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miRNAs and NAFLD: from pathophysiology to therapy

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Abbreviation list

DNL – *de novo* lipogenesis

ER – endoplasmic reticulum

HCC – hepatocellular carcinoma

miRNA – micro ribonucleic acid

mRNA – messenger RNA

NAFLD – non-alcoholic fatty liver disease

NASH – non-alcoholic steatohepatitis

RISC – RNA-induced silencing complex

TF – transcription factor

TS – tumour suppressor

UPR – unfolded protein response

UTR – untranslated region

ABSTRACT

Non-alcoholic fatty liver disease (NAFLD) is associated with a thorough reprogramming of hepatic metabolism. Epigenetic mechanisms, in particular those associated with deregulation of the expressions and activities of microRNAs (miRNAs), play a major role in metabolic disorders associated with NAFLD and their progression towards more severe stages of the disease. In this review, we discuss the recent progresses addressing the role of the many facets of complex miRNA regulatory networks in the development and progression of NAFLD. The basic concepts and mechanisms of miRNAs-mediated gene regulation, as well as the various setbacks encountered in basic and translational research in this field are debated. miRNAs identified so far, whose expressions/activities are deregulated in NAFLD, and which contribute to the outcomes of this pathology are further reviewed. Finally, the potential therapeutic usages in a short to medium term of miRNAs-based strategies in NAFLD, in particular to identify non-invasive biomarkers, or to design pharmacological analogs/inhibitors having a broad range of actions on hepatic metabolism, are highlighted.

With the worldwide obesity pandemic, insulin resistance, diabetes and non-alcoholic fatty liver disease (NAFLD) have become major public health issues. Hepatic steatosis arising from non-viral, non-genetic and non-alcoholic origin is the main characteristic of the NAFLD spectrum of metabolic disorders, which can further progress to inflammation (non-alcoholic steatohepatitis, NASH), fibrosis, and finally cirrhosis and hepatocellular carcinoma (HCC) as end stage diseases [1,2]. NAFLD is associated with a thorough reprogramming of hepatic metabolism resulting from deregulation of key molecular mechanisms governing the latter, as well as associated gene expression. Alterations of epigenetic mechanisms dictating gene expression, which include DNA methylation, histone acetylation and non-coding RNA-dependent regulation, contribute importantly to these hepatic metabolic disorders. Among the latter, microRNAs (miRNAs) play a major role in NAFLD and its progression towards more severe stages of the disease, therefore having a great potential to affect patient outcome [3]. Many facets of miRNA regulatory networks remain obscure in heterogenous and multifactorial metabolic diseases, such as NAFLD and its complications. However, despite the high complexity of miRNA biology, several of them have already been identified as important actors in NAFLD and as reliable circulating biomarkers for non-invasive diagnosis between different stages of the disease [4]. Deepening our understanding on the complex roles of specific miRNAs in NAFLD should open promising perspectives in the close future for therapeutic strategies based on pharmacological targeting of miRNAs.

MiRNA biogenesis, expression and functions

The human genome contains over 2000 miRNAs, whose primary functions are to inhibit gene expression [5]. miRNAs synthesis starts with the transcription of primary miRNAs (pri-miRNAs), which are then cleaved by a nuclear enzymatic protein complex (Drosha/DGCR8) to generate pre-miRNA molecules [6,7]. Pre-miRNAs are then translocated to the cytoplasm and processed into mature miRNA duplexes of around 20-25 nucleotides by the Dicer enzyme. The guide strand of the duplex is incorporated into the RNA-induced silencing complex (RISC), while the passenger strand is frequently degraded [6,7]. Nevertheless, this latter step of miRNAs biogenesis is highly versatile, as passenger strands can also be incorporated into RISC and trigger gene silencing [8]. Mature miRNAs bind to complementary seed sequences in the 3'-

UTR of messenger RNAs (mRNAs), leading either to their degradation, or inhibition of their translation by restraining their access to the translational machinery. The end result of both mechanisms is inhibition of gene expression. Importantly, one miRNA has the capacity to bind numerous 3'-UTRs of different mRNAs, and one mRNA can be targeted by multiple miRNAs as well. While this represents the dogmatic view of miRNA-mRNA interactions, other mechanisms have been reported, such as miRNAs binding to the 5'-UTR or coding sequence of mRNAs [9]. Furthermore, some miRNAs may not be intended to inhibit, but rather to enhance gene expression, providing an additional layer of complexity in the miRNA molecular functions [10]. Finally, other mechanisms have been described for miRNAs, including direct binding to Toll-like receptors [11], or mitochondrial transcripts [12], which furtherly outlines our currently limited understanding of miRNA biology.

Complexity and biases of miRNAs-based studies

The expressions and functions of specific miRNAs can be highly different between distinct cell types [13,14]. Indeed, direct transcriptional regulation of miRNAs or of protein-coding genes, in which miRNAs are encoded, depends on the availability of cell-specific transcription factors (TFs) under the control of various environmental factors, but also of the energetic, differentiation and proliferative status of the cells [15]. *Vice versa*, the transcriptome of a specific cell type is conversely shaped by miRNAs expression and activity levels. A corollary of these observations is that miRNA-dependent functions observed in pathologic and cancerous cells/tissues may not be translatable to primary cells or tissues, even from same origin [16]. Moreover, rodent disease models are widely used to assess functions and therapeutic potentials of miRNAs, yet miRNAs in humans frequently have different target genes, or could be under the control of different regulatory mechanisms [17]. Finally, pharmacological targeting by miRNA inhibitors/activators in cells/tissues of interest may be, to some extent, unspecific and can lead to inconclusive interpretations of related studies, due to off-target events. Given all these potential pitfalls, data should be interpreted with high degree of caution, depending on the experimental approaches used to investigate miRNA functions.

Increasing evidence also indicates that the expression and activity of miRNAs do not always correlate [15]. A wide variety of mechanisms, including competitive binding of proteins or RNAs to miRNAs, as well as differences in the relative stoichiometries of cellular miRNAs or mRNAs, are responsible for this paradox as illustrated in **Fig.1**. Finally, miRNAs can undergo sequence editing during processing (e.g., A-to-I), which affects their binding characteristics and governs the choice of the strand (guide *versus* passenger) incorporated in the RISC complex

[18,19]. Supporting the complexity of these elaborated miRNA-based regulatory mechanisms, over 4000 editing sites have been found in pri-miRNAs and mature miRNAs with great differences between human and animals [18].

In conclusion, it is clear that investigating the cell/tissue-specific miRNA expression or circulating miRNA signature likely represents valuable diagnostic tools featuring specific diseases. However, considering only alterations of miRNAs expression to evaluate their pathophysiological roles in tissues and complex diseases such as NAFLD is far from sufficient.

MiRNAs-dependent epigenetic reprogramming in NAFLD

Various metabolic pathways deregulated in NAFLD converge in the aberrant accumulation of lipids into hepatocytes. These include i) boosted *de novo* lipogenesis (DNL), ii) increased uptake of lipids found in excess in the blood, iii) diminished hepatic export of lipids, or iv) impaired lipid oxidation [20,21]. All these metabolic processes are tightly regulated by specific miRNAs **(Fig.2)**. Since elevated glucose levels fuel DNL, miRNA-dependent alterations of hepatic glycolysis, gluconeogenesis and glycogen metabolism are also key pathological mechanisms contributing to NAFLD development. Finally, deregulated cellular processes, such as autophagy or endoplasmic reticulum (ER)-stress and the unfolded protein response (UPR) were also recently implicated in steatosis development and shown to be under the control of miRNAs [22–24] **(Fig.2)**.

Pathophysiological miRNA-dependent regulation of hepatic lipid metabolism

Several miRNAs were suggested to exert tight control on various aspects of the hepatic lipid metabolism [3,25]. Herein, we will focus on four specific miRNAs, i.e., miR-122, miR-33, miR-34a and miR-21, which are well acknowledged for their important regulatory functions in hepatic metabolism and their high therapeutic potential in fatty liver disease (FLD). A non-exhaustive list of other miRNAs with deregulated hepatic expression and/or altered circulating levels in NAFLD is provided in **Table 1**.

MiR-122 accounts for almost 70% of all miRNA copies expressed in the liver [26]. Current evidence indicates that it represents a major regulator of hepatic lipid metabolism. In mice, hepatic miR-122 inhibition leads to i) indirect downregulation of lipogenic enzymes (e.g. FASN, ACC), increased fatty acid β-oxidation and a decreased accumulation of intracellular

triglycerides [27], and ii) decreased cholesterol synthesis [28], through mechanisms still poorly understood **(Fig.2)**. Strikingly, although miR-122 expression is downregulated in hepatic tissues of patients with NAFLD/NASH [29], circulating levels of this miRNA are increased, with evidence indicating that hepatic miR-122 secretion is promoted by fatty acid-dependent mechanisms [30] **(Table 1)**.. A recent meta-analysis further reported that miR-122 allows a good diagnostic accuracy in distinguishing NAFLD from NASH [4]. Based on these studies, miR-122 appears undoubtedly as an important regulator of hepatic lipid metabolism and illustrates well the cautiousness principle assuming that circulating levels of miRNAs do not always reflect their tissue expression and/or activity, as shown in NAFLD patients.

Although *miR-34a* is weakly expressed in hepatocytes, it appears to tightly regulate the lipid metabolism. This miRNA is also significantly upregulated in the plasma and liver of NASH patients, qualifying it as a good and reliable biomarker of this disease stage [4,31] **(Table 1)**. In human hepatic cells and in mouse models of steatosis, inhibition of miR-34a allowed to demonstrate that this miRNA specifically targets PPARα and Sirtuin 1 (SIRT1), thereby restraining fatty acid catabolism and favouring steatosis development [32]. Interestingly, miR-34a inhibition also promoted AMP-activated protein kinase α (AMPKα) activity, a major metabolic switch antagonizing lipogenesis [32]. Finally, miR-34a was also shown in mice to exert broad control over lipid storage by specifically targeting the hepatocyte nuclear factor 4 (HNF4), a key transcription factor protecting from steatosis development through the transcriptional control of several genes implicated in lipid catabolism [33].

MiR-33 is also upregulated in hepatic tissues and the blood circulation of patients with NAFLD, and especially NASH [34] **(Table 1)**. This miRNA attracted attention since its two isoforms a and b are encoded in the introns of two key lipogenic transcription factors, SREBP2 (sterol regulatory element-binding protein 2) and SREBP1 respectively. MiR-33 regulates both cholesterol and fatty acids metabolism in human hepatic cell lines by targeting cholesterol efflux regulatory proteins (ABCA1 and ABCG1) and regulators of fatty acid β-oxidation (CPT1A and AMPKα) [35,36]. Based on these studies, miR-33 inhibitors are regarded as potential therapeutic weapons against cardiovascular diseases and atherosclerosis [35]. However, miR-33 was also reported to protect from obesity and hepatic steatosis in mice [37]. Interestingly, the molecular mechanisms linking obesity and miR-33 are still debated, since different mouse models of miR-33 deletion have led to the observation of different mechanisms of action, i.e., as miR-33-mediated inhibition of the lipogenic TF SREBP1 [38] or a miR-33-dependent restriction of food intake through still unclear molecular mechanisms [37].

Discrepant results between different studies on this miRNA could also originate from the inhibition of different isoforms of miR-33. Of note, injections of miR-33 inhibitors in mice at protective doses against atherosclerosis did not foster obesity development in these animals, unlike miR-33 gene ablation, therefore supporting the potential safety of therapeutic interventions with miR-33 inhibitors [39].

