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TOPIC HIGHLIGHT

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MicroRNAs as controlled systems and controllers in non-alcoholic fatty liver disease

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is a multi-faceted condition including simple steatosis alone or associated with inflammation and ballooning (non-alcoholic steatohepatitis) and eventually fibrosis. The NAFLD incidence has increased over the last twenty years becoming the most frequent chronic liver disease in industrialized countries. Obesity, visceral adiposity, insulin resistance, and many other disorders that characterize metabolic syndrome are the major predisposing risk factors for NAFLD. Furthermore, different factors, including genetic background, epigenetic mechanisms and environmental factors, such as diet and physical exercise, contribute to NAFLD development and progression. Several lines of evidence demonstrate that specific microRNAs expression profiles are strongly associated with several pathological conditions including NAFLD. In NAFLD, microRNA deregulation in response to intrinsic genetic or epigenetic factors or environmental factors contributes to metabolic dysfunction. In this review we focused on microRNAs role both as controlled and controllers molecules in NAFLD development and/or their eventual value as non-invasive biomarkers of disease.

Key words: Liver steatosis; Fibrosis; Non-alcoholic fatty liver disease; Non-alcoholic steatohepatitis; MicroRNAs

Core tip: Genetic background, epigenetic mechanisms and environmental factors contribute to Non-alcoholic fatty liver disease (NAFLD) development and progression. Among epigenetic mechanisms microRNAs are the most studied in NAFLD, contributing to metabolic dysfunction and liver damage. As during the last five years several authors investigated the role of microRNAs as potential therapeutic targets and/or non-invasive biomarkers we believe that this mini-review could be a good tool for researchers that critically approach this topic.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a multifactorial condition encompassing a wide spectrum of liver damage, ranging from simple steatosis non-alcoholic fatty liver (NAFL) to steatohepatitis non-alcoholic steatohepatitis (NASH). While NAFL is considered a relatively benign condition with little risk of progression, NASH, described as the inflammatory phase of steatosis, is

sometimes associated with fibrosis and may potentially progress to irreversible cirrhosis and in some cases to hepatocellular carcinoma (HCC)[1]. The interesting particularity of this pathology is that, even though the histopathological pattern of NAFLD/NASH resembles that observed during alcohol-induced liver damage, the disease occurs in subjects with no significant or even absent alcohol consumption. Furthermore, it also occurs in the absence of other underlying aetiology such as virus infection, autoimmune hepatitis, drug- induced and inherited metabolic disorders[2].

Alarming epidemiological data shows that the incidence of NAFLD has increased over the last twenty years becoming the most common cause of chronic liver disease worldwide[3-5]. Noteworthy, several studies now reveal the NAFLD prevalence in Western nations fluctuates between 20% and 30%, going up to 90% in obese individuals, while NASH affects approximately 2%-3% of the general population, rising up to 37% in the obese individuals[6,7].

It is now widely accepted that the major predisposing risk factors of NAFLD are represented by obesity, visceral adiposity, insulin resistance (IR) and many other disorders that define the metabolic syndrome[7]. Moreover, several clinical and experimental lines of evidence report a reciprocal relationship between NAFLD and diabetes onset[8].

On the other hand, different factors including genetic background, epigenetic mechanisms and environmental factors, as diet and physical exercise, participate in NAFLD development. Additionally, adipose tissue and gut may intervene in establishing a complicated cross-talk causing the production and the release of circulating endotoxins, adipokines and pro-inflammatory cytokines, which in turn contribute to IR and free fatty acids (FFAs) accumulation bursting oxidative stress and hepatocellular damage[9,10]. To further complicate this scenario, NAFLD is still a condition under-diagnosed and there is currently no specific drug therapy approved. In fact, the mainstay of NAFLD treatment is lifestyle modification in term of weight loss and diet changes. However, the majority of paediatric and adult patients have difficulty to achieve and to maintain weight loss, thus reducing the beneficial long-term effects of lifestyle intervention[11,12]. In the latest years, it has been widely documented that anomalous microRNA (miRNA or miR) profiles are strongly associated with several pathological conditions including some kind of cancers and liver, heart, kidney and autoimmune diseases[12]. Furthemore, the deregulation of microRNA expression in response to intrinsic factors (genetic or epigenetic) or extrinsic factors (environmental challenge or stress conditions) may also contribute to metabolic dysfunction[13]. Therefore, it is not surprising that numerous recent evidence reports altered expression patterns of microRNAs in NAFLD, suggesting their role in the pathogenesis of the disease.

Given its importance, in this review we will discuss the recent emerging implication of microRNAs both as controlled and controllers in NAFLD development and/or their eventual prognostic value of disease progression.

MICRORNA CONTROLLED SYSTEMS AND FUNCTION

In the world of non-coding RNAs (ncRNAs), microRNAs are small endogenous single-stranded RNA molecules acting in transcriptional and post-transcriptional regulation of gene expression. Conserved across diverse species including animals, plants and even viruses, microRNAs exert their function of base-pairing with complementary sequences within mRNA targets, usually resulting in gene silencing through translational repression or mRNA stability alteration[14]. By the way, a positive regulation (transcriptional and translational activation) mediated by microRNAs is also possible[15-19]. Since the discovery of microRNAs in *C. elegans* over ten years ago[20], much attention has been paid to deeply understand microRNAs biology and regulatory function. At present, the latest versions of the miR dedicated Database (http://www.mirbase.org) list over 24521 entries and the number of publications dealing with microRNAs is growing day by day[21].

To date, it is estimated that these small molecules are likely to regulate approximately 30% of the human protein-coding genome[22] and are now recognized as key regulator of almost every cellular process including proliferation, apoptosis, differentiation and cellular growth[23,24]. Furthermore, in the latest years, mis-expression of microRNAs has been linked to numerous disease states and for this reason, microRNA-based therapies are under investigation[13].

