiously, even slight changes in abundances of even a few miRNAs can substantially alter cellular physiology and contribute to the development of a disease. Consistent with this notion, alterations in miRNAs profiles have recently been described in various types of acute and chronic liver diseases including alcoholic and non-alcoholic fatty liver diseases, viral hepatitis, primary biliary cirrhosis, liver fibrosis, and hepatocellular carcinoma (Table 1).

In this review, we will discuss the current knowledge of the roles for miRNAs in liver disease and novel tools to change the abundance of miRNAs in the liver in animals.

MicroRNAs as disease-specific targets for modulating liver diseases

The importance of miRNAs in liver diseases has been the focus of many recent investigations. The physiological importance of miRNA in the liver has been recognized in regulation of metabolic pathways, immunity, viral hepatitis, cancer, and liver fibrosis [2,3,7]. Among "organ-specific" miRNAs, miR-122 uniquely represents 70% of the total miRNAs in hepatocytes. Curiously, the pri- and pre-miRs of miR-122 are regulated in a circadian manner [8]. This is a very surprising observation given the finding that the turnover of mature miR-122 is several weeks in the liver, pointing to possible functional roles for the precursor molecules of miR-122. The targets of miR-122 include genes of cholesterol and lipid metabolism, and it also plays an important host factor in HCV infection [8,9]. Other miRNAs were found abnormally expressed in various forms of liver diseases in the liver or in the circulation [2,3,7]. Indeed, the concept that miRNAs could serve as potential serum/plasma biomarkers of disease is gaining attention in liver disease.

MicroRNA silencing in viral hepatitis

Perhaps the most compelling evidence for the therapeutic feasibility of miRNA inhibition comes from studies in hepatitis C virus

Keywords: MicroRNA; Fatty liver; Viral hepatitis; Liver fibrosis; RNA interference; LNA; Nanoparticle.
Received 9 August 2011; received in revised form 18 January 2012; accepted 25 January 2012

* Corresponding author. Address: Department of Medicine, University of Massachusetts Medical School, 364 Plantation Street, Worcester, MA 01605, United States. Tel.: +1 508 856 5275; fax: +1 508 856 4770.
E-mail address: gyongyi.szabo@umassmed.edu (G. Szabo).