MiR-21 is highly increased in the liver and plasma of patients with NASH [29,40] **(Table 1)**. This miRNA is a typical representative of stress-induced miRNAs, strongly expressed in the liver, but remaining inactivated in normal physiological conditions [41]. Thousands of reports have outlined the oncogenic role of miR-21, but recent works have also highlighted its key functions in hepatic metabolism and inflammation [41]. In human hepatic cells, miR-21 expression/activity was shown to be induced by unsaturated fatty acids [42], or by steatogenic strains of HCV (our unpublished results). In hepatic cells, miR-21 targets important factors restraining hepatic steatosis development, such as phosphatase and tensin homolog (PTEN), which inhibits DNL and fatty acid uptake [43] or PPARα, which triggers lipid oxidation [44]. We recently also demonstrated that hepatocytes-specific miR-21 ablation in mice restrains steatosis development induced by an obesogenic diet, through upregulation of multiple miR-21 targets involved in lipid metabolism [41]. Other miR-21-dependent mechanisms, such as i) modulation of the HBP1-p53-SREBP1 signalling axis promoting diet-induced steatosis [42]; ii) targeting of HMGCR, which regulates both triglycerides and cholesterol metabolism [45]; and iii) regulation of fatty acid-binding protein 7 (FABP7), which modulates lipid trafficking in hepatocytes [46], were also recently reported, testifying for the broad range of miR-21 functions, not only in carcinogenesis, but also in the metabolic homeostasis.

miRNAs in NAFLD: more than just regulators of lipid metabolism

Hepatic carbohydrate metabolism

Carbohydrate and lipid metabolism pathways are tightly interlinked *via* common biochemical substrates, and need to be considered together regarding NAFLD development and the role of miRNAs in this disease. Several miRNAs were reported to modulate the glycogen metabolism, thereby potentially diverting glucose towards DNL, as observed in insulin resistance [47] **(Fig.2)**. miR-122 was reported to target glycogen synthase 1 (GYS1), thereby restraining glycogen synthesis [27], while miR-20-5p drove hepatic glycogen synthesis through indirect mechanisms involving p53 and PTEN [48]. Moreover, miR-29 decreased glycogen content and glucose uptake in primary human skeletal muscle cells, also indirectly, by attenuating insulin

signalling [49]. Interestingly, miR-20 and miR-29a serum levels in NAFLD patients are significantly up- and down-regulated, respectively, pointing also to these specific miRNAs as potential biomarkers [50,51] **(Table 1)**. Upregulation of hepatic glycolysis is likewise frequently observed in NAFLD patients and may contribute importantly to DNL by providing metabolic substrates in excess [52]. In this regard, miR-122 was shown to target the glycolytic enzyme aldolase A in hepatic cells, suggesting that miR-122 downregulation with NAFLD could be in part responsible for upregulation of glycolysis [53] **(Fig.2)**. Other studies, mostly using cancer cells, have pointed to specific miRNAs potentially regulating glycolysis. This is indeed the case for miR-34a, which targets lactate dehydrogenase A (LDHA), a key enzyme in glycolysis [54], or miR-125b, which is significantly upregulated in NASH patients [55] and targets hexokinase II (HKII), therefore inhibiting glycolysis in HCC cells [56] **(Fig.2)**. Whether these miRNAs modulate glycolysis in NAFLD/NASH and whether they exert a protective or deleterious role in these diseases remains to be established. Finally, miR-33 was also shown to inhibit gluconeogenesis, a pathway up-regulated in insulin-resistant NAFLD patients, *via* direct targeting of phosphoenolpyruvate carboxykinase (PCK1) and glucose-6-phosphatase (G6PC), two key enzymes in the *de novo* glucose biosynthesis [57].

Stress-activated pathways

ER stress-associated UPR regulation by miRNA - NAFLD entails important cellular stress and lipotoxicity, which lead to the activation of stress-activated pathways, such as the unfolded protein response (UPR) triggered by endoplasmic reticulum (ER) stress. Hepatic steatosis disturbs normal ER functions and thus triggers the three axes of the UPR, which include inositol-requiring enzyme 1 (IRE1), PRKR-like ER kinase (PERK) and activating transcription factor 6 (ATF6) [22]. Interestingly, the UPR was shown to activate directly lipogenesis, thus creating a vicious circle promoting lipid accumulation [22]. Among the pleiotropic cellular actions of miR-122, this miRNA also negatively regulates the UPR in human HCC cells **(Fig.2),** and its downregulation, as observed in NAFLD, results in decreased apoptosis [58]. Interestingly, the UPR can in turn further modulate miRNAs expression, as exemplified by miR-30c-2-3p, which is induced by PERK, leading to the targeting and subsequent downregulation of X-box-binding protein-1 (XBP-1) expression [59]. In addition, IRE1, a key endoribonuclease in the UPR, was shown to induce the selective degradation of miRNAs in mouse embryonic fibroblasts, resulting in apoptosis activation [60]. High apoptotic levels observed in NAFLD significantly contribute to progression towards NASH [61], further highlighting the importance of miRNAs controlling the UPR.

MiRNA-dependent regulation of autophagy - Autophagy deeply impacts hepatocyte lipid homeostasis by reducing in particular cellular fat content [23]. Nevertheless, autophagic regulation by miRNAs in the liver is poorly studied, yet data in hepatic cancer cells could lay the bases in this field. Indeed, miR-375, which is upregulated in the liver and serum of NAFLD patients **(Table 1)**, was shown to behave as an important inhibitor of autophagy in HCC cells by targeting the autophagy-related protein 7 (ATG7) [62]. Other miRNAs were reported to modulate autophagy, such as miR-224, which downregulates ATG5, or miR-101 and miR-199, which target the mTOR kinase, a key signalling effector promoting autophagy [24]. Future studies are now required to better understand the role of miRNAs in the regulation of cellular stress responses and the relevance of these mechanisms for NAFLD, which remain likely underestimated, despite the recent progresses discussed above.

MiRNA-dependent shaping of malignancy in NAFLD

The incidence of HCC in non-cirrhotic patients with NAFLD is on the rise, breaking the dogma that cirrhosis is a necessary pre-requisite to develop HCC [1,63]. The molecular mechanisms associated with cancer development in the absence of cirrhosis remain mostly obscure, but accumulating evidence indicates that non-genomic alterations of cancer-related factors in NAFLD could generate a favourable microenvironment promoting hepatic tumorigenesis.

Supporting this concept, PTEN, a key tumour suppressor (TS), whose partial loss of expression/activity is sufficient to induce malignancies in various organs, is significantly downregulated with steatosis in both animal models and humans [64,65]. miR-21 was repeatedly reported to target PTEN expression in hepatic cancer cells, supporting its reputation of a potent oncogenic miRNA (oncomiRs) in other cancers [43]. Thus, besides its role in hepatic metabolism and NAFLD/NASH development, upregulation of miR-21 in FLD may also promote tumorigenesis. Nevertheless, and as illustrated in **Figure 3**, the multitude of validated miR-21 targets, which are intricately linked to the control of metabolism, immunity and carcinogenesis, still raise important debates about the pathophysiological *in vivo* role of miR-21 as a cancer-promoting factor in the liver. In contrast to miR-21, miR-122 was shown to have a tumour-suppressive role in HCC through different mechanisms including i) an indirect inhibition of the oncogene c-Myc as reported in mice [66] and ii) its potential role in promoting hepatocytes differentiation in human liver [67]. Consistent with its role as a TS, miR-122 is frequently downregulated in HCC and miR-122 downregulation correlates with poor prognosis [67]. Finally, a reciprocal regulation of key miRNAs in NAFLD by deregulated cancer-related factors may also occur. For example, miR-34a expression was reported to be under the control

of the TS p53 in the liver of diet-induced obese mice, suggesting that inhibition of p53 and thereby miR-34a downregulation have a protective role against NAFLD-associated complications [68]. Moreover, the relevance of this pathway was recently further supported by studies in rats showing that activation of p53/miR-34a pathway triggers liver fibrosis [69].

Here again, a clear understanding of how miRNAs deregulated in NAFLD contribute to priming hepatocytes for malignancy, either directly or indirectly, for example by providing a favourable environment for carcinogenesis, necessitates further investigations. Deepening our knowledge in this field could provide both fundamental scientists and clinicians with breakthrough concepts to understand and prevent/treat HCC in non-cirrhotic patients with NAFLD.

Therapeutic potential of targeting miRNAs for NAFLD

Unravelling pathophysiological functions and therapeutic potential of miRNAs and their targets

High-throughput technologies, such as miRNA microarrays and miRNA sequencing, have been determinant to identify deregulated miRNAs in tissues or the blood circulation, that are likely involved in disease development or presenting a therapeutic potential [70]. Nevertheless, and as described above, variations in the expression of a miRNA do not necessarily infer differences in its activity or support its relevance in a disease. Therefore, a growing number of additional tools, such as miRNA sensors and decoys, are being currently developed to evaluate miRNAs bioavailability and activity in pathophysiological conditions, as well as their therapeutic potential [71]. In this regard, the gold standard methodology to confirm pathophysiological miRNA/mRNA interactions takes advantage of luciferase reporter gene-based assays. Relevant cells, e.g., hepatic cells for NAFLD, are engineered to express 3'-UTR sequences (or other related sequences) of mRNA targets of interest, coupled to a reporter gene, such as luciferase, whose activity is modulated by synthetic nucleotides mimicking or inhibiting specific miRNAs [72]. Alternatively, pull-down assay of labelled miRNAs (e.g., biotinylated miRNAs), or immunoprecipitation of AGO complexes (RISC-associated proteins binding to miRNAs), followed by RNA sequencing are other valuable strategies for high throughput targets identification [73]. Various publicly available software (e.g., TargetScan, MirWalk, miRDB, miRGator, miRTar) are also frequently used to bioinformatically predict potential mRNA targets of miRNAs and *vice versa*, by aligning pre-defined seed sequences of the miRNAs with

mRNA sequences [74]. Although bioinformatic algorithms that allow the detection of miRNAs/mRNAs interactions often lead to relevant results, these predictions are to be considered with caution and need to be confirmed through experimental approaches, such as those described above. Conversely, available bioinformatic approaches are currently limited in their predictive power as illustrated in **Figure 4**, which highlights the elevated number of miR-122, miR-34a, miR-33 and miR-21 targets, which were experimentally validated, but not predicted by bioinformatic tools.