The human genome encodes hundreds of microRNA genes classified as intergenic, intronic or exonic. Independently of their genomic location, microRNA genes can be found either as single genes or as clusters of genes under the control of their own promoter or the promoter of protein-coding genes[14].

The majority of microRNAs are transcribed by RNA polymerase Ⅱ (Pol Ⅱ) or occasionally RNA polymerase Ⅲ to generate a long hairpin precursor called pri-miRNA[25,26].

Pri-miRNAs are both capped at the 5' end and polyadenylated at the 3' end like other protein coding primary transcripts[27-29] but they have an unique hairpin structure that allow the microRNA biogenesis machinery to distinguish them from the various RNA stem-loop-like structures present in the nucleus[26].

In the canonical pathway, the precursor pri-miRNA is processed through a two-step mechanism by the ribonuclease (RNase) Ⅲ-family proteins Drosha and Dicer[30,31]. Actually, once the pri-miRNA is synthesized, it is cleaved by the nuclear Drosha, that together with the cofactor DGCR8 composes the microprocessor complex[32,33].

Many other proteins, such as helicases and heterogeneous nuclear ribonucleoproteins (hnRNPs) may take part to help, thus creating a complex regulatory network[34].

The cleavage gives rise to a hairpin structure of 60-110 nt, termed pre-miRNA. Pre-miRNA is then exported out from the nucleus to the cytoplasm by the cargo transporter Exportin-5/Ran-GTP complex, which also protects microRNAs from degradation[35,36]. However, some exceptions exist. In fact, it has been shown that intronic microRNAs (mirtrons) can bypass the Drosha cleavage step, undergoing splicing and debranching instead[37].

In either case, once in the cytosol, the RNaseⅢ enzyme Dicer-1 further processes pre-miRNA, producing an imperfect about 20-bp double-stranded miRNA duplex (miRNA/*miRNA)[31,38-41]. The duplex is composed by the guide strand and the passenger (or miRNA*) strand. The guide strand, generally the one with the lowest thermodynamic stability at its 5' end, is preferentially incorporated into the miRNA-induced silencing complex (miRISC)[33]. On the contrary, the passenger is excluded from the RISC and it is degraded[33,42,43]. Anyway, it should be kept in mind that sometimes miRNA* strands can be loaded into miRISC to function as microRNAs and that each strand of the duplex can be tissue-specific[44].

In mammals, the miRISC is mainly composed by the Argonaute (AGO) proteins, as core components, by the GW182 family proteins and by other accessory proteins. The formation and activity of the miRISC implies many additional factors, some of which play an important regulatory function[45]. As part of miRISC, the miRNA can induce translational repression or deadenylation and degradation of target mRNA usually through antisense base-pairing in the 3'-UTR of target mRNAs[46].

Whereas the full mechanism of microRNA-mediated translational repression is still poorly understood, more details are known about mRNA deadenylation[45-47]. In mammals and Drosophila the carboxy-terminal domain of GW182 proteins plays a central role interacting with the poly(A) binding protein (PABP) and recruiting the deadenylases CCR4 and CAF1 indeed[47,48].

In addition, in *D. melanogaster,* the C-terminal and the Ago-interacting N-terminal GW182 domains, contain other regions that are active in translational repression, opening numerous regulatory mechanisms[49].

Interestingly, in plants a typical miRISC-mediated gene regulation is guided by a perfect match between microRNAs and target RNAs that lead to an endonucleolytic cleavage and then to degradation. Even if rare, this mechanism can be found in animals too. However, microRNAs in plants may imperfectly base-pair to mRNAs, as occurs in mammals, and repress translation in this way[50].

MICRORNA AS REGULATORS AND CONTROLLERS IN NAFLD

Experimental evidence

Recently, it has been demonstrated that variations in the expression levels of some micro-RNAs are related to the pathogenesis of NAFLD and its progression to NASH. In fact, some of them are key regulators of glucose, cholesterol and lipid metabolism and their deregulation may result in intra-hepatic excessive accumulation of triglycerides and fatty acids[51].

One of the first microRNAs correlating with lipid metabolism and homeostasis is miR-122, the most abundant in the liver[52]. In fact, an important murine study showed that inhibition of miR-122 using antagomirs results in a 25%-30% reduction of plasma cholesterol levels and in an altered expression of several hepatic genes involved in the biosynthesis of cholesterol, as the 3-hydroxy-3-methylglutaryl-Coa synthase 1 (Hmgcs1), 3-hydroxy-3-methylglutaryl-Coa reductase (Hmgcr) and the 7-dehydrocholesterol reductase (Dhcr7)[53]. Furthermore, Esau *et al*[54] highlighted how the antisense-based silencing of miR-122 was able to induce a significant decrease in fatty acids and cholesterol biosynthesis and an increase in the fatty acids β-oxidation pathway in mice fed a high fat diet. Interestingly, targeting miR-122 by antisense inhibitors with "locked nucleic acid" (LNA) technique correlated with decreased circulating cholesterol levels in monkeys[55].

Recently, it has been found that miR-122 is involved also in the propagation of hepatitis C virus (HCV) suggesting that the silencing of this microRNA may represent a safe therapeutic approach for the treatment of both chronic HCV infection and diseases related to the alteration of cholesterol and lipid metabolism[56].

