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Obesity Comorbidity/Etiology and Pathophysiology

Adipose may actively delay progression of NAFLD by releasing tumor-suppressing, anti-fibrotic miR-122 into circulation

A. Baranova[1,2], D. Maltseva[3,4] and A. Tonevitsky[4,5]

[1]School of Systems Biology, George Mason University, Fairfax, VA, USA; [2]Research Center for Medical Genetics, Moscow, Russia; [3]Department of Fundamental Medicine, School of Biomedicine, Far Eastern Federal University (FEFU), Vladivostok, Russia; [4]Scientific Research Center Bioclinicum (SRC Bioclinicum), Moscow, Russia; and [5]Higher School of Economics, Moscow, Russia

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Address for correspondence: Prof. A Baranova, School of Systems Biology, George Mason University, 4400 University Dr., Fairfax, VA, USA.
E-mail: abaranov@gmu.edu

Summary

Nonalcoholic fatty liver disease (NAFLD) is the most common liver pathology. Here we propose tissue-cooperative, homeostatic model of NAFLD. During early stages of NAFLD the intrahepatic production of miR-122 falls, while the secretion of miRNA-containing exosomes by adipose increases. Bloodstream carries exosome to the liver, where their miRNA cargo is released to regulate their intrahepatic targets. When the deterioration of adipose catches up with the failing hepatic parenchyma, the external supply of liver-supporting miRNAs gradually tapers off, leading to the fibrotic decompensation of the liver and an increase in hepatic carcinogenesis. This model may explain paradoxical observations of the disease-associated decrease in intrahepatic production of certain miRNAs with an increase in their levels in serum. Infusions of miR-122 and, possibly, some other miRNAs may be efficient for preventing NAFLD-associated hepatocellular carcinoma. The best candidates for exosome-wrapped miRNA producer are adipose tissue-derived mesenchymal stem cells (MSCs), known for their capacity to shed large amounts of exosomes into the media. Notably, MSC-derived exosomes with no specific loading are already tested in patients with liver fibrosis. Carrier exosomes may be co-manufactured along with their cargo. Exosome-delivered miRNA cocktails may augment functioning of human organs suffering from a variety of chronic diseases.

Keywords: NAFLD, miR-122, adipose, homeostasis.

Non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease (NAFLD) is a chronic liver pathology with the risk factors almost identical to that of the metabolic syndrome (MS) (1,2). Moreover, NAFLD is considered to be the hepatic manifestation of MS (3,4). Simple, non-inflammatory steatosis is the most common form of NAFLD, which remains stable in a majority of individuals, or may even resolve (5,6). However, in ~5% to 20% of individuals with NAFLD, steatosis eventually develops into non-alcoholic steatohepatitis (NASH), and subsequently, with fibrotic progression, into liver cirrhosis (3,7,8). Importantly, fibrosis progression may be seen not only in NASH but also in non-inflammatory NAFLD (9). In patients with fatty liver, but no lobular inflammation or hepatocellular ballooning, the annual fibrosis progression rate is about one stage of progression over 14.3 years; patients with NASH attain next fibrotic grade, on average, in 7.1 years (10). Both NAFLD variants, the fibrotic and the not yet fibrotic, predispose to the development of hepatocellular carcinoma (HCC) (3,11), with the estimated annual HCC incidence in the progressive form of NAFLD being at about 0.3% (12).

The pathophysiological complexity of the non-alcoholic fatty liver disease involves inter-organ communications

It seems that all four pathophysiological processes involved in the pathogenesis of NAFLD and its sequelae, namely, the accumulation of intrahepatic fat, the inflammation of the liver parenchyma, the fibrosis and the tumorigenesis, may

proceed simultaneously, within the same organ. While hastening each other and serving as mutual confounders, these processes develop by deregulation of distinct regulatory networks (13,14). Moreover, all four pathophysiological components of NASH are influenced by the cellular and molecular changes taking place in distant tissues and organs. A major extrahepatic player in the progression of NAFLD is cytokine-producing and adipokine-producing visceral adipose tissue (15–17). Other named important contributors are the gut (18), the gastric tissue (19), the muscle (20,21), the thyroid (22) and even the brain (23).