To decipher the roles and functions of miRNAs *in vivo*, three main experimental approaches have been developed in rodents: a) genetic engineering of constitutive or conditional knock-out animals, b) modulation of miRNA expression by viral transductions, or c) administration of synthetic oligonucleotides mimicking or inhibiting endogenous miRNAs. **Table 2** summarizes important studies performed *in vivo* through these different approaches to investigate the role of key miRNAs relevant for NAFLD. All methods display both advantages and disadvantages. Genetic engineering of miRNA knock-out mouse models leads to the loss of both the guide and passenger miRNA strands, with the impossibility to distinguish a specific role for each strand. Gene knock-out also results in the complete loss of miRNA expression, in contrast to the usual 2- to 10-fold changes observed in pathophysiological conditions. Finally, if the coding sequence for the knocked-out miRNA lies within a host gene, the expression of this latter may also be affected. In contrast, and highly relevant for NAFLD in particular, a significant interest of genetic approaches resides in the possibility to generate cell/tissue-specific knock-out or overexpression of miRNAs, therefore avoiding numerous adverse effects often associated with viral transductions or pharmacological targeting of miRNAs. Indeed, lentiviral, adenoviral (AV) and adeno-associated viral (AAV) transduction can be employed for delivery of miRNAs analogs/antagonists *in vivo* [75,76]. Lentiviral vectors have the advantage of triggering a stable genomic insertion of the transgene, but potential insertional mutagenesis is a high-risk factor. AV/AAV mediated-delivery provides a transgene remaining in an episomal form, which can be lost with cell division, yet the risk of insertional mutagenesis is low. Relevant for NAFLD research, genetic engineering of these viruses with specific promoters or appropriated tropism of the viral capsid proteins, can provide a liver-specific expression of the miRNA transgenes. However, immune responses against these vectors cannot always be excluded. Finally, synthetic oligonucleotides mimicking (mimics) or inhibiting (antimiRs) endogenous miRNAs can be administered *in vivo*, with a high efficiency **(Table 2)**. These pharmacological compounds allow to reasonably control the level of inhibition/activation for specific miRNAs,

as well as the timing of these interventions, in addition to opening valuable therapeutic perspectives for complex metabolic diseases, such as NAFLD. Unfortunately, important drawbacks of pharmacological mimics/antimiRs call for cautiousness. Indeed, an efficient effect of mimics/antimiRs requires supra-physiological concentrations that can saturate the RISC complex in target cells, leading to important and unwanted off-target effects [77]. Furthermore, targeting specifically one cell type or organ with these pharmacological compounds remains challenging, although various delivery systems, such as liposomal solutions, lipid conjugates and polymers have been developed for the liver [78,79]. Interestingly, nanoparticles combined with engineered proteins able to target specific cell types for delivery were also successfully used for miRNA conveyance to acute myeloid leukaemia cells specifically [80]. Such approaches could also be adapted for hepatic miRNAs delivery in the future. Chemical modification techniques on oligonucleotide sequences have likewise been developed to prevent degradation of these short double-stranded RNAs by RNAses, as well as to enhance their binding affinity [81]. For mimics, chemical modifications can interfere with recognition of the synthetic miRNA by the RISC complex and only 2′-fluoro (2′-F) modifications were reported to display protection against nucleases, while still allowing recognition by RISC [82]. Of note, optimization through chemical modifications of the binding/activity of mimics can also drastically improve the stability and affinity of these compounds, potentially leading to unexpected and unwanted cellular effects as compared to those of endogenous miRNAs. AntimiRs, on the contrary, are more suitable for chemical modifications in order to improve their functional affinity and stability. These include 2′-O-methyl (2′-O-Me)-cholesterol-conjugated oligonucleotides (antagomiRs), 2′-O-methoxyethyl (2′-MOE)-conjugated oligonucleotides, or locked nucleic acid (LNA) presenting a modified structure of the sugar of the oligonucleotides [82,83]. It is important to underline that different strategies investigating the role of the same miRNA in specific pathologies (e.g. genetic deletion and administration of inhibitory synthetic nucleotides) may yield to discrepant results and conclusions about the relevance of this miRNA for therapeutic purpose. A striking example of this paradox is illustrated by studies investigating the role of miR-21 in cardiac stress protection through either administration of antimiRs or genetic deletion in mice [84].

Based on the above elements, it is clear that before stepping up to clinical trials for miRNA-based therapies, a thorough examination of the role and relevance of miRNAs of interest needs to be performed in various *in vivo* models and through different methodical approaches.

MiRNAs as suitable biomarkers for NAFLD/NASH

The lack of exploitable biomarkers to diagnose different stages of NAFLD with a non-invasive strategy is currently one of the biggest challenges that clinicians are facing, since, to date, liver biopsies remain the gold standard diagnosis method. Recent evidence indicates that the levels of specific miRNAs in the serum of patients with NAFLD/NASH may be significantly altered, depending of the stage of the disease **(Table 1)** [4,55,85]. In particular, miR-122 and miR-34a serum levels correlate with damage-associated liver enzymes and lipids in the serum, as well as with hepatic inflammation and fibrosis stage, confirming their relevance as circulating biomarkers able to accurately discriminate NAFLD from NASH in patients [4,50,86]. Other circulating miRNAs, such as miR-16, miR-19a/b, miR-21, miR-125, miR-375 and miR-192, were also suggested to represent promising new blood biomarkers for NAFLD/NASH **(Table 1)** [3,4,50,55,87], but further validation of the relevance of these miRNAs as trustworthy biomarkers for NAFLD/NASH is now required in large human cohorts. Last, it is noteworthy that miRNA-based biomarkers in the circulation could be highly relevant in other diseases with NAFLD-like characteristics, such as glycogen storage disease type I, which can progress towards HCC, but for which no currently available serum biomarkers are pertinent [88].

MiRNAs analogues or inhibitors as therapeutic agents for NAFLD/NASH

Currently, no clinical trials have been designed to test miRNA-based therapies specifically for NAFLD/NASH. However, encouraging data with miR-122 and miR-34 pharmacological inhibitors in other liver pathologies are precluding the design of future trials in NAFLD/NASH. Besides diagnostic value, both miR-122 inhibition or mimicking is of therapeutic relevance [89]. miR-122 silencing was indeed effective against HCV infection in African green monkeys [90] and in chimpanzees [91]. A miR-122 antisense LNA (Miravirsen) is currently in phase II clinical trial, since the ability of these inhibitors was confirmed to reduce viral RNA loads in HCV-infected patients, without inducing viral resistance [92,93]. In addition, N-acetylgalactosamine-conjugated miR-122 inhibitors (RG-101) were also efficient in significantly decreasing and even clearing viral load in patients [94]. On the other hand, for NAFLD and HCC, therapeutic approaches with miR-122 mimics may improve the outcome of patients, by refraining NAFLD progression to NASH, or decreasing HCC aggressiveness. However, no clinical trials have been currently achieved to test this hypothesis.

For HCC and other solid tumours treatments, another clinical trial was established using the compound MRX34, a liposomal mimic of miR-34 [95]. However, while first results of this trial

were encouraging, showing in particular an efficient downregulation of cancer-promoting factors targeted by miR-34, serious immune-related adverse events have called upon a halt in this clinical trial. The failure of this trial and other experimental evidence indicate that two major issues need to be solved in order to improve the efficiency and safety of miRNA-based therapies. First, the specific delivery of miRNAs mimics/inhibitors to the diseased cells/organs of interest needs to be improved in order to prevent unwanted off-target effects in healthy organs. Further, illustrating this issue are anti-miR-33-based therapies, which yielded promising results in mice to treat cardiovascular pathologies, but which could also potentially promoted obesity and metabolic dysfunctions [37]. Similarly, although the pharmacological inhibition of miR-21 may ameliorate NAFLD/NASH or restrict hepatocyte proliferation in cancer [41,96], it may also induce a deficient immune response [97]. Second, the therapeutic dosage of pharmacological mimics/antimiRs is a delicate equilibrium between the efficient doses leading to appreciable clinical outcomes and toxic doses inducing adverse effects, in particular through the disruption of an optimal physiological stoichiometry between all miRNAs in cells/tissues as highlighted in **Fig.1**. These important issues are part of the next challenges that clinicians and scientists need to address to progress with miRNA-based therapies for NAFLD/NASH and other diseases.

Other therapeutically relevant miRNAs for NAFLD/NASH

Several other miRNAs have been identified, in addition to those extensively discussed in previous sections, as potential therapeutic targets for NAFLD/NASH. One interesting candidate is miR-132, whose inhibition in obese mice triggers up-regulation of several of its specific targets, i.e., PTEN, SIRT1 and FOXO3 (forkhead box O3), which act in concert to alleviate diet-induced hepatic steatosis [98]. Other miRNAs, including miR-217, miR-181a and miR-29 were also reported to govern expression of SIRT1, a key regulator of autophagy and lipogenesis [99–102]. The impact of pharmacological modulation of miRNAs expression/activity on metabolic pathways can be further amplified when targeted miRNAs regulate important TFs and their co-factors, which have broad impacts on metabolic homeostasis. This is the case for miRNAs regulating i) lipogenic TFs, such as SREPB1, ChREBP, LXR and PPARγ, ii) important co-factors or repressors of lipogenic TFs such as PGC1α and NCOR respectively, and iii) TFs promoting lipid catabolism, such as PPARα. **Figure 5** summarizes miRNAs previously described to modulate expression and activity of these key players in glucose/lipid metabolism. Expression of many of those miRNAs illustrated in **Figure 5** were reported to have an altered expression in the liver, of either rodent models of obesity and steatosis, or of patients

with NAFLD. Others were identified in studies investigating the lipid metabolism in adipose tissues from obese mice/humans. For the latter, whether they are also effective in regulating hepatic lipid metabolism remains to be established. Future studies should further assess the relevance and feasibility of therapeutic strategies based on pharmacological mimicking or inhibition of these miRNAs for NAFLD/NASH treatments. In an attempt to clarify this question, in **Table 3** we have summarized published data concerning the regulation of the expression of these miRNAs in different pathological conditions, as well as their nutritional and hormonal regulation. Interestingly, almost all of these miRNAs seem to be under nutritional/hormonal control. However, a part of these studies highlights findings in organs other than the liver, such as skeletal muscle, heart and adipose tissue. While these data represent an important base for new therapeutic strategies, the relevance of these findings in to be taken with caution in NAFLD, since, as mentioned before, these mechanisms can be tissue-specific.

Concluding remarks

Since their discovery in the early 1990s, significant progresses in our understanding of the role and regulation of miRNAs have been achieved, opening new perspectives for diagnostics and therapeutic interventions in complex and multifactorial diseases such as hepatic metabolic disorders and cancers. Standardizations of circulating miRNA expression profiles as biomarkers for non-invasive NAFLD/NASH diagnostics are being currently evaluated and could lead to routine clinical practices in the short-term, following validation of these biomarkers in extended cohorts of patients. However, prior to concrete clinical applications for miRNAs-based therapies in the treatment of NAFLD/NASH, several important questions and technical challenges remain to be addressed. These include in particular i) understanding and mastering of the interaction networks between miRNAs, their target mRNAs, protein factors (e.g., RBPs) and other non-coding RNAs (e.g., lncRNAs), in order to assess miRNA activity ii) to decipher the specific roles of guide and passenger strands of miRNAs of interest, and iii) to achieve an efficient and specific targeting of miRNA mimics or inhibitors to hepatic cells/tissues. Solving these issues should provide clinicians with an extensive arsenal of therapeutic weapons to treat not only hepatic diseases, but also numerous other pathologies still poorly curable.

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References

1 Baffy G, Brunt EM, Caldwell SH. Hepatocellular carcinoma in non-alcoholic fatty liver disease: an emerging menace. *J Hepatol* 2012;**56**:1384–91. doi:10.1016/j.jhep.2011.10.027

2 Abd El-Kader SM, El-Den Ashmawy EMS. Non-alcoholic fatty liver disease: The diagnosis and management. *World J Hepatol* 2015;**7**:846–58. doi:10.4254/wjh.v7.i6.846

3 Sobolewski C, Calo N, Portius D, *et al.* MicroRNAs in fatty liver disease. *Semin Liver Dis* 2015;**35**:12–25. doi:10.1055/s-0034-1397345

4 Liu C-H, Ampuero J, Gil-Gómez A, *et al.* miRNAs in patients with non-alcoholic fatty liver disease: A systematic review and meta-analysis. *J Hepatol* 2018;**69**:1335–48. doi:10.1016/j.jhep.2018.08.008

5 Hammond SM. An overview of microRNAs. *Adv Drug Deliv Rev* 2015;**87**:3–14. doi:10.1016/j.addr.2015.05.001

6 Nakanishi K. Anatomy of RISC: how do small RNAs and chaperones activate Argonaute proteins? *Wiley Interdiscip Rev RNA* 2016;**7**:637–60. doi:10.1002/wrna.1356

7 Pratt AJ, MacRae IJ. The RNA-induced Silencing Complex: A Versatile Gene-silencing Machine. *J Biol Chem* 2009;**284**:17897–901. doi:10.1074/jbc.R900012200

8 Mah SM, Buske C, Humphries RK, *et al.* miRNA*: a passenger stranded in RNA-induced silencing complex? *Crit Rev Eukaryot Gene Expr* 2010;**20**:141–8.