Further, the hepatic expression pattern of some crucial microRNAs has been analyzed in two interesting different animal models of NASH. In C57BL/6J and DBA/2J mice fed with methyl-deficient diet, the hepatic over-expression of miR-34a, miR-155, miR-200b and miR-221 and a concomitant down-regulation of miR-29c, miR-122, miR-192 and miR-203 was reported[57]. The microRNAs analysis on rats fed with hypercaloric diet enriched in fat (HFD) and fructose (HFD-HFr), highlighted a down-regulation of miR-27a, miR-122, miR-451 and an up-regulation of miR-200a, miR-200b and miR-429[58].

Moreover, in a recent paper of our group, mir-200b, together with miR-155, have been also linked with enhancer of zeste homolog 2 (EZH2) expression in both *in vivo* and *in vitro* models of NAFLD[59].

An additional microRNA involved in the regulation of lipid metabolism is miR-15b. Indeed, its potential contribution has been revealed through two studies: the first conducted on HFD fed rats and the second on the L02 hepatocyte cell line treated with palmitate. The data obtained by real-time PCR showed increases of miR-15b hepatic expression in both *in vivo* and *in vitro* NAFLD models compared to the controls. To further investigate miR-15b roles, miR-15b was over-expressed in QSG7701 hepatic cells, causing an increase in triglyceride levels and a decrease in glucose consumption. Therefore, these data collectively suggest that augmented expression of miR-15b can lead to an alteration in glucose and lipid metabolism, possibly increasing the risk of NAFLD onset[60]. An *in vitro* study conducted by Zheng *et al*[61] highlighted

Table 1 miRs deregulated in experimental models of non-alcoholic fatty liver disease

Ref.	miR	miR regulation	Experimental model
[55]	miR-122	Up-regulation	*In vivo:* Silencing by antisense-base technology in a diet induced obesity mouse model
[56]	miR-122	Up-regulation	*In vivo:* Inhibition by antisense oligonucleotides "locked nucleic acid" (LNA) in monkeys
[59]	miR-34a miR155 miR-200b miR-221 miR-29c	Up-regulation	*In vivo*: C57BL/6J and DBA/2J methyl-deficient diet mice fed
	miR-122 miR-192 miR-203 miR-200a	Down-regulation	
[60]	miR-200b miR-429 miR-27	Up-regulation	*In vivo*: HFD/HFr fed rats
	miR-122 miR-451	Down-regulation	
[61]	miR 200b miR 155	Up-regulation	*In vivo:* HFD/HFr fed rats *In vitro*: HepG2 hepatoma cell line upon FFA stimulation
[62]	miR-15b	Up-regulation	*In vivo*: HFD fed rats *In vitro*: L02 human hepatocyte cell line treated with palmitate
[63]	miR-10b	Up-regulation	*In vitro:* Steatotic model of human L02 hepatocytes
[66]	miR-103 mi-R107	Up-regulation	*In vivo:* Dietary obese rats
[71,72]	miR-34a	Up-regulation	*In vivo:* Dietary obese mice

HFD/HFr: Hypercaloric diet enriched in fat/fructose.

the miR-10b involvement in NAFLD pathogenesis. In fact, miR-10b was found altered in steatotic hepatic cells. More precisely, when over-expressed, miR-10b induces an increase in the cellular lipid content and in the triglycerides levels acting on peroxisome proliferator-activated receptor-α (PPARα), a nuclear receptor involved in the catabolism of fatty acids. Other post-transcriptional regulators of lipid metabolism seem to be miR-33a and miR-33b, intronic microRNAs, located within SREBF2 and SREBF1 genes, respectively, both implicated in the positive expression of cholesterogenic and lipogenic genes[62].

Notably, several studies reported a tight co-expression of miR-33a/b with their host genes, with whom they act in concert to promote hepatic lipogenesis[63]. However, other microRNAs, such as miR-103 and miR-107, which are up-regulated in diet-induced obese rats, have been associated with metabolic disorders related to IR development. Interestingly, given that the antisense-mediated silencing of miR-103 and 107 improved insulin sensitivity, these two microRNAs might be used as therapeutic targets to improve obesity IR-related[64]. Moreover, miR-33a/b play also a key role in post-transcriptional repression of ATP-binding cassette transporter sub-family A member 1 (ABCA1), which is essential for the binding of cholesterol to apolipoprotein A1 (APOA1) during HDL formation. The mechanisms involves the direct binding of miR-33 to the 3' UTR of ABCA1 gene[65]. Interestingly, it has been reported that miR-33a/b are involved in lipid and glucose homeostasis also interacting with the deacetylases NAD+ dependent sirtuin 6 (SIRT6), which in turn regulates the expression of Sterol Regulatory Element-Binding Protein (SREBP) target genes[66]. In fact, it has been demonstrated that SIRT6 knockout is associated with the development of liver steatosis in mice[67]. In line with previous published papers, among SIRT6 regulators, the NAD+-dependent deacetylase sirtuin (SIRT1) seem to play an important function in mice. SIRT1 complexes with several factors such as FOXO3a and NRF1 on the SIRT6 promoter regulates positively SIRT6 expression, which, in turn, negatively regulates glycolysis, triglyceride synthesis, and fat metabolism acting on the transcription of many genes involved in these processes[67]. According to the literature, SIRT1 is further regulated at post-transcription level by miR-34a. Cantó *et al*[68], reviewed that miR-34a inhibits the expression of SIRT1 and prevents the activation of PPARα and of liver X receptor (LXR), a key regulator of energy homeostasis. In addition, the repression of SIRT1 results in inhibition of both SREBP and nuclear factor-κB (NF-κB), a critical mediator of inflammation. Moreover, recent research has shown that miR-34a reduces levels of NAD+ and SIRT1 activity by acting directly on the nicotinamidide phosphoribosyl transferase (NAMPT) required for NAD+ biosynthesis, resulting in a decrease in β-oxidation and in an increase in lipogenesis. Besides, miR-34a silencing in obese mice leads to the restoration of NAMPT/NAD+ levels relieving hepatic steatosis and inflammation[69]. Notably, miR-34a also acts on the repression of β-Klotho, a coreceptor of the fibroblast growth factor 19 (FGF19), implicated in the regulation of glucose metabolism in the post-prandial response under physiological conditions. This evidence comes from a paper showing that antisense inhibition of miR-34a in obese mice is able to restore both β-Klotho levels and FGF19 target gene expression improving obesity[70]. Studies reporting experimental evidence of microRNA deregulation during NAFLD are summarized in Table 1.