In each pair of communicating organs, the long-distance conversation is maintained by a set of soluble messengers. When secreted into circulation, these messengers are carried to their target tissues where they are either internalized or bind to surface receptors and elicit the transduction of respective signals. In the case of the thyroid, a classical endocrine organ, the inter-tissue communication relies on the secretion of the soluble thyroid hormones thyroxine (T4) and 3,3′,5-triiodo-L-thyronine (T3), which stimulate hepatic lipid synthesis, oxidation and autophagy (24). These hormones bind to specific thyroid hormone receptors (TR), of which the TRβ isoform is liver specific (25). In the case of the brain, a link has been suggested between the deregulation of the hypothalamic–pituitary–adrenal axis to the subclinical hypercortisolemia, and to the liver histopathology (26,27). Both the thyroid and the brain influence the liver through classical endocrine mechanisms by producing hormones being transported by the circulatory system to target distant organs.

The visceral and subcutaneous adipose tissue compartments are now considered endocrine organs (28) because they release multiple bioactive substances known as 'adipose-derived secreted factors', or 'adipokines'. A partial, incomplete list of adipose-derived cytokines and adipokines includes tumor necrosis factor-α, interleukin 6, plasminogen activator inhibitor 1, adiponectin, visfatin and other well-characterized molecules known to be involved in NAFLD (29–31). Recently, skeletal muscles were also added to the ranks of endocrine-active tissues (32). Muscles produce multiple myokines, primarily represented by insulin-secretion-promoting interleukin 6 (33), and myostatin, a negative regulator of muscle growth that plays an important role in the development of insulin resistance (34). On top of this, dyslipidemia-preventing myonectin and irisin were also recently discovered (35). As insulin sensitivity and dyslipidemia are well known as key factors in the pathophysiology for NAFLD, a reasonable inference is that muscle is likely a factor in the development of NAFLD.

Similarly, one may propose that gastric tissue is also an endocrine gland on the basis that it secretes a number of soluble molecules involved in metabolic and inflammatory pathways. This organ secretes acetylated and des-acetylated forms of ghrelin, and obestatin (36,37). The gut communicates to the liver through the alteration of the levels of small metabolites produced by residing microbiome, and through increasing the permeability of its walls to various injurious substances (38). In addition, intestines secrete a variety of physiologically active gut peptides such as secretins, cholecystokinin, substance P and others (39,40). Importantly, gastric-derived and intestine-derived substances have been also suggested as contributors to either the initiation or the progression of NAFLD.

Given this complexity, it is not surprising that organ interplay may complicate the development of the blood-based, non-invasive diagnostic and prognostic tests necessary for the risk stratification of patients with NAFLD.

miRNAs are proposed both as biomarkers and as regulators of non-alcoholic fatty liver disease phenotypes

A majority of proposed candidate biomarkers for non-invasive assessment of NAFLD were selected among either soluble proteins (41,42) or small metabolites (43–45). Recently, short, noncoding RNAs, also known as microRNAs (miRNAs), have been associated with histological features of NAFLD (46,47). These intracellularly produced molecules may be shed into circulation where they remain stable. Importantly, circulating miRNAs are detectable by a polymerase chain reaction, thus providing for much higher sensitivity than protein biomarkers.

Shortly after the discovery of these molecules, the remarkable diagnostic capacity of circulating miRNAs became evident in relation to liver diseases. In their groundbreaking animal work, Wang and colleagues reported significant changes in the levels of many cell-free miRNAs in the blood of mice treated with acetaminophen, a liver-injuring agent (48). For two liver-specific miRNAs, miR-122 and miR-192, elevations in their levels were dose dependent; moreover, these increases preceded changes in the activity of alanine aminotransferase (48). Soon after, Laterza *et al.* observed that blood plasma concentrations of miR-122, miR-133a and miR-124 reflect the respective degrees of liver, muscle and brain tissue injuries (49). In human studies, Zhang *et al.* have further noted correlations between increases in serum levels of miR-122 and other miRNAs and the histological stage of NAFLD (50), while Xu and colleagues expanded these findings into HCC and chronic hepatitis (51). Moreover, a miRNA panel consisting of miR-122, miR-192, miR-21, miR-223, miR-26a, miR-27a and miR-801 was shown to effectively diagnose HCC with a high degree of accuracy, even in patients with underlying non-malignant liver pathologies (52).