9 Lee I, Ajay SS, Yook JI, *et al.* New class of microRNA targets containing simultaneous 5'-UTR and 3'-UTR interaction sites. *Genome Res* 2009;**19**:1175–83. doi:10.1101/gr.089367.108

10 Xiao M, Li J, Li W, *et al.* MicroRNAs activate gene transcription epigenetically as an enhancer trigger. *RNA Biol* 2016;**14**:1326–34. doi:10.1080/15476286.2015.1112487

11 Fabbri M, Paone A, Calore F, *et al.* A new role for microRNAs, as ligands of Toll-like receptors. *RNA Biol* 2013;**10**:169–74. doi:10.4161/rna.23144

12 Das S, Bedja D, Campbell N, *et al.* miR-181c Regulates the Mitochondrial Genome, Bioenergetics, and Propensity for Heart Failure In Vivo. *PLOS ONE* 2014;**9**:e96820. doi:10.1371/journal.pone.0096820

13 Sood P, Krek A, Zavolan M, *et al.* Cell-type-specific signatures of microRNAs on target mRNA expression. *PNAS* 2006;**103**:2746–51. doi:10.1073/pnas.0511045103

14 Ludwig N, Leidinger P, Becker K, *et al.* Distribution of miRNA expression across human tissues. *Nucleic Acids Res* 2016;**44**:3865–77. doi:10.1093/nar/gkw116

15 Zhang J, Le TD, Liu L, *et al.* Inferring condition-specific miRNA activity from matched miRNA and mRNA expression data. *Bioinformatics* 2014;**30**:3070–7. doi:10.1093/bioinformatics/btu489

16 Rupaimoole R, Calin GA, Lopez-Berestein G, *et al.* MicroRNA deregulation in cancer cells and the tumor microenvironment. *Cancer Discov* 2016;**6**:235–46. doi:10.1158/2159-8290.CD-15-0893

17 Roux J, Gonzàlez-Porta M, Robinson-Rechavi M. Comparative analysis of human and mouse expression data illuminates tissue-specific evolutionary patterns of miRNAs. *Nucleic Acids Res* 2012;**40**:5890–900. doi:10.1093/nar/gks279

18 Li L, Song Y, Shi X, *et al.* The landscape of miRNA editing in animals and its impact on miRNA biogenesis and targeting. *Genome Res* 2018;**28**:132–43. doi:10.1101/gr.224386.117

19 Pinto Y, Buchumenski I, Levanon EY, *et al.* Human cancer tissues exhibit reduced A-to-I editing of miRNAs coupled with elevated editing of their targets. *Nucleic Acids Res* 2018;**46**:71–82. doi:10.1093/nar/gkx1176

20 Samuel VT, Shulman GI. Nonalcoholic Fatty Liver Disease as a Nexus of Metabolic and Hepatic Diseases. *Cell Metabolism* 2018;**27**:22–41. doi:10.1016/j.cmet.2017.08.002

21 Postic C, Girard J. Contribution of de novo fatty acid synthesis to hepatic steatosis and insulin resistance: lessons from genetically engineered mice. *J Clin Invest* 2008;**118**:829–38. doi:10.1172/JCI34275

22 Han J, Kaufman RJ. The role of ER stress in lipid metabolism and lipotoxicity. *J Lipid Res* 2016;**57**:1329–38. doi:10.1194/jlr.R067595

23 Czaja MJ. Function of Autophagy in Nonalcoholic Fatty Liver Disease. *Dig Dis Sci* 2016;**61**:1304–13. doi:10.1007/s10620-015-4025-x

24 Kim KM, Kim SG. Autophagy and microRNA dysregulation in liver diseases. *Arch Pharm Res* 2014;**37**:1097–116. doi:10.1007/s12272-014-0439-9

25 Aryal B, Singh AK, Rotllan N, *et al.* microRNAs and lipid metabolism. *Curr Opin Lipidol* 2017;**28**:273–80. doi:10.1097/MOL.0000000000000420

26 Chang J, Guo J-T, Jiang D, *et al.* Liver-specific microRNA miR-122 enhances the replication of hepatitis C virus in nonhepatic cells. *J Virol* 2008;**82**:8215–23. doi:10.1128/JVI.02575-07

27 Esau C, Davis S, Murray SF, *et al.* miR-122 regulation of lipid metabolism revealed by in vivo antisense targeting. *Cell Metabolism* 2006;**3**:87–98. doi:10.1016/j.cmet.2006.01.005

28 Krützfeldt J, Rajewsky N, Braich R, *et al.* Silencing of microRNAs *in vivo* with 'antagomirs.' *Nature* 2005;**438**:685–9. doi:10.1038/nature04303

29 Cheung O, Puri P, Eicken C, *et al.* Nonalcoholic steatohepatitis is associated with altered hepatic MicroRNA expression. *Hepatology* 2008;**48**:1810–20. doi:10.1002/hep.22569

30 Chai C, Rivkin M, Berkovits L, *et al.* Metabolic Circuit Involving Free Fatty Acids, microRNA 122, and Triglyceride Synthesis in Liver and Muscle Tissues. *Gastroenterology* 2017;**153**:1404–15. doi:10.1053/j.gastro.2017.08.013

31 Liu X-L, Pan Q, Zhang R-N, *et al.* Disease-specific miR-34a as diagnostic marker of non-alcoholic steatohepatitis in a Chinese population. *World J Gastroenterol* 2016;**22**:9844–52. doi:10.3748/wjg.v22.i44.9844

32 Ding J, Li M, Wan X, *et al.* Effect of miR-34a in regulating steatosis by targeting PPARα expression in nonalcoholic fatty liver disease. *Scientific Reports* 2015;**5**:13729. doi:10.1038/srep13729

33 Xu Y, Zalzala M, Xu J, *et al.* A Metabolic Stress-inducible miR-34a-HNF4α Pathway Regulates Lipid and Lipoprotein Metabolism. *Nat Commun* 2015;**6**:7466. doi:10.1038/ncomms8466

34 Auguet T, Aragonès G, Berlanga A, *et al.* miR33a/miR33b* and miR122 as Possible Contributors to Hepatic Lipid Metabolism in Obese Women with Nonalcoholic Fatty Liver Disease. *International Journal of Molecular Sciences* 2016;**17**:1620. doi:10.3390/ijms17101620

35 Rayner KJ, Suárez Y, Dávalos A, *et al.* MiR-33 contributes to the regulation of cholesterol homeostasis. *Science* 2010;**328**:1570–3. doi:10.1126/science.1189862

36 Dávalos A, Goedeke L, Smibert P, *et al.* miR-33a/b contribute to the regulation of fatty acid metabolism and insulin signaling. *PNAS* 2011;**108**:9232–7. doi:10.1073/pnas.1102281108

37 Price NL, Singh AK, Rotllan N, *et al.* Genetic Ablation of miR-33 Increases Food Intake, Enhances Adipose Tissue Expansion, and Promotes Obesity and Insulin Resistance. *Cell Reports* 2018;**22**:2133–45. doi:10.1016/j.celrep.2018.01.074

38 Horie T, Nishino T, Baba O, *et al.* MicroRNA-33 regulates sterol regulatory element-binding protein 1 expression in mice. *Nature Communications* 2013;**4**:2883. doi:10.1038/ncomms3883

39 Karunakaran D, Richards L, Geoffrion M, *et al.* Therapeutic inhibition of miR-33 promotes fatty acid oxidation but does not ameliorate metabolic dysfunction in diet-induced obesity. *Arterioscler Thromb Vasc Biol* 2015;**35**:2536–43. doi:10.1161/ATVBAHA.115.306404

40 Becker PP, Rau M, Schmitt J, *et al.* Performance of Serum microRNAs -122, -192 and -21 as Biomarkers in Patients with Non-Alcoholic Steatohepatitis. *PLoS ONE* 2015;**10**:e0142661. doi:10.1371/journal.pone.0142661

41 Calo N, Ramadori P, Sobolewski C, *et al.* Stress-activated miR-21/miR-21* in hepatocytes promotes lipid and glucose metabolic disorders associated with high-fat diet consumption. *Gut* 2016;**65**:1871–81. doi:10.1136/gutjnl-2015-310822

42 Wu H, Ng R, Chen X, *et al.* MicroRNA-21 is a potential link between non-alcoholic fatty liver disease and hepatocellular carcinoma via modulation of the HBP1-p53-Srebp1c pathway. *Gut* 2016;**65**:1850–60. doi:10.1136/gutjnl-2014-308430

43 MENG F, HENSON R, WEHBE-JANEK H, *et al.* MicroRNA-21 Regulates Expression of the PTEN Tumor Suppressor Gene in Human Hepatocellular Cancer. *Gastroenterology* 2007;**133**:647–58. doi:10.1053/j.gastro.2007.05.022

44 Loyer X, Paradis V, Hénique C, *et al.* Liver microRNA-21 is overexpressed in non-alcoholic steatohepatitis and contributes to the disease in experimental models by inhibiting PPARα expression. *Gut* 2016;**65**:1882–94. doi:10.1136/gutjnl-2014-308883

45 Sun C, Huang F, Liu X, *et al.* miR-21 regulates triglyceride and cholesterol metabolism in non-alcoholic fatty liver disease by targeting HMGCR. *Int J Mol Med* 2015;**35**:847–53. doi:10.3892/ijmm.2015.2076

46 Ahn J, Lee H, Jung CH, *et al.* Lycopene inhibits hepatic steatosis via microRNA-21-induced downregulation of fatty acid-binding protein 7 in mice fed a high-fat diet. *Mol Nutr Food Res* 2012;**56**:1665–74. doi:10.1002/mnfr.201200182

47 Krssak M, Brehm A, Bernroider E, *et al.* Alterations in postprandial hepatic glycogen metabolism in type 2 diabetes. *Diabetes* 2004;**53**:3048–56.

48 Fang W, Guo J, Cao Y, *et al.* MicroRNA-20a-5p contributes to hepatic glycogen synthesis through targeting p63 to regulate p53 and PTEN expression. *J Cell Mol Med* 2016;**20**:1467–80. doi:10.1111/jcmm.12835

49 Massart J, Sjögren RJO, Lundell LS, *et al.* Altered miRNA-29 Expression in Type 2 Diabetes Influences Glucose and Lipid Metabolism in Skeletal Muscle. ;:43.

50 Jampoka K, Muangpaisarn P, Khongnomnan K, *et al.* Serum miR-29a and miR-122 as Potential Biomarkers for Non-Alcoholic Fatty Liver Disease (NAFLD). *Microrna* 2018;**7**:215–22. doi:10.2174/2211536607666180531093302

51 Ye D, Zhang T, Lou G, *et al.* Plasma miR-17, miR-20a, miR-20b and miR-122 as potential biomarkers for diagnosis of NAFLD in type 2 diabetes mellitus patients. *Life Sci* 2018;**208**:201–7. doi:10.1016/j.lfs.2018.07.029

52 Wruck W, Adjaye J. Meta-analysis reveals up-regulation of cholesterol processes in non-alcoholic and down-regulation in alcoholic fatty liver disease. *World J Hepatol* 2017;**9**:443–54. doi:10.4254/wjh.v9.i8.443

53 Castoldi M, Vujic Spasic M, Altamura S, *et al.* The liver-specific microRNA miR-122 controls systemic iron homeostasis in mice. *J Clin Invest* 2011;**121**:1386–96. doi:10.1172/JCI44883

54 Zhang H-F, Wang Y-C, Han Y-D. MicroRNA-34a inhibits liver cancer cell growth by reprogramming glucose metabolism. *Molecular Medicine Reports* 2018;**17**:4483–9. doi:10.3892/mmr.2018.8399

55 Pirola CJ, Fernández Gianotti T, Castaño GO, *et al.* Circulating microRNA signature in non-alcoholic fatty liver disease: from serum non-coding RNAs to liver histology and disease pathogenesis. *Gut* 2015;**64**:800–12. doi:10.1136/gutjnl-2014-306996

56 Jiang J-X, Gao S, Pan Y-Z, *et al.* Overexpression of microRNA-125b sensitizes human hepatocellular carcinoma cells to 5-fluorouracil through inhibition of glycolysis by targeting hexokinase II. *Molecular Medicine Reports* 2014;**10**:995–1002. doi:10.3892/mmr.2014.2271

57 Ramírez CM, Goedeke L, Rotllan N, *et al.* MicroRNA 33 Regulates Glucose Metabolism. *Molecular and Cellular Biology* 2013;**33**:2891–902. doi:10.1128/MCB.00016-13

58 Yang F, Zhang L, Wang F, *et al.* Modulation of the Unfolded Protein Response Is the Core of MicroRNA-122-Involved Sensitivity to Chemotherapy in Hepatocellular Carcinoma. *Neoplasia* 2011;**13**:590–600.