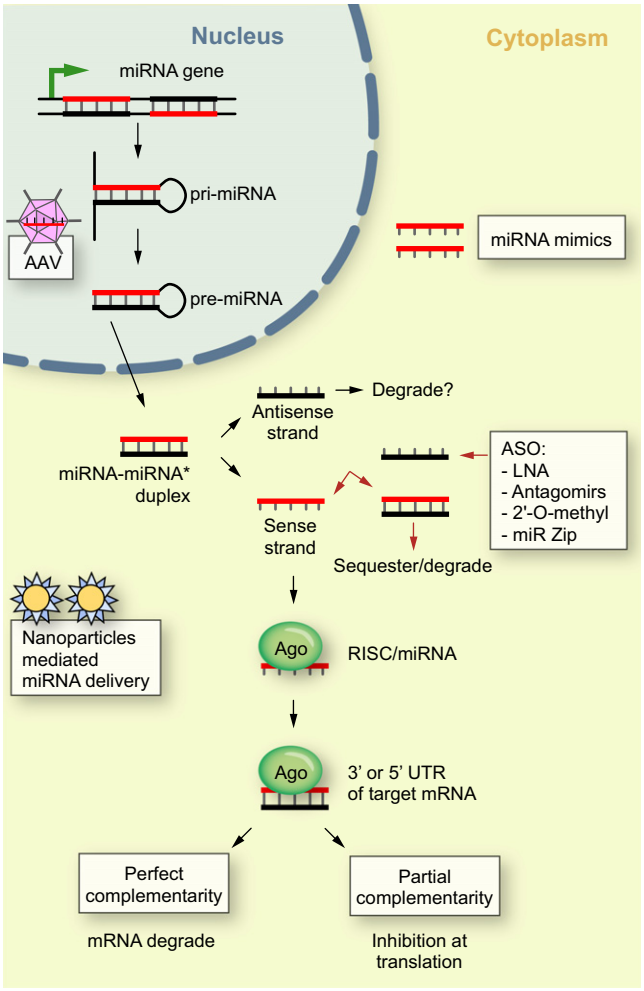


Fig. 1. miRNA biogenesis and therapeutic delivery. miRNA are transcribed as pri-miRNA in the nucleus and exported to the cytoplasm as pre-miRNA and later processed as mature miRNAs. Sense or guide strand is loaded into the Ago complex, which is named RNA inducing silencing complex (RISC). Depending on complementary of sense strand to target gene, either the target mRNA is degraded (100% complementary) or translation is inhibited (partial complementary). miRNA function can be inhibited by using antisense oligonucleotides (ASO) such as lock nucleic acid (LNA), antagomirs, miR ZIp, 2′-O-methyl, etc. Nanoparticles or adeno-associated vectors (AVV) can be used for tissue-specific anti-miRNA delivery particularly in *in vivo* systems. For overexpression of a particular miR, miRNA mimics can be used directly or delivery can be mediated by AAV or nanoparticles.

infection. The role of miR-122 has received attention in HCV infection as miR-122 has been shown to enhance the RNA abundance of HCV by targeting the viral 5′ non-coding region [10]. While the exact role of miR-122 in HCV replication is still not fully understood, *in vivo* studies in chronic HCV infected chimpanzees showed a potent inhibition of circulating HCV virus levels after administration of modified, anti-sense miR-122 molecules, so-called antagomirs [11]. HCV RNA levels were decreased in the animal receiving the highest dose of the miR-122 antagomir and this was associated with improvements in liver histology. Importantly, there was no evidence of viral breakthrough based on deep sequencing analysis of the viral 5′ non-coding region (NCR) [11]. These observations support the feasibility of disease manipulation in HCV infection with a miRNA-silencing approach. miR-122 has numerous target genes of which hemoxygenase-1 (HO-1), an inducible enzyme involved in oxidative stress and bilirubin metabolism, has been shown to affect both HCV and HBV virus levels [12]. It was shown that miR-122 inhibition with a miR-122 antagomir significantly increased HBsAg and HBeAg secretion in HuH7 cells [12]. Interestingly, miR-122 overexpression in HepG2 cells resulted in a marked reduction of HBsAg and HBeAg expression. This was associated with suppression of HO-1 and decreased HBV replication suggesting that miR-122 is antiviral for HBV, but proviral for HCV [12]. Further understanding of the role of miR-122 in hepatocytes and its role in the host–virus interactions will help reconcile these observations.

MicroRNA targets in fatty liver disease

Both alcoholic and non-alcoholic fatty livers (NAFLD) show dysregulation of miRNAs in animal models and in human samples [3,13]. In human NAFLD, 23 miRNAs regulating cell proliferation, apoptosis, inflammation, oxidative stress and metabolism were either overexpressed or underexpressed [14]. Changes in miRNAs in the metabolic syndrome that underpin NAFLD and non-alcoholic steatohepatitis (NASH) have recently been reviewed in relevance to potential therapeutic strategies [15].

In alcoholic liver disease, the role of inflammation and Kupffer cells (KCs) activation resulting in increased TNFα production has been long established. The Szabo laboratory has recently shown that upregulation of miR-155 in KCs after chronic alcohol feeding contributes to sensitization of KCs to gut-derived LPS [16]. Specifically, alcohol upregulated miR-155 via NF-κB activation, leading to stabilization of TNFα mRNA. Further studies are needed to evaluate the *in vivo* relevance of these findings by targeting miR-155 in KCs.

Liver fibrosis

Liver fibrosis is a complex process where activation of stellate cells and expression of matrix metalloproteinases and tissue inhibitors of metalloproteinase play important roles. A differential expression of miRNA profiles was reported in stellate cells from fibrotic rat livers [17]. In depth investigation of miR-150 and miR-194 revealed significantly reduced levels in fibrosis and found that both of these miRNAs inhibit stellate cell activation via inhibition of c-myb and rac1 expression. They also demonstrated that overexpression of miR-150 or miR-194 could reverse the activated phenotype of stellate cells in a human stellate cell line thereby identifying miR-150 and miR-194 as the potential therapeutic targets in fibrosis [17]. The family of miRNA-29 (miR-29a, 29b1, 29b2, and 29c) also plays a role in mouse models of fibrosis (carbon tetrachloride or bile duct ligation models) and as well in human [18]. Mechanistically, it was shown that treatment of hepatic stellate cells with TGFβ suppressed miR-29 expression suggesting that part of the fibrogenic effects of TGFβ is mediated via miR-29 downregulation [18]. TGFβ is also a direct target of miR-29 and thus, miR-29 may act as an amplifier for liver fibrosis. In another study, expression of miR-199a, 199a*, 200a, and 200b was found to be correlated with the degree of progression of liver fibrosis in chronic hepatitis C patients [19]. Overexpression of these miRNAs in LX-2 cells (hepatic stellate cell line) resulted in the induction of fibrotic genes such as *TIMP1*, *MMP13*, and alpha1 procollagen, indicating a role of these miRs in