Table 2 miRs deregulated in human non-alcoholic fatty liver disease

Ref.	miR	miR regulation	Source	Phenotype
[74]	miR-34a	Up-regulation	Blood	Only miR-122 is correlated with the severity of liver steatosis
	miR-122			
	miR-21			
	miR-451			
[75]	miR-122	Up-regulation	Blood	Positive correlation with fibrosis, inflammation and hepatic steatosis
	miR-34a			
	miR-16			
[77]	miR-122	Up-regulation	Blood/liver	From mild to severe hepatosteatosis
	miR-122	Down-regulation		End-stage liver fibrosis
[79]	miR-15b	Up-regulation	Blood	Positive correlation with NAFLD parameters
[80]	miR-34a	Up-regulation	Liver	Association between NASH and altered hepatic miRNA expression
	miR-146	Down-regulation	Liver	
	miR-122			

NAFLD: Non-alcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis.

microRNA as biomarkers in NAFLD

Since serum miRNAs have been recognized as diagnostic and prognostic markers for lung, prostate, breast, ovarian, and liver cancers, the overall idea is to identify the altered microRNA expression profiles potentially associated with the pathogenesis of NAFLD and eventually to evaluate serum levels of microRNAs as potential non-invasive biomarkers for the diagnosis and monitoring of disease[71]. The microRNAs suggested as potential biomarkers for human NAFLD are reported in Table 2.

A recent study conducted by Yamada *et al*[72] on Japanese subjects, adds evidence on the possibility to use circulating microRNAs as biomarkers for NAFLD. In this study, serum levels of five selected miRNAs (miR-145, miR-451, miR-122, miR-34a and miR-21), well known to be implicated in cholesterol and fatty acid homeostasis in liver tissue, were evaluated in individuals with hepatic steatosis assessed by ultrasound scan. Here the authors found increased levels of miR-34a, miR-122, miR-21 and miR-451 in NAFLD patients compared to non-NAFLD participants. These findings are in line with other previous human studies showing that altered expression of circulating levels of these and other microRNAs are related to NAFLD pathogenesis. Indeed, in a human study by Cermelli *et al*[73] serum levels of miR-122 and miR-34a were found significantly increased in patients with NASH, showing a positive correlation with fibrosis and inflammation stage. The same study also found a positive correlation between increased circulating levels of miR-16 and simple hepatic steatosis. The possible explanation of miR-34 mis-expression lies on the fact that miR-34a acts by inducing pro-apoptotic genes and *p53* transcription, thus sustaining the liver damage occurring during NAFLD[74]. In a more recent human study in NAFLD patients, serum and hepatic miR-122 levels were found increased moving from mild to severe steatosis. In contrast, a significant inverse correlation with progression of fibrotic liver damage was observed[75]. This particular expression trend of miR-122 could be explained by molecular mechanisms occurring during the progression of the disease. Persistent liver injury results in loss of hepatocytes, the main miR-122 producing cells, and thus may determine an inadequate miR-122 expression[73]. Another mechanism supporting the link between miR-122 and fibrotic liver damage is the inhibitory effect of this miR on hepatic stellate cell activation and collagen deposition[76]. Taken together, this evidence suggests that the measure of serum miR-122 levels can provide a useful predictive marker of liver fibrosis in patients with NAFLD.

In a recent cohort study of patients with NAFLD, the increase of body mass index, blood glucose or alanine transferases levels, were interestingly associated with a parallel increase in miR-15b serum levels, suggesting a potential role also for miR-15b as a diagnostic candidate[62]. However, comparing studies on circulating levels and hepatic expression of microRNAs in NAFLD it is possible to find interesting differences. Indeed, in a study of hepatic microRNA expression patterns, miR-34a and miR-146b were significantly increased in livers of NASH subjects while decreased expression of miR-122 was found in the majority of patients[77]. In Table 3 the most important NAFLD-related miRNAs with known and validated target genes are reported.

CONCLUSION

Despite numerous research efforts, NAFLD molecular pathogenesis is only partially understood. Recent evidence indicates, that during NAFLD development and progression, some tissue-specific or circulating microRNAs may play crucial roles in the post-transcriptional regulation of genes that regulate critical process in liver damage associated with this disease. In this picture, therefore, the study of microRNA could assume a dual significance. On one hand, the comprehension of regulatory upstream/downstream factors associated with these microRNAs might provide a source of novel potential therapeutic targets to block NAFLD progression. On the other hand, the analysis of the microRNA expression pattern in NAFLD might highlight which of them have a strong correlation with liver damage and possess adequate characteristics as

Table 3 Key miRs involved in non-alcoholic fatty liver disease and their target genes

Ref.	miR	Target genes
[55,56]	miR-122	Hmgcs1, Hmgcr, Dhcr7
[70-72]	miR-34a	SIRT1, NAMPT, β-Klotho coreceptor
[63]	miR-10b	PPAR-α
[66]	miR-33a/b	SIRT6
		ABCA1

non-invasive biomarkers.