59 Byrd AE, Aragon IV, Brewer JW. MicroRNA-30c-2* limits expression of proadaptive factor XBP1 in the unfolded protein response. *J Cell Biol* 2012;**196**:689–98. doi:10.1083/jcb.201201077

60 Upton J-P, Wang L, Han D, *et al.* IRE1α Cleaves Select microRNAs During ER Stress to Derepress Translation of Proapoptotic Caspase-2. *Science* 2012;**338**:818–22. doi:10.1126/science.1226191

61 Kanda T, Matsuoka S, Yamazaki M, *et al.* Apoptosis and non-alcoholic fatty liver diseases. *World J Gastroenterol* 2018;**24**:2661–72. doi:10.3748/wjg.v24.i25.2661

62 Chang Y, Yan W, He X, *et al.* miR-375 inhibits autophagy and reduces viability of hepatocellular carcinoma cells under hypoxic conditions. *Gastroenterology* 2012;**143**:177-187.e8. doi:10.1053/j.gastro.2012.04.009

63 Baffy G. Hepatocellular Carcinoma in Non-alcoholic Fatty Liver Disease: Epidemiology, Pathogenesis, and Prevention. *J Clin Transl Hepatol* 2013;**1**:131–7. doi:10.14218/JCTH.2013.00005

64 Sanchez-Pareja A, Clément S, Peyrou M, *et al.* Phosphatase and tensin homolog is a differential diagnostic marker between nonalcoholic and alcoholic fatty liver disease. *World J Gastroenterol* 2016;**22**:3735–45. doi:10.3748/wjg.v22.i14.3735

65 Vinciguerra M, Veyrat-Durebex C, Moukil MA, *et al.* PTEN down-regulation by unsaturated fatty acids triggers hepatic steatosis via an NF-kappaBp65/mTOR-dependent mechanism. *Gastroenterology* 2008;**134**:268–80. doi:10.1053/j.gastro.2007.10.010

66 Wang B, Hsu S, Wang X, *et al.* Reciprocal Regulation of miR-122 and c-Myc in Hepatocellular Cancer: Role of E2F1 and TFDP2. *Hepatology* 2014;**59**:555–66. doi:10.1002/hep.26712

67 Coulouarn C, Factor VM, Andersen JB, *et al.* Loss of miR-122 expression in liver cancer correlates with suppression of the hepatic phenotype and gain of metastatic properties. *Oncogene* 2009;**28**:3526–36. doi:10.1038/onc.2009.211

68 Derdak Z, Villegas KA, Harb R, *et al.* Inhibition of p53 attenuates steatosis and liver injury in a mouse model of non-alcoholic fatty liver disease. *J Hepatol* 2013;**58**:785–91. doi:10.1016/j.jhep.2012.11.042

69 Tian X-F, Ji F-J, Zang H-L, *et al.* Activation of the miR-34a/SIRT1/p53 Signaling Pathway Contributes to the Progress of Liver Fibrosis via Inducing Apoptosis in Hepatocytes but Not in HSCs. *PLOS ONE* 2016;**11**:e0158657. doi:10.1371/journal.pone.0158657

70 Coenen-Stass AML, Magen I, Brooks T, *et al.* Evaluation of methodologies for microRNA biomarker detection by next generation sequencing. *RNA Biol* 2018;**15**:1133–45. doi:10.1080/15476286.2018.1514236

71 Mullokandov G, Baccarini A, Ruzo A, *et al.* High-throughput assessment of microRNA activity and function using microRNA sensor and decoy libraries. *Nat Methods* 2012;**9**:840–6. doi:10.1038/nmeth.2078

72 Jin Y, Chen Z, Liu X, *et al.* Evaluating the MicroRNA Targeting Sites by Luciferase Reporter Gene Assay. *Methods Mol Biol* 2013;**936**:117–27. doi:10.1007/978-1-62703-083-0_10

73 Tan SM, Lieberman J. Capture and Identification of miRNA Targets by Biotin Pulldown and RNA-seq. *Methods Mol Biol* 2016;**1358**:211–28. doi:10.1007/978-1-4939-3067-8_13

74 Riffo-Campos ÁL, Riquelme I, Brebi-Mieville P. Tools for Sequence-Based miRNA Target Prediction: What to Choose? *Int J Mol Sci* 2016;**17**. doi:10.3390/ijms17121987

75 Yang N. An overview of viral and nonviral delivery systems for microRNA. *Int J Pharm Investig* 2015;**5**:179–81. doi:10.4103/2230-973X.167646

76 Herrera-Carrillo E, Liu YP, Berkhout B. Improving miRNA Delivery by Optimizing miRNA Expression Cassettes in Diverse Virus Vectors. *Hum Gene Ther Methods* 2017;**28**:177–90. doi:10.1089/hgtb.2017.036

77 Khan AA, Betel D, Miller ML, *et al.* Transfection of small RNAs globally perturbs gene regulation by endogenous microRNAs. *Nat Biotechnol* 2009;**27**:549–55. doi:10.1038/nbt.1543

78 Baumann V, Winkler J. miRNA-based therapies: Strategies and delivery platforms for oligonucleotide and non-oligonucleotide agents. *Future Med Chem* 2014;**6**:1967–84. doi:10.4155/fmc.14.116

79 Daige CL, Wiggins JF, Priddy L, *et al.* Systemic delivery of a miR34a mimic as a potential therapeutic for liver cancer. *Mol Cancer Ther* 2014;**13**:2352–60. doi:10.1158/1535-7163.MCT-14-0209

80 Jiang X, Bugno J, Hu C, *et al.* Eradication of Acute Myeloid Leukemia with FLT3 Ligand-Targeted miR-150 Nanoparticles. *Cancer Res* 2016;**76**:4470–80. doi:10.1158/0008-5472.CAN-15-2949

81 Lennox KA, Behlke MA. Chemical modification and design of anti-miRNA oligonucleotides. *Gene Therapy* 2011;**18**:1111–20. doi:10.1038/gt.2011.100

82 van Rooij E, Kauppinen S. Development of microRNA therapeutics is coming of age. *EMBO Mol Med* 2014;**6**:851–64. doi:10.15252/emmm.201100899

83 Lundin KE, Højland T, Hansen BR, *et al.* Chapter Two - Biological Activity and Biotechnological Aspects of Locked Nucleic Acids. In: Friedmann T, Dunlap JC, Goodwin SF, eds. *Advances in Genetics*. Academic Press 2013. 47–107. doi:10.1016/B978-0-12-407676-1.00002-0

84 Morrisey EE. The magic and mystery of miR-21. *J Clin Invest* 2010;**120**:3817–9. doi:10.1172/JCI44596

85 Celikbilek M, Baskol M, Taheri S, *et al.* Circulating microRNAs in patients with non-alcoholic fatty liver disease. *World J Hepatol* 2014;**6**:613–20. doi:10.4254/wjh.v6.i8.613

86 Cermelli S, Ruggieri A, Marrero JA, *et al.* Circulating microRNAs in patients with chronic hepatitis C and non-alcoholic fatty liver disease. *PLoS ONE* 2011;**6**:e23937. doi:10.1371/journal.pone.0023937

87 López-Riera M, Conde I, Quintas G, *et al.* Non-invasive prediction of NAFLD severity: a comprehensive, independent validation of previously postulated serum microRNA biomarkers. *Sci Rep* 2018;**8**. doi:10.1038/s41598-018-28854-4

88 Kishnani PS, Austin SL, Abdenur JE, *et al.* Diagnosis and management of glycogen storage disease type I: a practice guideline of the American College of Medical Genetics and Genomics. *Genetics in Medicine* 2014;**16**:e1. doi:10.1038/gim.2014.128

89 Thakral S, Ghoshal K. miR-122 is a unique molecule with great potential in diagnosis, prognosis of liver disease, and therapy both as miRNA mimic and antimir. *Curr Gene Ther* 2015;**15**:142–50.

90 Elmén J, Lindow M, Schütz S, *et al.* LNA-mediated microRNA silencing in non-human primates. *Nature* 2008;**452**:896–9. doi:10.1038/nature06783

91 Lanford RE, Hildebrandt-Eriksen ES, Petri A, *et al.* Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection. *Science* 2010;**327**:198–201. doi:10.1126/science.1178178

92 Janssen HLA, Reesink HW, Lawitz EJ, *et al.* Treatment of HCV infection by targeting microRNA. *N Engl J Med* 2013;**368**:1685–94. doi:10.1056/NEJMoa1209026

93 Chakraborty C, Sharma AR, Sharma G, *et al.* Therapeutic miRNA and siRNA: Moving from Bench to Clinic as Next Generation Medicine. *Molecular Therapy - Nucleic Acids* 2017;**8**:132–43. doi:10.1016/j.omtn.2017.06.005

94 van der Ree MH, de Vree JM, Stelma F, *et al.* Safety, tolerability, and antiviral effect of RG-101 in patients with chronic hepatitis C: a phase 1B, double-blind, randomised controlled trial. *Lancet* 2017;**389**:709–17. doi:10.1016/S0140-6736(16)31715-9

95 Beg MS, Brenner AJ, Sachdev J, *et al.* Phase I study of MRX34, a liposomal miR-34a mimic, administered twice weekly in patients with advanced solid tumors. *Invest New Drugs* 2017;**35**:180–8. doi:10.1007/s10637-016-0407-y

96 Yan-nan B, Zhao-yan Y, Li-xi L, *et al.* MicroRNA-21 accelerates hepatocyte proliferation in vitro via PI3K/Akt signaling by targeting PTEN. *Biochem Biophys Res Commun* 2014;**443**:802–7. doi:10.1016/j.bbrc.2013.12.047

97 He W, Wang C, Mu R, *et al.* MiR-21 is required for anti-tumor immune response in mice: an implication for its bi-directional roles. *Oncogene* 2017;**36**:4212–23. doi:10.1038/onc.2017.62

98 Hanin G, Yayon N, Tzur Y, *et al.* miRNA-132 induces hepatic steatosis and hyperlipidaemia by synergistic multitarget suppression. *Gut* 2018;**67**:1124–34. doi:10.1136/gutjnl-2016-312869

99 Yin H, Hu M, Zhang R, *et al.* MicroRNA-217 promotes ethanol-induced fat accumulation in hepatocytes by down-regulating SIRT1. *J Biol Chem* 2012;**287**:9817–26. doi:10.1074/jbc.M111.333534

100 Zhou B, Li C, Qi W, *et al.* Downregulation of miR-181a upregulates sirtuin-1 (SIRT1) and improves hepatic insulin sensitivity. *Diabetologia* 2012;**55**:2032–43. doi:10.1007/s00125-012-2539-8

101 Kurtz CL, Fannin EE, Toth CL, *et al.* Inhibition of miR-29 has a significant lipid-lowering benefit through suppression of lipogenic programs in liver. *Sci Rep* 2015;**5**:12911. doi:10.1038/srep12911

102 Chang H-C, Guarente L. SIRT1 and other sirtuins in metabolism. *Trends Endocrinol Metab* 2014;**25**:138–45. doi:10.1016/j.tem.2013.12.001

Figure legends

Figure 1: Molecular mechanisms modulating the repressor activity of miRNAs

A) RNA-binding proteins (RBPs) can influence the repressor activity of miRNAs through different mechanisms. Indeed, RBPs can promote the repressor activity of miRNAs by binding target mRNAs and favouring a conformation suitable for miRNA-mRNA binding and repression of transcription (a) (PMID 25048093). On the contrary, as shown in (b) RBPs can also inhibit miRNAs activities by binding the miRNAs themselves, therefore impeding their interactions with target mRNAs. A typical example illustrating this mechanism is the sequestering of miR-21 by the RNA-binding protein HuR, which prevents miR-21 to repress its targets (PMID 26189797). Alternatively, RBPs can occupy the miRNA-binding sequence or induce non-permissive conformations of the target mRNA for recognition by specific miRNAs by binding outside of the 3'UTR, both mechanisms resulting in inhibition of the repressor activity of miRNAs (PMID 25048093).