Table 1. Aberrant microRNAs expression in liver disease and reported therapeutic approaches.

Disease	miRNAs	Expression	Delivery approach
Hepatitis C	miR-296, miR-351, miR-155, miR-146	Up	LNA, AAV, antagomirs, 2'-O-Me, antisense oligonucleotides*
	miR-29, miR-221, mir-222, miR-181a	Down	miRNA mimics*
Hepatitis B	miR-373, miR-370, miR-523	Up	Antisense oligonucleotides*, LNA*
	miR-122, miR-223, miR-29c, mir-199b, miR-198	Down	miRNA mimics*
NASH	miR-155, miR-34a, miR-146b, miR-200b,	Up	2′-O-Me (antagomirs), antisense oligonucleotides*
	miR-122, miR-617, miR-451	Down	miRNA mimics*
ALD	miR-155, miR-705, miR-1224, miR-212	Up	Antisense oligonucleotides*
	miR-182, miR-183, miR-199	Down	miRNA mimics*
Drug-induced liver injury	miR-466 family, miR-710, miR-328, miR-721, miR-1224	Up	LNA, AAV, antagomirs, nanoparticles (potential approaches)
	miR-122, miR-101a, miR-29 family, miR-101b, miR-21, miR-19b	Down	miRNA mimics, AAV (potential approaches)
HCC	miR-21, miR-221, miR-22, miR-15, miR-517a	Up	AAV, LNA, antisense oligonucleotides*
	miR-122, miR-29 family, miR-26a, miR-124, let-7 family members, miR-199a/b-3p	Down	miRNA mimics*, AAV
Liver fibrosis	miR-199a, miR-200, miR-21, let-7 family members, mir-223, miR-34c	Up	Antisense oligonucleotides*
	miR-29 family, miR-122, miR-146a, miR-15b, miR-150, miR-194	Down	miRNA mimics*

*_In vitro_ studies.
LNA, lock nucleic acid; AAV, adeno-associated vector.

the initiation of liver fibrosis [19]. The role of miRNAs in hepatic stellate cell differentiation has been reviewed elsewhere [7]. From the potential therapeutic point of view, it is tempting to speculate that overexpression or inhibition of miRNAs could attenuate liver fibrosis. However, tissue and cell-specific delivery of miRNA duplexes remains to be a technical challenge and has been elegantly reviewed [20].

HCC and other cancers in the liver

Numerous studies indicate that loss of miRNAs can contribute to liver tumorigenesis. It was shown that ectopic expression of a single miRNA, miR-26a, could reverse HCC progression in a mouse model of liver cancer [21]. In another study, myc-induced liver tumors in mice resulted in concomitant downregulation of selective miRNAs, including miR-15a/16, miR-26a, miR-24a, miR-150, and miR-195 [22]. MiR-26a regulates the expression of cyclin D2 and E2 and induces G1 arrest of human liver cancer cells; thus, disturbed expression of miR-26a dysregulates cell cycle and proliferation as seen in liver cancer cells but inhibition of miR-26a through therapeutic modulation could prevent these devastating effects. Indeed, overexpression of miR-26a in human liver tumor cells resulted in an increased number of cells in G1 stage and fewer cells in the proliferative S cycle [21]. *In vivo* data was consistent with this result, as forced expression of miR-26a with an AAV2 vector prevented development of liver tumors [21].

Experimental data suggest that, in addition to HCC, efficient *in vivo* targeting can also attenuate metastatic tumors in the liver. The pro-metastatic miRNA-182 was the target of therapeutic intervention. *In vitro* inhibition of miR-182 results in apoptosis and represses invasion in melanoma metastasis. Anti-miR-182 oligonucleotide, synthesized with 2′ sugar modifications and a phosphothioate backbone, was used in a mouse model of melanoma and it was found that anti-miR-182 treatment resulted in a lower burden of liver metastasis [23].