REFERENCES

1 **De Minicis S**, Day C, Svegliati-Baroni G. From NAFLD to NASH and HCC: pathogenetic mechanisms and therapeutic insights. *Curr Pharm Des* 2013; **19**: 5239-5249 [PMID: 23394093 DOI: 10.2174/1381612811319290006]
2 **Brunt EM**. Pathology of nonalcoholic fatty liver disease. *Nat Rev Gastroenterol Hepatol* 2010; **7**: 195-203 [PMID: 20195271 DOI: 10.1038/nrgastro.2010.21]
3 **Browning JD**, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, Grundy SM, Hobbs HH. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology* 2004; **40**: 1387-1395 [PMID: 15565570 DOI: 10.1002/hep.20466]
4 **Bellentani S**, Scaglioni F, Marino M, Bedogni G. Epidemiology of non-alcoholic fatty liver disease. *Dig Dis* 2010; **28**: 155-161 [PMID: 20460905 DOI: 10.1159/000282080]
5 **Milić S**, Stimac D. Nonalcoholic fatty liver disease/steatohepatitis: epidemiology, pathogenesis, clinical presentation and treatment. *Dig Dis* 2012; **30**: 158-162 [PMID: 22722431 DOI: 10.1159/000336669]
6 **Barshop NJ**, Francis CS, Schwimmer JB, Lavine JE. Nonalcoholic fatty liver disease as a comorbidity of childhood obesity. *Ped Health* 2009; **3**: 271-281 [PMID: 20556232 DOI: 10.2217/phe.09.21]
7 **Fabbrini E**, Sullivan S, Klein S. Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications. *Hepatology* 2010; **51**: 679-689 [PMID: 20041406 DOI: 10.1002/hep.23280]
8 **Williams KH**, Shackel NA, Gorrell MD, McLennan SV, Twigg SM. Diabetes and nonalcoholic Fatty liver disease: a pathogenic duo. *Endocr Rev* 2013; **34**: 84-129 [PMID: 23238855 DOI: 10.1210/er.2012-1009]
9 **Day CP**. Genetic and environmental susceptibility to non-alcoholic fatty liver disease. *Dig Dis* 2010; **28**: 255-260 [PMID: 20460920 DOI: 10.1159/000282098]
10 **Nobili V**, Svegliati-Baroni G, Alisi A, Miele L, Valenti L, Vajro P. A 360-degree overview of paediatric NAFLD: recent insights. *J Hepatol* 2013; **58**: 1218-1229 [PMID: 23238106 DOI: 10.1016/j.jhep.2012.12.003]
11 **Corrado RL**, Torres DM, Harrison SA. Review of treatment options for nonalcoholic fatty liver disease. *Med Clin North Am* 2014; **98**: 55-72 [PMID: 24266914 DOI: 10.1016/j.mcna.2013.09.001]
12 **Nobili V**, Alisi A, Raponi M. Pediatric non-alcoholic fatty liver disease: preventive and therapeutic value of lifestyle intervention. *World J Gastroenterol* 2009; **15**: 6017-6022 [PMID: 20027672 DOI: 10.3748/wjg.15.6017]
13 **Sayed D**, Abdellatif M. MicroRNAs in development and disease. *Physiol Rev* 2011; **91**: 827-887 [PMID: 21742789 DOI: 10.1152/physrev.00006.2010]
14 **Ul Hussain M**. Micro-RNAs (miRNAs): genomic organisation, biogenesis and mode of action. *Cell Tissue Res* 2012; **349**: 405-413 [PMID: 22622804 DOI: 10.1007/s00441-012-1438-0]
15 **Vasudevan S**, Steitz JA. AU-rich-element-mediated upregulation of translation by FXR1 and Argonaute 2. *Cell* 2007; **128**: 1105-1118 [PMID: 17382880]
16 **Vasudevan S**, Tong Y, Steitz JA. Switching from repression to activation: microRNAs can up-regulate translation. *Science* 2007; **318**: 1931-1934 [PMID: 18048652 DOI: 10.1126/science.1149460]
17 **Ørom UA**, Nielsen FC, Lund AH. MicroRNA-10a binds the 5'UTR of ribosomal protein mRNAs and enhances their translation. *Mol Cell* 2008; **30**: 460-471 [PMID: 18498749 DOI: 10.1016/j.molcel.2008.05.001]
18 **Henke JI**, Goergen D, Zheng J, Song Y, Schüttler CG, Fehr C, Jünemann C, Niepmann M. microRNA-122 stimulates translation of hepatitis C virus RNA. *EMBO J* 2008; **27**: 3300-3310 [PMID: 19020517 DOI: 10.1038/emboj.2008.244]
19 **Lee S**, Vasudevan S. Post-transcriptional stimulation of gene expression by microRNAs. *Adv Exp Med Biol* 2013; **768**: 97-126 [PMID: 23224967 DOI: 10.1007/978-1-4614-5107-5_7]
20 **Lee RC**, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell* 1993; **75**: 843-854 [PMID: 8252621 DOI: 10.1016/0092-8674(93)90529-Y]
21 **Kozomara A**, Griffiths-Jones S. miRBase: integrating microRNA annotation and deep-sequencing data. *Nucleic Acids Res* 2011; **39**: D152-D157 [PMID: 21037258 DOI: 10.1093/nar/gkq1027]
22 **Lewis BP**, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell* 2005; **120**: 15-20 [PMID: 15652477 DOI: 10.1016/j.cell.2004.12.035]
23 **Ghildiyal M**, Zamore PD. Small silencing RNAs: an expanding universe. *Nat Rev Genet* 2009; **10**: 94-108 [PMID: 19148191 DOI: 10.1038/nrg2504]
24 **Bartel DP**. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell* 2004; **116**: 281-297 [PMID: 14744438 DOI: 10.1016/S0092-8674(04)00045-5]
25 **Cullen BR**. Transcription and processing of human microRNA precursors. *Mol Cell* 2004; **16**: 861-865 [PMID: 15610730 DOI: 10.1016/j.molcel.2004.12.002]
26 **Lee Y**, Kim M, Han J, Yeom KH, Lee S, Baek SH, Kim VN. MicroRNA genes are transcribed by RNA polymerase II. *EMBO J* 2004; **23**: 4051-4060 [PMID: 15372072 DOI: 10.1038/sj.emboj.7600385]
27 **Berezikov E**. Evolution of microRNA diversity and regulation in animals. *Nat Rev Genet* 2011; **12**: 846-860 [PMID: 22094948 DOI: 10.1038/nrg3079]
28 **Bracht J**, Hunter S, Eachus R, Weeks P, Pasquinelli AE. Trans-splicing and polyadenylation of let-7 microRNA primary transcripts. *RNA* 2004; **10**: 1586-1594 [PMID: 15337850 DOI: 10.1261/rna.7122604]
29 **Cai X**, Hagedorn CH, Cullen BR. Human microRNAs are processed from capped, polyadenylated transcripts that can also function as mRNAs. *RNA* 2004; **10**: 1957-1966 [PMID: 15525708 DOI: 10.1261/rna.7135204]
30 **Lee Y**, Ahn C, Han J, Choi H, Kim J, Yim J, Lee J, Provost P, Rådmark O, Kim S, Kim VN. The nuclear RNase III Drosha initiates microRNA processing. *Nature* 2003; **425**: 415-419 [PMID: 14508493 DOI: 10.1038/nature01957]
31 **Gregory RI**, Yan KP, Amuthan G, Chendrimada T, Doratotaj B, Cooch N, Shiekhattar R. The Microprocessor complex mediates the genesis of microRNAs. *Nature* 2004; **432**: 235-240 [PMID: 15531877 DOI: 10.1038/nature03120]
32 **Landthaler M**, Yalcin A, Tuschl T. The human DiGeorge syndrome critical region gene 8 and Its D. melanogaster homolog are required for miRNA biogenesis. *Curr Biol* 2004; **14**: 2162-2167 [PMID: 15589161 DOI: 10.1016/j.cub.2004.11.001]
33 **Han J**, Lee Y, Yeom KH, Kim YK, Jin H, Kim VN. The Drosha-DGCR8 complex in primary microRNA processing. *Genes Dev* 2004; **18**: 3016-3027 [PMID: 15574589 DOI: 10.1101/gad.1262504]
34 **Winter J**, Jung S, Keller S, Gregory RI, Diederichs S. Many