B) Competing RNAs such as long non-coding RNAs (lncRNAs), small non-coding RNAs (sncRNA including microRNAs and small nucleolar RNAs), circular RNAs or pseudogenes can hold sequences complementary to the miRNA and thus sequester/decoy the miRNA from binding to its target mRNA, leaving it unrepressed (PMID 24523727).

C) Stoichiometry of target mRNAs expression can influence miRNA activity. When a target mRNA-X is over-expressed, it can sequester most copies of the miRNA in the cell, therefore strongly influencing the effect of this miRNA on secondary targets, even in the absence of alterations of miRNA expression (PMID 21802130).

D**) Stoichiometry of miRNAs** can also influence the respective activity of specific miRNAs. When miR-X competes with miR-Y for the same target, overexpression of miR-X will displace miR-Y from its primary target and leave it available to bind to secondary mRNAs targets (PMID 21802130).

Figure 2: miRNAs governing the glucose/lipid metabolism and stress-induced pathways in the liver

Schematic representation of miRNAs deregulated in NAFLD and contributing to steatosis development by altering the hepatic glucose and lipid metabolism, autophagy, the ER-stress and the UPR pathways.

Figure 3: Pleiotropic roles of miR-21 in hepatic metabolic homeostasis and tumorigenesis.

Although miR-21 is upregulated in NAFLD/NASH and contributes to the development of fatty liver disease, many studies have identified it as a potent oncogenic miRNA (oncomiR) targeting key tumour suppressors (PMID 27699004). However, numerous experimentally validated miR-21 targets are also well-characterized oncogenes suggesting that depending on the context (e.g., cells/tissues, tumour microenvironment, mutations, metabolic homeostasis, etc.), miR-21 may also have a tumour suppressive activity. The multitude of miR-21 targets involved in intricate processes tightly regulating metabolism and progression/regression of tumorigenesis render difficult to predict the outcomes of potential therapeutic strategies based on inhibition or activation of miR-21. Illustrating this complexity and the miR-21 pleiotropic roles, a list of experimentally validated human miR-21-5p targets was retrieved from the miRWalk database (V2.0) and then cross-referenced (Venn Diagram, upper left panel) with HCC-related genes obtained from the MetaCore^{TM} software and a list of glucose/lipid metabolism-related genes (Gene Ontology, glucose and lipid metabolic processes). 23 genes were overlapping between validated miR-21 targets and genes involved in metabolism (upper right panel in green). An overlap of 109 genes was found between validated miR-21 targets and HCC-related factors. The 109 HCC-related factors were then classified as oncogenes (lower left panel in red), or as tumour suppressors (lower right panel in blue), based on literature. References (PMIDs) for the classification of each gene are mentioned below the gene name. It is noteworthy that some miR-21 targets classified as tumour suppressors or oncogenes are involved in hepatic metabolism (e.g., *PTEN, BRCA1, HPGD, AKT2, PPARα, MYC* and *CLU* in green in lower panels), thereby emphasizing the contribution of metabolic reprogramming to hepatic tumorigenesis.

Figure 4: Predicted and validated human miR-122-5p, miR-33a-5p, miR-34a-5p and miR-21-5p targets involved in glucose and lipid metabolism.

The miRWalk 2.0 database was used to retrieve the lists of predicted and validated targets of: (**A**) miR-122-5p, (**B**) miR-33a-5p, (**C**) miR-34a-5p and (**D**) miR-21-5p. Predicted targets were

obtained using 12 different algorithms (i.e., miRWalk2.0, MicroT4, miRanda, miRBridge, miRDB, miRMap, miRNAMap, PICTAR2, PITA, RNA22, RNAhybrid and TargetScan). Only candidates predicted by at least five different algorithms are represented. Predicted and validated targets of each miRNA were then compared with glucose/lipid metabolism-related genes obtained with the MetaCore™ software (Enrichment with Gene Ontology, biological processes, lipid/glucose metabolism). Genes involved in lipid/glucose metabolism and targeted by each specific miRNA are displayed on right panels.

Figure 5: miRNAs targeting of key metabolic transcription factors and regulators

In order to develop an efficient miRNA-based strategy, targeting transcription factors involved in hepatic glucose and lipid metabolism would likely have a broader systemic effect on NAFLD than choosing miRNAs targeting specific metabolic enzymes. As depicted in the figure, miR-192 and miR-29, which are both deregulated in the liver of patients with NAFLD, were reported to target SREBP1, thereby downregulating hepatic lipogenesis in rodents (PMID 28483554, 28664184). ChREBP is regulated by miR-1322 in hepatic cells (PMID 30079502). LXR, which can regulate the activity of both SREBP1 and ChREBP, was also found to be targeted by miRNAs such as miR-1, miR-155, miR-206 and miR-613 (PMID 23499676, 23991091, 24603323, 23496987). PPARγ, a fourth lipogenic transcription factor, was found to be regulated by miR-27a, miR-34a, miR-128 and miR130 (PMID 28167956). Of note, besides lipogenesis, PPARγ is also involved in hepatic stellate cells activation repression, thereby negatively regulating hepatic fibrosis. Given that hepatic fibrosis is a common complication in later stages of NAFLD, these miRNAs could be employed to prevent fibrosis development as well, aside from lipid metabolism modulation. Of interest are also miRNAs targeting co-factors (e.g., PGC1α) or repressors (e.g., NCOR) of PPARγ. Few miRNAs have been shown to regulate the expression of these proteins, including miR-696 and miR-130a for PGC1α (PMID 27432632, 25595716), miR-16 and miR-100 for NCOR2 (PMID 22292036, 24244722). Several other miRNAs indicated in the figure were shown to target PPARγ in murine and human adipose tissue, including miR-27b, miR-540, miR-302a, miR-138, and miR-548d but their role in the liver remains currently not investigated. Another strategy to alleviate steatosis in the liver is to activate lipid oxidation by de-repressing expression of PPARα, a key lipid oxidation factor. PPARα was shown to be targeted by miR-9, miR-10b, miR-21, miR-33 and miR-199a (PMID 25592151, 19780876, 21636785, 24100264, 25312970). Finally, as for PPARγ, miRNAs targeting PPARα in adipose tissue of obese mice/humans, e.g., miR-106b-93

(PMID 23954633), are also indicated. Arrows on the miRNAs indicate up- or down-regulation of the miRNA in NAFLD; ML: mouse liver; HL: human liver; MAT: mouse adipose tissue; HAT: human adipose tissue; H-HCC: human HCC samples; ND: Not determined. The PMID of references of interest are indicated.

BOX 1

- The main role of miRNAs is to induce mRNA degradation or to inhibit their translation, but new functions have been recently uncovered.
- Both the guide and passenger strand of miRNAs can be functionally active in cells.
- The expression of a miRNA does not necessarily correlate with its activity in pathophysiological conditions.
- miRNA activity can be modulated by various mechanisms including competing endogenous RNAs, RNA-binding proteins, miRNAs editing and the stoichiometry of miRNAs/mRNAs.
- Both the expression and the activity of specific miRNAs needs to be evaluated to understand their pathophysiological roles.

BOX 2

- miRNAs regulate various aspects of the trafficking, anabolism and catabolism of lipids in hepatic cells.
- Other pathways contributing to steatosis development, e.g. the carbohydrate metabolism and stress-activated pathways, are also under the control of miRNAs.
- Deregulated miRNAs expression/activity with NAFLD can importantly contribute to alterations of cancer-related factors in the liver.
- Alterations of miR-122, miR-33, miR-34a and miR-21 expression/activity are key mechanisms contributing to NAFLD development and progression to more severe stages.

BOX 3

- Currently available bioinformatic tools have a limited predictive power to identify all relevant pathophysiological targets of specific miRNAs.
- *In vivo* modulation of miRNAs expression/activity can be accomplished through either genetic engineering of knockout/transgenic animals, or transduction of viral vector systems, or pharmacological delivery of synthetic modified nucleotides inhibiting or mimicking endogenous miRNAs.
- Different *in vivo* approaches to decipher the pathophysiological role of a specific miRNA can lead to different conclusions due to the peculiarity of available methods.
- Circulating levels of miR-122 and miR-34a are suitable biomarkers for NAFLD/NASH. Other miRNAs of interest as biomarkers need further validation in large human cohorts.

- No clinical trials using miRNAs-based therapies have been currently designed for NAFLD specifically.
- Optimization of hepatic delivery systems, chemical modifications, and pharmacological dosages of miRNA analogues/inhibitors are required to design successful clinical trials for miRNAs-based therapies in NAFLD.
- Analogues/inhibitors of miRNAs targeting metabolic transcription factors having a broad systemic effect on hepatic metabolism are of particular interest as therapeutic tools for NAFLD.

Table 1 : Deregulated miRNAs in hepatic tissues and blood circulation of patients with NAFLD/NASH

Liver tissue miRNAs			Circulating miRNAs		
miRNA	Expression	Reference (PMID)	miRNA	Levels	Reference (PMID)
miR-122	↓	19030170	miR-122	↑	24973316, 21886843, 27956809, 26565986, 29848284
miR-34a	↑	19030170, 30142428	miR-34a	↑	21886843, 23727030, 27956809
miR-33	↑	27669236	miR-33	↑	27669236
miR-21	↑	19030170, 26338827	miR-21	↑	23727030
miR-192	↑	24973316, 30142428	miR-192	↑	24973316, 27956809, 26565986
miR-375	↓	19030170, 30142428, 26874844	miR-375	↑	24973316
miR-146b	↑	19030170, 28119530	miR-146b	↓	25232454, 27493762
miR-221/222	↑	19030170 22267590	miR-221/222	↑	30544653
miR-132	↑	28381526	miR-132	↓	27493762
miR-181b	↑	19030170	miR-181d	↓	25232454
miR-422	↓	28119530	miR-197	↓	25232454
miR-139	↓	28119530	miR-29a	↓	29848284

Table 2 : Experimental *in vivo* approaches to investigate the role of miR-122, miR-34a, miR-33 and miR-21 in NAFLD.