Methods to modulate the abundance of miRNAs in the liver

The above examples reveal that modulation of microRNA expression in the liver can have dramatic consequences on liver pathology and disease. Thus, it is very desirable to be able to express miRNAs and to sequester miRNAs in the liver.

Adeno-associated virus (AAV)-mediated gene delivery

It is known that adeno-associated virus (AAV) vectors can be used to express genes or small non-coding RNAs in the liver. AAV vectors can be designed to express precursors or small interfering inhibitors of miRNAs with relatively good specificity in hepatocyte delivery. Among AAV serotypes, the use of AAV8 was found to be advantageous since this envelope does not seem to be associated with human AAV infections and thus immunological memory should not be a concern when using it clinically [24]. In contrast, the use of AAV2 for *in vivo* therapy was limited because of early immunologic memory of AAV infections [25]. More recently, AAV9 vector was shown to have liver specificity. Expression of miR-122 target sequences into the 3′-UTR of AAV vectors revealed liver-specific delivery without affecting other organs, including the heart [24]. Yet, AAV-expressed genes are presumably unmodified and, thus, their affinity to target RNAs and their turnover cannot easily be manipulated.

For decades, researchers in both academia and industry have studied methods to deliver small, efficacious oligonucleotides to specific target organs in animals. While it has been difficult to target oligonucleotides to any organ, recent advantages have shown that the liver can take up, especially single-stranded oligonucleotides either unformulated or in particle-associated complexes.

Delivery of antisense oligonucleotides (antagomirs) to the liver

Antagomirs are nucleic acid sequences with antisense polarity to miRNAs. They sequester miRNAs in small duplexes that do not induce innate immune responses. To enhance stability in serum and in cells, antagomirs can be further chemically modified by introducing different moieties such as "2′-O-methoxyethyl groups" and phosphothioate linkages. Their uptake by the liver can also be enhanced by adding cholesterol moieties [26].

One of the champion antagomirs are locked nucleic acids (LNA) that consist of a class of bicyclic conformational analogues of RNA. LNA modifications comprise one or more nucleotide building blocks in which a methylene bridge connects the ribose moiety either in the C3′-endo or C2′-endo conformation [27]. Addition of LNA to DNA or RNA not only enhances the affinity for complementary sequences but also increases the stability *in vivo* [27]. LNAs directed against miR-122, which are applied to non-human primates [11] and humans by either intravenous or subcutaneous injection, locate quickly to the liver where they sequester miR-122 for as long as seven weeks, after which the LNAs get secreted from the organism. Importantly, cholesterol abundance dropped in treated animals without any adverse effects on liver function [11]. This finding suggests that LNAs can be used to functionally sequester the highly abundant, low-turnover guide strand of miR-122, without any off-targets effects from the passenger miR-122 strand. In general, the off-target effects can be specific (non-targeted mRNA) and non-specific (immune activation) and are reviewed in detail in [20].

Delivery of small duplex RNAs using cationic lipid-based and nanoparticle-based delivery systems

While delivery of small, single-stranded RNA to the liver is now possible, the delivery of small interfering RNAs or duplex miRNA mimetics to the liver is very cumbersome. In contrast to LNAs, duplex RNAs need to be delivered by cationic lipids or nanoparticles. However, exit from the delivery vehicles or the cellular endosomes is very inefficient. While off-target effects of the passenger strand can be minimized by chemical modification of the RNA duplex, activation of toll-like receptors and other innate immune responses needs to be carefully monitored.

A recent report described a method to deliver miR-133b mimics into lung tissue. To increase the effectiveness of cellular uptake, cationic lipids were used to condense miRNAs to form lipoplexes [28]. Pre-miR-133b containing lipoplexes were delivered into mice via tail vein to enhance miR-133b levels in lung and around 30% pre-miR-133b lipoplexes were found in lung tissue. Yet, despite increased accumulation of pre-miR-133b lipoplexes in the lung, the complexes were also found in other organs such as kidney, liver, and spleen.