roads to maturity: microRNA biogenesis pathways and their regulation. *Nat Cell Biol* 2009; **11**: 228-234 [PMID: 19255566 DOI: 10.1038/ncb0309-228]
35 **Yi R**, Qin Y, Macara IG, Cullen BR. Exportin-5 mediates the nuclear export of pre-microRNAs and short hairpin RNAs. *Genes Dev* 2003; **17**: 3011-3016 [PMID: 14681208 DOI: 10.1101/gad.1158803]
36 **Bohnsack MT**, Czaplinski K, Gorlich D. Exportin 5 is a RanGTP-dependent dsRNA-binding protein that mediates nuclear export of pre-miRNAs. *RNA* 2004; **10**: 185-191 [PMID: 14730017 DOI: 10.1261/rna.5167604]
37 **Berezikov E**, Chung WJ, Willis J, Cuppen E, Lai EC. Mammalian mirtron genes. *Mol Cell* 2007; **28**: 328-336 [PMID: 17964270 DOI: 10.1016/j.molcel.2007.09.028]
38 **Hutvágner G**, McLachlan J, Pasquinelli AE, Bálint E, Tuschl T, Zamore PD. A cellular function for the RNA-interference enzyme Dicer in the maturation of the let-7 small temporal RNA. *Science* 2001; **293**: 834-838 [PMID: 11452083]
39 **Ketting RF**, Fischer SE, Bernstein E, Sijen T, Hannon GJ, Plasterk RH. Dicer functions in RNA interference and in synthesis of small RNA involved in developmental timing in C. elegans. *Genes Dev* 2001; **15**: 2654-2659 [PMID: 11641272 DOI: 10.1101/gad.927801]
40 **Provost P**, Dishart D, Doucet J, Frendewey D, Samuelsson B, Rådmark O. Ribonuclease activity and RNA binding of recombinant human Dicer. *EMBO J* 2002; **21**: 5864-5874 [PMID: 12411504 DOI: 10.1093/emboj/cdf578]
41 **Zhang H**, Kolb FA, Brondani V, Billy E, Filipowicz W. Human Dicer preferentially cleaves dsRNAs at their termini without a requirement for ATP. *EMBO J* 2002; **21**: 5875-5885 [PMID: 12411505 DOI: 10.1093/emboj/cdf582]
42 **Denli AM**, Tops BB, Plasterk RH, Ketting RF, Hannon GJ. Processing of primary microRNAs by the Microprocessor complex. *Nature* 2004; **432**: 231-235 [PMID: 15531879 DOI: 10.1038/nature03049]
43 **Krol J**, Loedige I, Filipowicz W. The widespread regulation of microRNA biogenesis, function and decay. *Nat Rev Genet* 2010; **11**: 597-610 [PMID: 20661255 DOI: 10.1038/nrg2843]
44 **Ro S**, Park C, Young D, Sanders KM, Yan W. Tissue-dependent paired expression of miRNAs. *Nucleic Acids Res* 2007; **35**: 5944-5953 [PMID: 17726050 DOI: 10.1093/nar/gkm641]
45 **Dostie J**, Mourelatos Z, Yang M, Sharma A, Dreyfuss G. Numerous microRNPs in neuronal cells containing novel microRNAs. *RNA* 2003; **9**: 180-186 [PMID: 12554860 DOI: 10.1101/gad.974702]
46 **Chekulaeva M**, Filipowicz W. Mechanisms of miRNA-mediated post-transcriptional regulation in animal cells. *Curr Opin Cell Biol* 2009; **21**: 452-460 [PMID: 19450959 DOI: 10.1016/j.ceb.2009.04.009]
47 **Fabian MR**, Sonenberg N, Filipowicz W. Regulation of mRNA translation and stability by microRNAs. *Annu Rev Biochem* 2010; **79**: 351-379 [PMID: 20533884 DOI: 10.1146/annurev-biochem-060308-103103]
48 **Eulalio A**, Tritschler F, Izaurralde E. The GW182 protein family in animal cells: new insights into domains required for miRNA-mediated gene silencing. *RNA* 2009; **15**: 1433-1442 [PMID: 19535464 DOI: 10.1261/rna.1703809]
49 **Chekulaeva M**, Filipowicz W, Parker R. Multiple independent domains of dGW182 function in miRNA-mediated repression in Drosophila. *RNA* 2009; **15**: 794-803 [PMID: 19304924 DOI: 10.1261/rna.1364909]
50 **Voinnet O**. Origin, biogenesis, and activity of plant microRNAs. *Cell* 2009; **136**: 669-687 [PMID: 19239888 DOI: 10.1016/j.cell.2009.01.046]
51 **Rottiers V**, Näär AM. MicroRNAs in metabolism and metabolic disorders. *Nat Rev Mol Cell Biol* 2012; **13**: 239-250 [PMID: 22436747 DOI: 10.1038/nrm3313]
52 **Lewis AP**, Jopling CL. Regulation and biological function of the liver-specific miR-122. *Biochem Soc Trans* 2010; **38**: 1553-1557 [PMID: 21118125 DOI: 10.1042/BST0381553]
