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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.

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

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* 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).

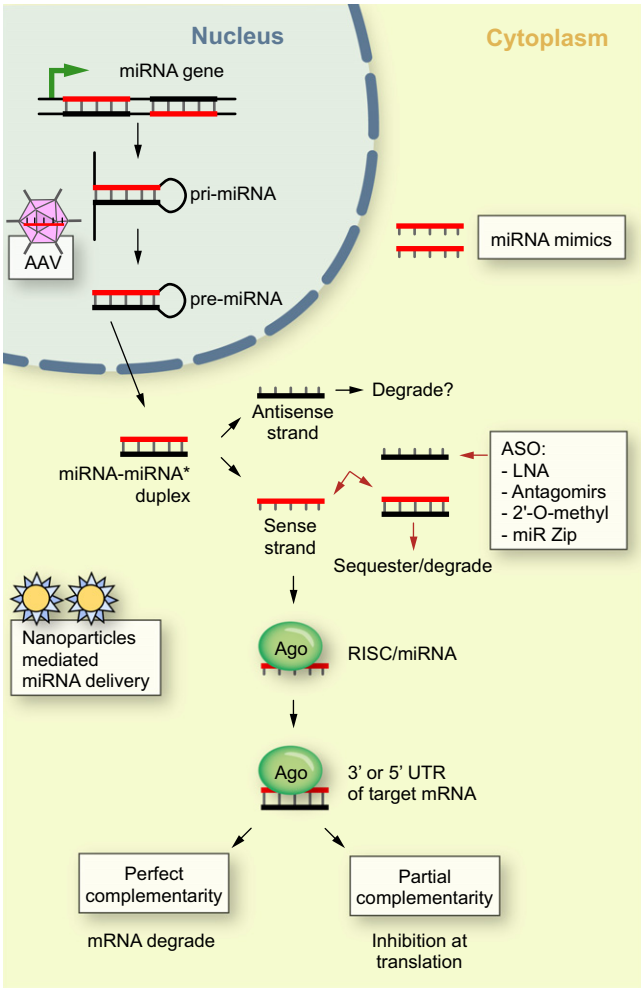

Fig. 1. miRNA biogenesis and therapeutic delivery. miRNA are transcribed as pri-miRNA in the nucleus and exported to the cytoplasm as pre-miRNA and later processed as mature miRNAs. Sense or guide strand is loaded into the Ago complex, which is named RNA inducing silencing complex (RISC). Depending on 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