The NAFLD-associated changes in the spectrum of extracellular miRNA species present in human blood have been the subject of more than one intensive investigation (50,53–63). Each of these reports showed that circulating miRNAs

reflect liver damage more precisely than do serum transaminases. In particular, serum levels of miR-122 were repeatedly reported as steadily increasing with the progression of the disease from simple steatosis to steatohepatitis (53–56). In fact, the top candidate position of miR-122 remained unchallenged in a number of follow-up studies highlighting additional serum miRNA candidates, including miR-34a, miR-16, miR-1290, miR-27b, miR-192 and miR-375 as promising biomarkers for staging of NAFLD (56,57,59).

To date, however, the appeal of circulating miRNA molecules for the diagnosis of various liver diseases including monitoring NAFLD progression into NASH and/or significant hepatic fibrosis has so far not resulted in diagnostic breakthrough. Ranked lists of miRNA candidates highlighted by studies cited earlier certainly revolve around same core group of miRNAs causally related to various pathophysiological aspects of NAFLD but fail to zero in on a consensus diagnostic panel. Most likely, this situation is due to the differences in the processing of collected serum samples, with larger or smaller portions of exosomal miRNAs being extracted in each protocol (64). It is expected that future studies of miRNA biomarkers of liver diseases would concentrate on using the state-of-the-art exosome or microvesicle (MV) preparation techniques rather than perform total nucleic acid extraction from the serum.

Beside technical difficulties with proper assessment of miRNA concentration in serum, plasma or various vesicular compartments, an assembly of NAFLD or NASH diagnosing panels is complicated by possibility that each pathophysiological process in the development of NAFLD may be governed or, at least, reflected by its own miRs. The patterns of miRNA expression and subsequent release to biological fluids are far from simple (65), possible owing to an intricate interplay between various pools of miRNAs and NAFLD-associated soluble proteins. Table 1 summarizes tissue sources of various miRNAs proposed as NAFLD and NASH biomarkers, and their putative physiological roles. As could be seen for this table, the production of a majority of these miRNA species is not restricted to liver, but rather ubiquitous, while the effects are pleiotropic, and, likely, tissue specific. Accordingly, we should assume that concentrations of individual miRNAs in serum may not be collinear with the temporal pattern of liver parenchyma deterioration.

To illustrate this point, provided subsequently is an analysis of the current state of the knowledge regarding intrahepatic and serum expression levels of the most studied NAFLD-associated noncoding RNA molecule miR-122.

miR-122: intracellular–extracellular seesaw expression paradox

According to a very thorough RNA-Seq-based study by Hou *et al.*, miR-122 is expressed almost exclusively in the liver, comprising more than 52% of the total pool of liver miRNAs (66). Overwhelming majority of published reports agree that the levels of miR-122 in sera of NAFLD individuals are elevated (53–57,59–62). If a majority of miRNAs present in circulation of individuals with NAFLD originate from the liver, it would be reasonable to expect an increase in the expression of miR-122 in hepatic parenchyma, along with a progression of NAFLD across its stages. However, in the diseased parenchyma of the livers of patients with NAFLD, intracellular levels of miR-122 are lower than those in the livers of healthy individuals (56,67,68) (Fig. 1A).

A paradox of the fall in the levels of miR-122 within the diseased tissue being accompanied by the increase in levels of same miRNA in the serum may have two possible explanations, either alternative or perhaps realized in combination. One of these explanations is that intracellular levels of hepatic miRNAs fall owing to stress-associated, possibly selective (69) excretion of these miRNAs with an increase in the production of exosomes or MVs, which is known to rise upon exposure to inflammatory or apoptotic signals of varying nature (70). Importantly, increased production of exosome and MVs may be accompanied by the suppression of the activity of macrophages, which normally clear up the MVs released by liver (71). Jointly, these two processes resolve the paradox outlined in the beginning of this paragraph (Fig. 1B).