53 **Krützfeldt J**, Rajewsky N, Braich R, Rajeev KG, Tuschl T, Manoharan M, Stoffel M. Silencing of microRNAs in vivo with 'antagomirs'. *Nature* 2005; **438**: 685-689 [PMID: 16258535 DOI: 10.1038/nature04303]
54 **Esau C**, Davis S, Murray SF, Yu XX, Pandey SK, Pear M, Watts L, Booten SL, Graham M, McKay R, Subramaniam A, Propp S, Lollo BA, Freier S, Bennett CF, Bhanot S, Monia BP. miR-122 regulation of lipid metabolism revealed by in vivo antisense targeting. *Cell Metab* 2006; **3**: 87-98 [PMID: 16459310 DOI: 10.1016/j.cmet.2006.01.005]
55 **Elmén J**, Lindow M, Schütz S, Lawrence M, Petri A, Obad S, Lindholm M, Hedtjärn M, Hansen HF, Berger U, Gullans S, Kearney P, Sarnow P, Straarup EM, Kauppinen S. LNA-mediated microRNA silencing in non-human primates. *Nature* 2008; **452**: 896-899 [PMID: 18368051 DOI: 10.1038/nature06783]
56 **Stenvang J**, Petri A, Lindow M, Obad S, Kauppinen S. Inhibition of microRNA function by antimiR oligonucleotides. *Silence* 2012; **3**: 1 [PMID: 22230293 DOI: 10.1186/1758-907X-3-1]
57 **Pogribny IP**, Starlard-Davenport A, Tryndyak VP, Han T, Ross SA, Rusyn I, Beland FA. Difference in expression of hepatic microRNAs miR-29c, miR-34a, miR-155, and miR-200b is associated with strain-specific susceptibility to dietary nonalcoholic steatohepatitis in mice. *Lab Invest* 2010; **90**: 1437-1446 [PMID: 20548288 DOI: 10.1038/labinvest.2010.113]
58 **Alisi A**, Da Sacco L, Bruscalupi G, Piemonte F, Panera N, De Vito R, Leoni S, Bottazzo GF, Masotti A, Nobili V. Mirnome analysis reveals novel molecular determinants in the pathogenesis of diet-induced nonalcoholic fatty liver disease. *Lab Invest* 2011; **91**: 283-293 [PMID: 20956972 DOI: 10.1038/labinvest.2010.166]
59 **Vella S**, Gnani D, Crudele A, Ceccarelli S, De Stefanis C, Gaspari S, Nobili V, Locatelli F, Marquez VE, Rota R, Alisi A. EZH2 down-regulation exacerbates lipid accumulation and inflammation in in vitro and in vivo NAFLD. *Int J Mol Sci* 2013; **14**: 24154-24168 [PMID: 24351808 DOI: 10.3390/ijms141224154]
60 **Zhang Y**, Cheng X, Lu Z, Wang J, Chen H, Fan W, Gao X, Lu D. Upregulation of miR-15b in NAFLD models and in the serum of patients with fatty liver disease. *Diabetes Res Clin Pract* 2013; **99**: 327-334 [PMID: 23287814 DOI: 10.1016/j.diabres.2012.11.025]
61 **Zheng L**, Lv GC, Sheng J, Yang YD. Effect of miRNA-10b in regulating cellular steatosis level by targeting PPAR-alpha expression, a novel mechanism for the pathogenesis of NAFLD. *J Gastroenterol Hepatol* 2010; **25**: 156-163 [PMID: 19780876 DOI: 10.1111/j.1440-1746.2009.05949.x]
62 **Horton JD**, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J Clin Invest* 2002; **109**: 1125-1131 [PMID: 11994399 DOI: 10.1172/JCI15593]
63 **Dávalos A**, Goedeke L, Smibert P, Ramírez CM, Warrier NP, Andreo U, Cirera-Salinas D, Rayner K, Suresh U, Pastor-Pareja JC, Esplugues E, Fisher EA, Penalva LO, Moore KJ, Suárez Y, Lai EC, Fernández-Hernando C. miR-33a/b contribute to the regulation of fatty acid metabolism and insulin signaling. *Proc Natl Acad Sci USA* 2011; **108**: 9232-9237 [PMID: 21576456 DOI: 10.1073/pnas.1102281108]
64 **Näär AM**. MiRs with a sweet tooth. *Cell Metab* 2011; **14**: 149-150 [PMID: 21803284 DOI: 10.1016/j.cmet.2011.07.005]
65 **Rayner KJ**, Suárez Y, Dávalos A, Parathath S, Fitzgerald ML, Tamehiro N, Fisher EA, Moore KJ, Fernández-Hernando C. MiR-33 contributes to the regulation of cholesterol homeostasis. *Science* 2010; **328**: 1570-1573 [PMID: 20466885 DOI: 10.1126/science.1189862]
66 **Goedeke L**, Vales-Lara FM, Fenstermaker M, Cirera-Salinas D, Chamorro-Jorganes A, Ramírez CM, Mattison JA, de Cabo R, Suárez Y, Fernández-Hernando C. A regulatory role for microRNA 33* in controlling lipid metabolism gene expression. *Mol Cell Biol* 2013; **33**: 2339-2352 [PMID: 23547260