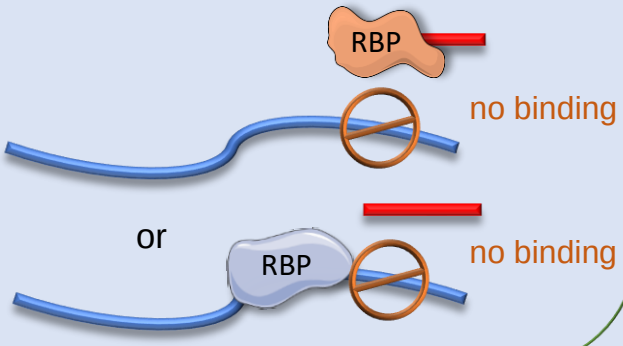
Pharmacological analogues (mimics) or inhibitors (antimiRs)			Constitutive/conditional knock-out animals and viral transductions		
miRNA	***mimic/antimiR***	***PMID***	***miRNA***	***Genetic manipulation***	***PMID***
miR-122 in mice	mimic (LNP-DP1)	23727126	miR-122 in mice	knock-out	28735896
	mimic (agomiR)	26933995		knock-out	22820290
	mimic (GPMQNs)	28114997		knock-out	22820284
	mimic (AAV)	22820288		liver-specific knock-out	24113455
	antimiR (LNA)	21364282		liver-specific and total knock-out	22820288
	antimiR (ASO)	16459310		liver-specific and total knock-out	28963035
	antimiR (antagomiR)	16258535	miR-34a in mice	knock-out	30342367
miR-122 in fish	antimiR (antagomiR)	27855320		knock-out	27635790
miR-122 in primates	antimiR (LNA)	18368051		knock-out	22844244
	antimiR (LNA)	19965718		knock-out	27377585
miR-34a in mice	mimic (agomiR)	29197627		knock-out	28533191
	antimiR (antagomiR)	24560136		Lentiviral miR-34 expression	22964582
miR-34a in fish	antimiR (antagomiR)	30115855	miR-33 in mice	knock-out	26538644
miR-34a in primates	mimic	24397447		knock-out	29091769
miR-33 in mice	antimiR	24753547		knock-out	29466739
	antimiR (LNA)	29643920		knock-out	24300912
	antimiR (ASO)	23702658		Knock-out	20855588
miR-21 in mice	antimiR (antagomiR)	26338827	miR-21 in mice	knock-out	26338827
	antimiR (LNA)	25141837		liver-specific and total knock-out	27222533
	antimiR (NP)	25652012	miR-21 in rats	Adenovirus-mediated miR-21 decoy	27226339

Table 3 : Summary of the nutritional, hormonal and pathological regulation of miRNAs which were found to target lipid metabolism-related transcription factors and co-factors.

A) RNA-binding proteins (RBP)

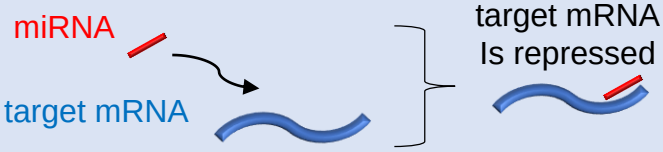
(a) promote miRNA-mediated mRNA transcription

(b) inhibit miRNA-mediated mRNA transcription



B) Competing endogenous RNA

Normal conditions

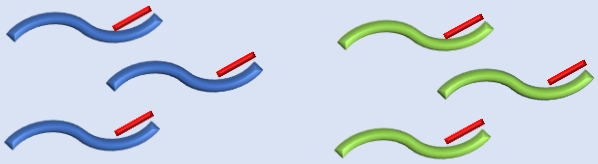


miRNA copies are sequestered by:

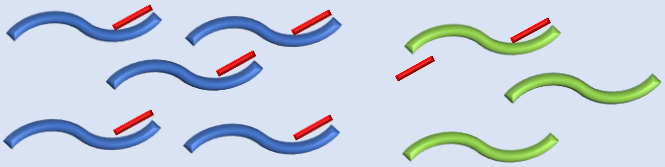
- lncRNA
- sncRNA
- circular RNA
- pseudogenes

C) Stoichiometry of target mRNAs

Normal conditions

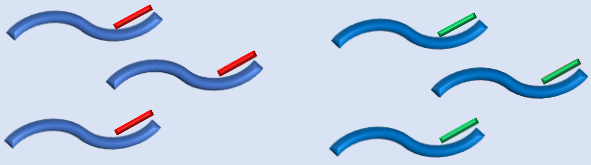


Target X is up-regulated

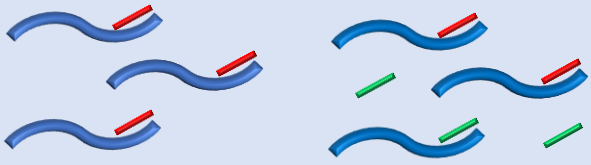


D) Stoichiometry of miRNA

Normal conditions



miR-X is up-regulated / increased activity



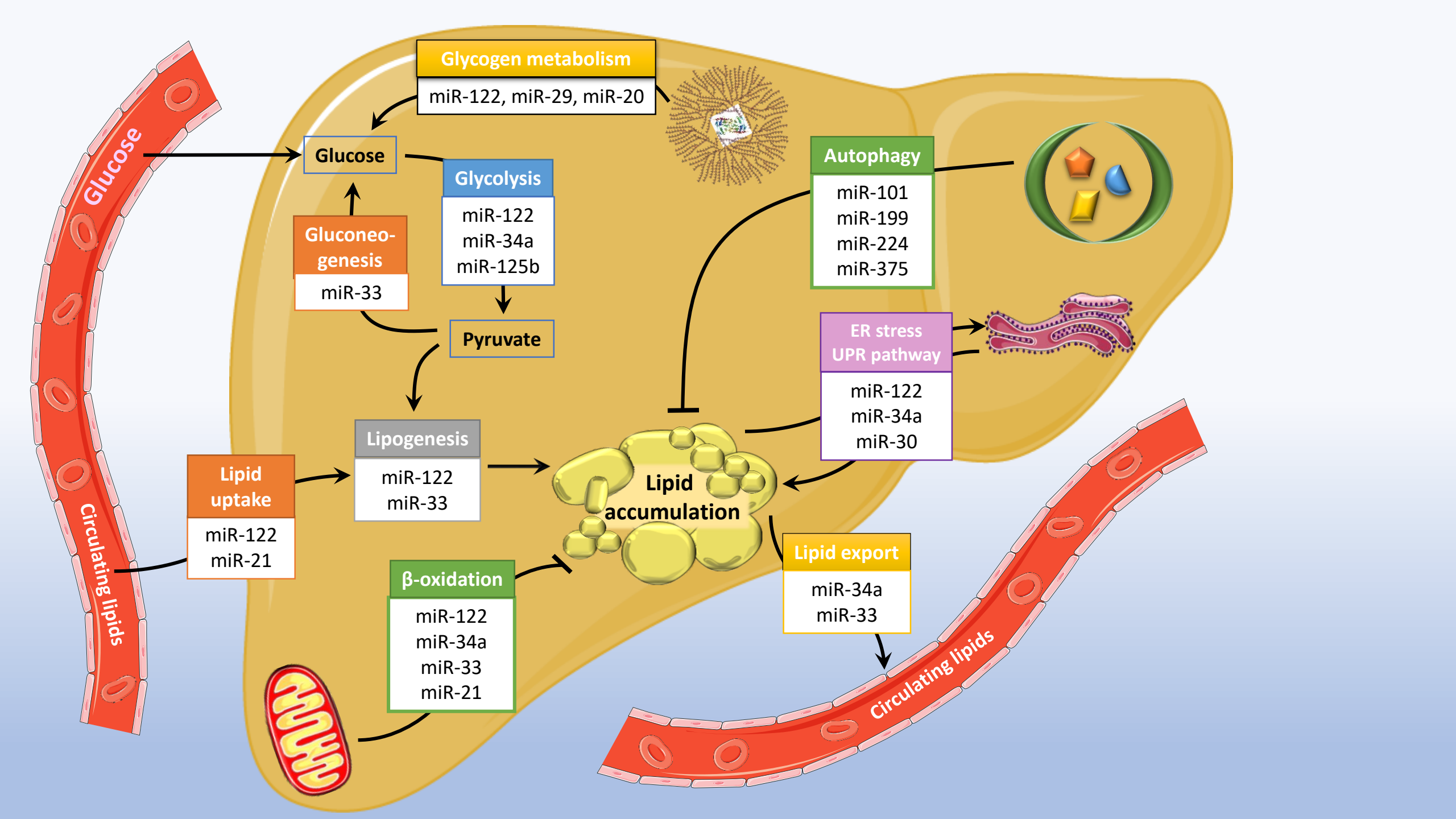
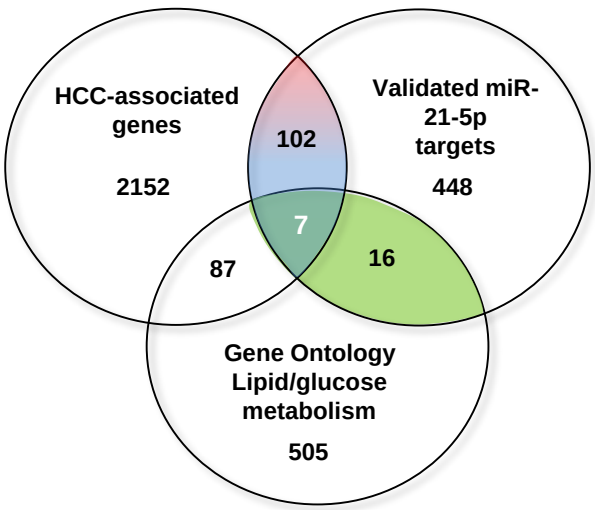
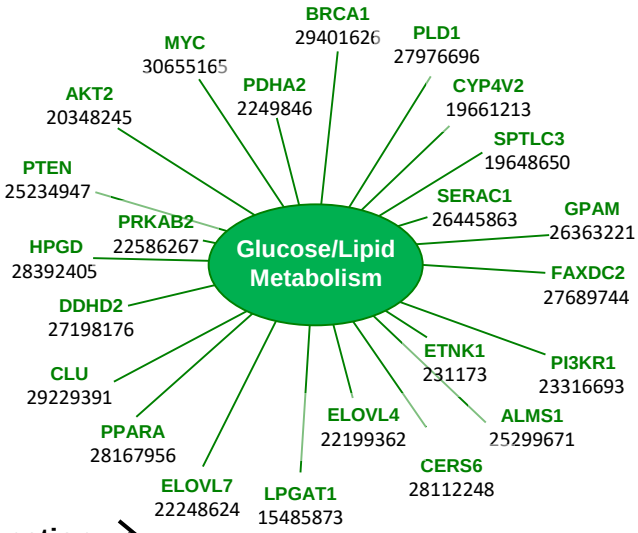

Glycogen metabolism
miR-122, miR-29, miR-20
Glucose
Glucose
Glycolysis
miR-122
miR-34a
miR-125b
Gluconeo-genesis
miR-33
Pyruvate
Autophagy
miR-101
miR-199
miR-224
miR-375
ER stress
UPR pathway
miR-122
miR-34a
miR-30
Lipogenesis
miR-122
miR-33
Lipid uptake
miR-122
miR-21
Lipid accumulation
β-oxidation
miR-122
miR-34a
miR-33
miR-21
Lipid export
miR-34a
miR-33
Circulating lipids
Circulating lipids