Nanoparticle-mediated miRNA delivery is gaining attention in the field. In the past, lipid nanoparticles (LNPs) have been successfully used to deliver RNA duplexes in the liver, including stellate cells [29]. Later on, anti-miR-132-containing nanoparticles specifically targeted to integrin $\alpha_V\beta_3$ receptor were delivered into mice to restore p120RasGAP expression in the tumor endothelium [30]. The advantage of this formulation is that it specifically targets the $\alpha_v\beta_3$ integrin-positive endothelial cells. *In vivo* silencing of miR-122 by interfering nanoparticles was recently reported in mice [31]. Recently PEGylated LPH (liposome-polycation-hyaluronic acid) nanoparticles along with modified cyclic RGD peptide (cRGD) were used for specific and efficient delivery of anti-mir-296 AMO in mice [32]. These examples show that miRNA duplexes can be delivered to specific tissues, yet off-target effects and effects on innate immune responses need to be examined as discussed above.

Remaining challenges

Despite the growing literature in miRNA field, miRNA-based therapies are still in infancy. There are several challenges in the field because of lack of tissue/cell specificity, suboptimal delivery systems, insufficient cellular uptake, off-target effects and cellular toxicity. While the development of novel therapeutic strategies mostly concentrates on silencing of miRNAs using various RNA interference approaches, restoration of insufficiently expressed miRNAs needs to be considered in the potential therapeutic armamentaria. In conclusion, while improvements in miRNA delivery systems are encouraging, more work is needed to evaluate their long-term *in vivo* efficacy, specificity, and systemic toxicity prior to future translation of these emerging technologies and strategies to direct patient care.

Financial support

Supported by NIAAA Grant # AA011576 (G.S.), NIAID Grant # AI069000 (P.S.).

Conflict of interest

The underlying research reported in this study was funded by the NIH Institutes of Health.

References

[1] Lee RC, Feinbaum RL, Ambros V. The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 1993;5:843–854.
[2] Bala S, Marcos M, Szabo G. Emerging role of microRNAs in liver diseases. World J Gastroenterol 2009;45:5633–5640.
[3] Kerr TA, Korenblat KM, Davidson NO. MicroRNAs and liver disease. Transl Res 2011;4:241–252.
[4] Davis BN, Hata A. Regulation of microRNA biogenesis: a miRiad of mechanisms. Cell Commun Signal 2009;7:18.
[5] Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell 2009;2:215–233.
[6] Garzon R, Liu S, Fabbri M, Liu Z, Heaphy CE, Callegari E, et al. MicroRNA-29b induces global DNA hypomethylation and tumor suppressor gene reexpression in acute myeloid leukemia by targeting directly DNMT3A and 3B and indirectly DNMT1. Blood 2009;25:6411–6418.
[7] Lakner AM, Bonkovsky HL, Schrum LW. microRNAs: fad or future of liver disease. World J Gastroenterol 2011;20:2536–2542.