DOI: 10.1128/MCB.01714-12]
67 **Kim HS**, Xiao C, Wang RH, Lahusen T, Xu X, Vassilopoulos A, Vazquez-Ortiz G, Jeong WI, Park O, Ki SH, Gao B, Deng CX. Hepatic-specific disruption of SIRT6 in mice results in fatty liver formation due to enhanced glycolysis and triglyceride synthesis. *Cell Metab* 2010; **12**: 224-236 [PMID: 20816089 DOI: 10.1016/j.cmet.2010.06.009]
68 **Cantó C**, Auwerx J. Targeting sirtuin 1 to improve metabolism: all you need is NAD(+)? *Pharmacol Rev* 2012; **64**: 166-187 [PMID: 22106091 DOI: 10.1124/pr.110.003905]
69 **Choi SE**, Fu T, Seok S, Kim DH, Yu E, Lee KW, Kang Y, Li X, Kemper B, Kemper JK. Elevated microRNA-34a in obesity reduces NAD+ levels and SIRT1 activity by directly targeting NAMPT. *Aging Cell* 2013; **12**: 1062-1072 [PMID: 23834033 DOI: 10.1111/acel.12135]
70 **Fu T**, Choi SE, Kim DH, Seok S, Suino-Powell KM, Xu HE, Kemper JK. Aberrantly elevated microRNA-34a in obesity attenuates hepatic responses to FGF19 by targeting a membrane coreceptor β-Klotho. *Proc Natl Acad Sci USA* 2012; **109**: 16137-16142 [PMID: 22988100 DOI: 10.1073/pnas.1205951109]
71 **Cortez MA**, Bueso-Ramos C, Ferdin J, Lopez-Berestein G, Sood AK, Calin GA. MicroRNAs in body fluids--the mix of hormones and biomarkers. *Nat Rev Clin Oncol* 2011; **8**: 467-477 [PMID: 21647195 DOI: 10.1038/nrclinonc.2011.76]
72 **Yamada H**, Suzuki K, Ichino N, Ando Y, Sawada A, Osakabe K, Sugimoto K, Ohashi K, Teradaira R, Inoue T, Hamajima N, Hashimoto S. Associations between circulating microRNAs (miR-21, miR-34a, miR-122 and miR-451) and non-alcoholic fatty liver. *Clin Chim Acta* 2013; **424**: 99-103 [PMID: 23727030 DOI: 10.1016/j.cca.2013.05.021]
73 **Cermelli S**, Ruggieri A, Marrero JA, Ioannou GN, Beretta L. Circulating microRNAs in patients with chronic hepatitis C and non-alcoholic fatty liver disease. *PLoS One* 2011; **6**: e23937 [PMID: 21886843 DOI: 10.1371/journal.pone.0023937]
74 **Hermeking H**. The miR-34 family in cancer and apoptosis. *Cell Death Differ* 2010; **17**: 193-199 [PMID: 19461653 DOI: 10.1038/cdd.2009.56]
75 **Miyaaki H**, Ichikawa T, Kamo Y, Taura N, Honda T, Shibata H, Milazzo M, Fornari F, Gramantieri L, Bolondi L, Nakao K. Significance of serum and hepatic microRNA-122 levels in patients with non-alcoholic fatty liver disease. *Liver Int* 2014; **34**: e302-e307 [PMID: 24313922 DOI: 10.1111/liv.12429]
76 **Li J**, Ghazwani M, Zhang Y, Lu J, Li J, Fan J, Gandhi CR, Li S. miR-122 regulates collagen production via targeting hepatic stellate cells and suppressing P4HA1 expression. *J Hepatol* 2013; **58**: 522-528 [PMID: 23178710 DOI: 10.1016/j.jhep.2012.11.011]
77 **Cheung O**, Puri P, Eicken C, Contos MJ, Mirshahi F, Maher JW, Kellum JM, Min H, Luketic VA, Sanyal AJ. Nonalcoholic steatohepatitis is associated with altered hepatic MicroRNA expression. *Hepatology* 2008; **48**: 1810-1820 [PMID: 19030170 DOI: 10.1002/hep.22569]

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