Figure 3



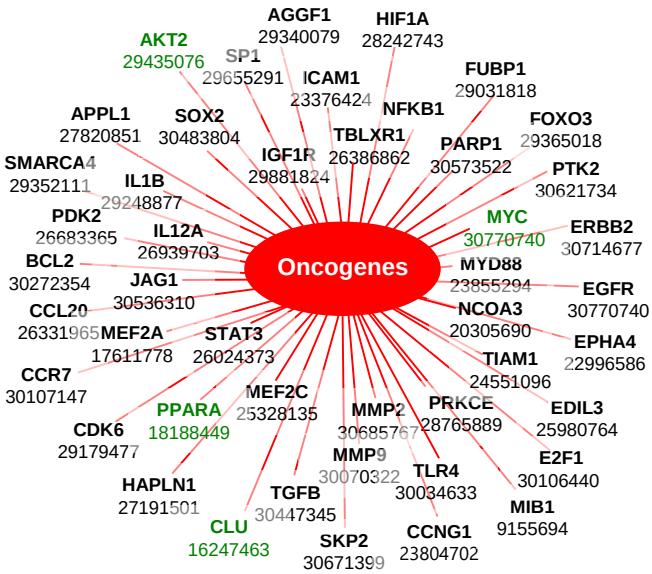


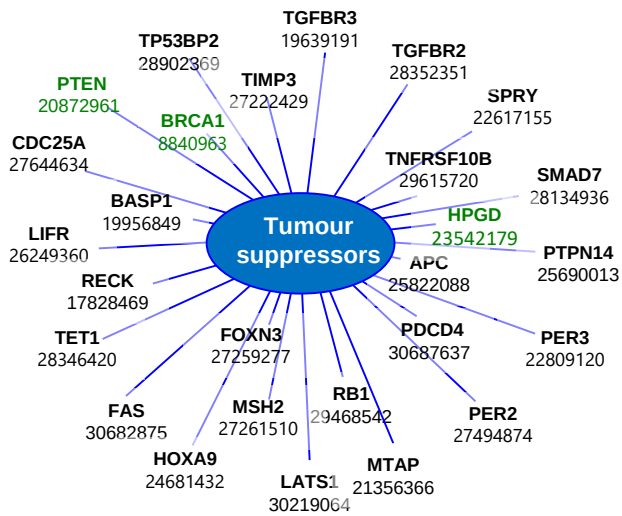
Metabolic function

miR-21-5p

Tumour suppressive function

Oncogenic function





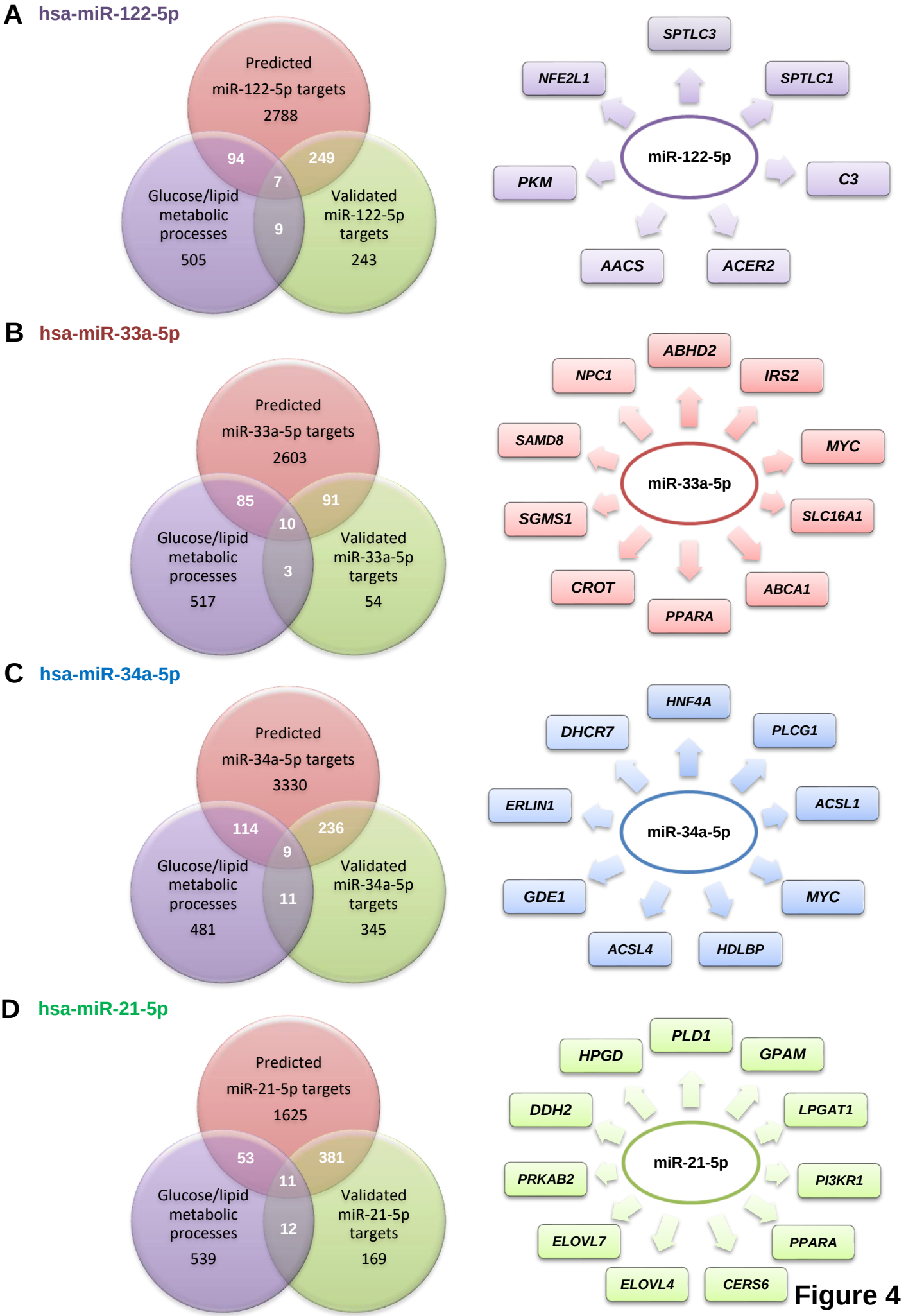
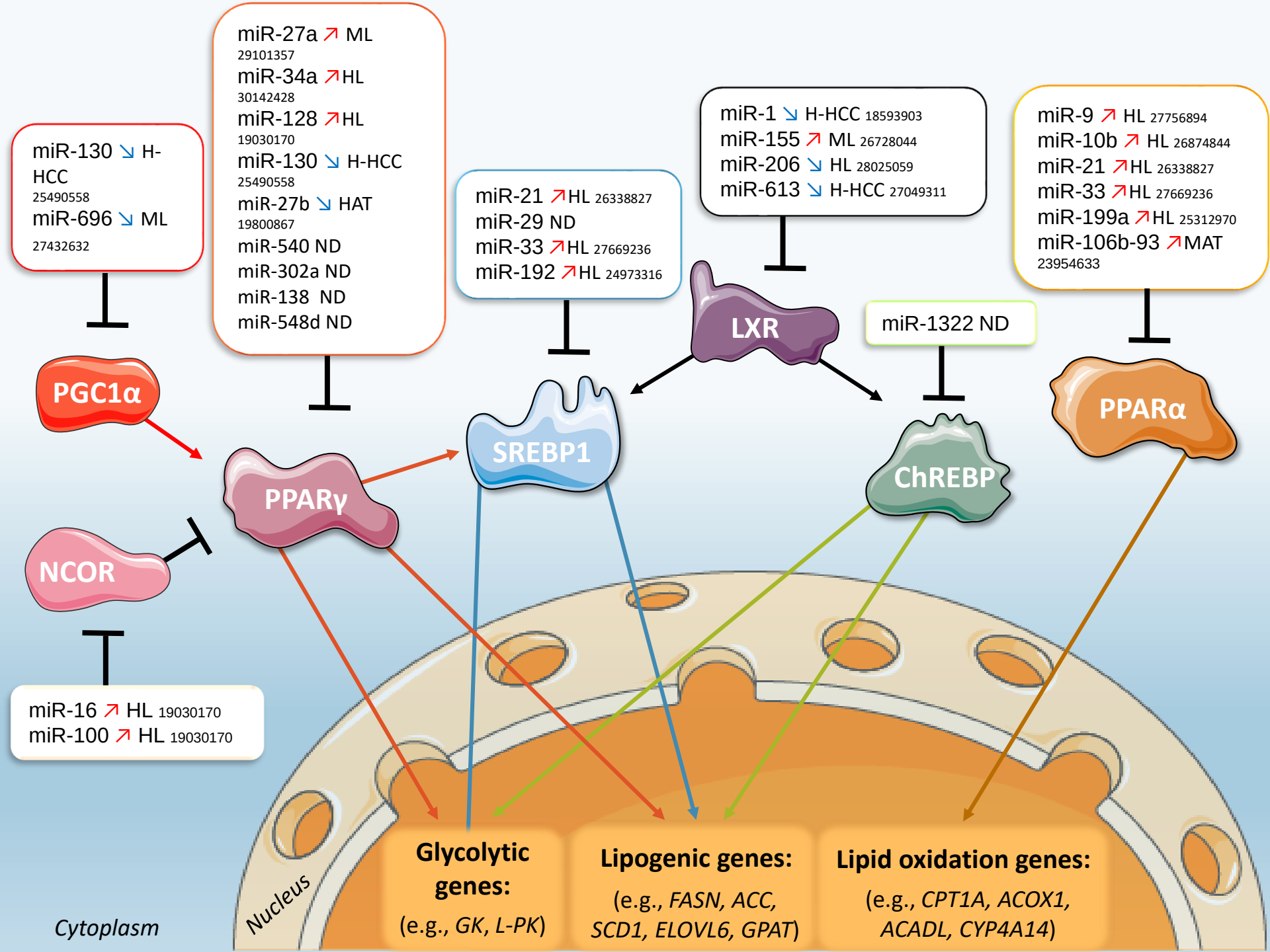

A
hsa-miR-122-5p
Predicted
miR-122-5p targets
2788
94
249
7
9
Glucose/lipid
metabolic
processes
505
Validated
miR-122-5p
targets
243
SPTLC3
NFE2L1
SPTLC1
miR-122-5p
PKM
C3
AACS
ACER2
B
hsa-miR-33a-5p
Predicted
miR-33a-5p targets
2603
85
91
10
3
Glucose/lipid
metabolic
processes
517
Validated
miR-33a-5p
targets
54
ABHD2
NPC1
IRS2
SAMD8
MYC
miR-33a-5p
SGMS1
SLC16A1
CROT
ABCA1
PPARA
C
hsa-miR-34a-5p
Predicted
miR-34a-5p targets
3330
114
236
9
11
Glucose/lipid
metabolic
processes
481
Validated
miR-34a-5p
targets
345
HNF4A
DHCR7
PLCG1
ERLIN1
ACSL1
miR-34a-5p
GDE1
MYC
ACSL4
HDLBP
D
hsa-miR-21-5p
Predicted
miR-21-5p targets
1625
53
381
11
12
Glucose/lipid
metabolic
processes
539
Validated
miR-21-5p
targets
169
PLD1
HPGD
GPAM
DDH2
LPGAT1
miR-21-5p
PRKAB2
PI3KR1
ELOVL7
PPARA
ELOVL4
CERS6

Figure 4


miR-130 ↘ H-HCC 25490558
miR-696 ↘ ML 27432632
miR-27a ↗ ML 29101357
miR-34a ↗HL 30142428
miR-128 ↗HL 19030170
miR-130 ↘ H-HCC 25490558
miR-27b ↘ HAT 19800867
miR-540 ND
miR-302a ND
miR-138 ND
miR-548d ND
miR-21 ↗HL 26338827
miR-29 ND
miR-33 ↗HL 27669236
miR-192 ↗HL 24973316
miR-1 ↘ H-HCC 18593903
miR-155 ↗ ML 26728044
miR-206 ↘ HL 28025059
miR-613 ↘ H-HCC 27049311
miR-9 ↗ HL 27756894
miR-10b ↗ HL 26874844
miR-21 ↗HL 26338827
miR-33 ↗HL 27669236
miR-199a ↗HL 25312970
miR-106b-93 ↗MAT 23954633
miR-1322 ND
PGC1α
PPARγ
NCOR
SREBP1
LXR
ChREBP
PPARα
miR-16 ↗ HL 19030170
miR-100 ↗ HL 19030170
Cytoplasm
Nucleus
Glycolytic genes:
(e.g., GK, L-PK)
Lipogenic genes:
(e.g., FASN, ACC, SCD1, ELOVL6, GPAT)
Lipid oxidation genes:
(e.g., CPT1A, ACOX1, ACADL, CYP4A14)