[8] Gatfield D, Le Martelot G, Vejnar CE, Gerlach D, Schaad O, Fleury-Olela F, et al. Integration of microRNA miR-122 in hepatic circadian gene expression. Genes Dev 2009;11:1313–1326.
[9] Roberts AP, Lewis AP, Jopling CL. miR-122 activates hepatitis C virus translation by a specialized mechanism requiring particular RNA components. Nucleic Acids Res 2011;39:7716–7729.
[10] Jopling CL, Yi M, Lancaster AM, Lemon SM, Sarnow P. Modulation of hepatitis C virus RNA abundance by a liver-specific MicroRNA. Science 2005;5740:1577–1581.
[11] Lanford RE, Hildebrandt-Eriksen ES, Petri A, Persson R, Lindow M, Munk ME, et al. Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection. Science 2010;5962:198–201.
[12] Qiu L, Fan H, Jin W, Zhao B, Wang Y, Ju Y, et al. MiR-122-induced down-regulation of HO-1 negatively affects miR-122-mediated suppression of HBV. Biochem Biophys Res Commun 2010;4:771–777.
[13] Cheung O, Sanyal AJ. Role of microRNAs in non-alcoholic steatohepatitis. Curr Pharm Des 2010;17:1952–1957.
[14] Cheung O, Puri P, Eicken C, Contos MJ, Mirshahi F, Maher JW, et al. Nonalcoholic steatohepatitis is associated with altered hepatic MicroRNA expression. Hepatology 2008;6:1810–1820.
[15] Czech MP, Aouadi M, Tesz GJ. RNAi-based therapeutic strategies for metabolic disease. Nat Rev Endocrinol 2011;8:473–484.
[16] Bala S, Marcos M, Kodys K, Csak T, Catalano D, Mandrekar P, et al. Up-regulation of microRNA-155 in macrophages contributes to increased tumor necrosis factor alpha (TNF{alpha}) production via increased mRNA half-life in alcoholic liver disease. J Biol Chem 2011;2:1436–1444.
[17] Venugopal SK, Jiang J, Kim TH, Li Y, Wang SS, Torok NJ, et al. Liver fibrosis causes downregulation of miRNA-150 and miRNA-194 in hepatic stellate cells, and their overexpression causes decreased stellate cell activation. Am J Physiol Gastrointest Liver Physiol 2010;1:G101–G106.
[18] Roderburg C, Urban GW, Bettermann K, Vucur M, Zimmermann H, Schmidt S, et al. Micro-RNA profiling reveals a role for miR-29 in human and murine liver fibrosis. Hepatology 2011;1:209–218.
[19] Murakami Y, Toyoda H, Tanaka M, Kuroda M, Harada Y, Matsuda F, et al. The progression of liver fibrosis is related with overexpression of the miR-199 and 200 families. PLoS One 2011;1:e16081.
[20] Singh S, Narang AS, Mahato RI. Subcellular fate and off-target effects of siRNA, shRNA, and miRNA. Pharm Res 2011;12:2996–3015.
[21] 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;6:1005–1017.
[22] Chang TC, Yu D, Lee YS, Wentzel EA, Arking DE, West KM, et al. Widespread microRNA repression by Myc contributes to tumorigenesis. Nat Genet 2008;1:43–50.
[23] Huynh C, Segura MF, Gaziel-Sovran A, Menendez S, Darvishian F, Chiriboga L, et al. Efficient in vivo microRNA targeting of liver metastasis. Oncogene 2011;12:1481–1488.
[24] Qiao C, Yuan Z, Li J, He B, Zheng H, Mayer C, et al. Liver-specific microRNA-122 target sequences incorporated in AAV vectors efficiently inhibits transgene expression in the liver. Gene Ther 2011;4:403–410.
[25] Mingozzi F, Maus MV, Hui DJ, Sabatino DE, Murphy SL, Rasko JE, et al. CD8(+) T-cell responses to adeno-associated virus capsid in humans. Nat Med 2007;4:419–422.
[26] Esau C, Davis S, Murray SF, Yu XX, Pandey SK, Pear M, et al. MiR-122 regulation of lipid metabolism revealed by in vivo antisense targeting. Cell Metab 2006;2:87–98.
[27] Elmen J, Lindow M, Silahtaroglu A, Bak M, Christensen M, Lind-Thomsen A, et al. Antagonism of microRNA-122 in mice by systemically administered LNA-antimiR leads to up-regulation of a large set of predicted target mRNAs in the liver. Nucleic Acids Res 2008;4:1153–1162.
[28] Wu Y, Crawford M, Yu B, Mao Y, Nana-Sinkam SP, Lee LJ. MicroRNA delivery by cationic lipoplexes for lung cancer therapy. Mol Pharm 2011;4: 1381–1389.
[29] Akinc A, Goldberg M, Qin J, Dorkin JR, Gamba-Vitalo C, Maier M, et al. Development of lipidoid-siRNA formulations for systemic delivery to the liver. Mol Ther 2009;5:872–879.
[30] Anand S, Majeti BK, Acevedo LM, Murphy EA, Mukthavaram R, Scheppke L, et al. MicroRNA-132-mediated loss of p120RasGAP activates the endothelium to facilitate pathological angiogenesis. Nat Med 2010;8:909–914.
[31] Su J, Baigude H, McCarroll J, Rana TM. Silencing microRNA by interfering nanoparticles in mice. Nucleic Acids Res 2011;6:e38.
[32] Liu XQ, Song WJ, Sun TM, Zhang PZ, Wang J. Targeted delivery of antisense inhibitor of miRNA for antiangiogenesis therapy using cRGD-functionalized nanoparticles. Mol Pharm 2011;1:250–259.