Here, we propose an alternative explanation to the paradox of opposing directionality in the intrahepatic and serum levels of same miRNA (Fig. 1C). This explanation implies that serum pools of miRNAs are a sum of the tissue-specific pools produced by more than one peripheral organ. This explanation also implies that relative contributions of these tissue-specific pools of miRNA may change with a progression of a disease. For example, while a majority of miR-122 molecules present in serum of healthy individual originate from the liver, in the patients with NASH, the levels of serum miRNAs would be defined by their extrahepatic production.

miR-122 in a larger context of endocrine signaling

In order to further understand the 'up in the serum, down in the liver' paradox, let us have a closer look onto the roles of miR-122 in human physiology by placing this molecule in larger context of endocrine signaling. It is well known that miRNAs secreted by one organ may then be internalized into the cells of other organs where they would regulate their respective physiological targets (72). In particular, miRNA-containing MVs serve as mediators of stem cell function, enabling and guiding their regenerative effects (73). According to endocrine regulation paradigm, organs communicate with each other by emitting molecular signals. These endocrine signals coordinate all aspects of the functioning of human body, from the development of the fetus to fine-tuning the metabolism in a

Table 1 Circulating miRNAs highlighted as possible biomarkers of NAFLD in previous studies

miRNA	Physiological role, if known	Tissue expression patterns
miR-122 (-5p and -3p)	Promotes differentiation of hepatocytes, regulates lipid metabolism and serves as liver-specific tumor suppressor	Predominantly liver, but also endotheliocytes, adipocytes, intestinal epithelial cells, fibroblasts, gallbladder and spleen
miR-15b (-5p and -3p)*	Regulates a cross-talk between hypoxia with angiogenesis while linking cell proliferation with	Ubiquitous, the levels of -5p form are highest in the muscle, bone, adipose and thyroid; -3p – in the veins and skin
miR-16 (-2-3p and -5p)*	metabolic needs	Ubiquitous, the levels of -2-3p form are highest in the muscle, bone, adipose, skin, spleen and thyroid; -5p – in the veins
miR-21 (-5p and -3p)	Amplifies TGF-β signaling and promotes fibrosis; master regulators of the metastatic program in many cancers	Ubiquitous, the levels of -5p form are highest in the arteries, fascia and nerve; -3p – in the spleen, lungs and colon
miR-34a (-5p and -3p)	Tumor suppressor, with some recent contradicting data	Ubiquitous, -3p evenly distributed; the levels of -5p form are highest in the epididymis, colon and brain
miR-451	Promotes erythropoiesis and progression of some tumors; limits CD4+ T cell proliferative responses to infection	miR-451a is expressed in endotheliocytes of the arteries and veins; miR-451b – in the spleen
miR-1290	Promotes proliferation and migration of cells	Ubiquitous; the levels are highest in the epididymis and colon
miR-27b (-5p and -3p)	May promote or inhibit proliferation depending on context; regulates browning of visceral adipocytes	Ubiquitous, -3p has the highest levels in the thyroid, skin, heart and muscle; the levels of -5p form are highest in the arteries, bladder and nerves
miR-192 (-5p and -3p)	Suppresses lipid synthesis; inhibits cell proliferation	-3p form is ubiquitous, the levels are highest in the arteries, bladder and nerves; -5p form is specific for the colon and intestine, with some expression in the liver and thyroid
miR-19a	Promotes cell migration, cancer metastasis and stemness	Ubiquitous, with highest levels in the veins, thyroid, liver and lungs
miR-19b (-1-5p and -3p)	Promotes atherosclerosis, may promote or inhibit proliferation depending on context	Ubiquitous, the levels of -3p form are highest in the veins
miR-125b (-1-3p, -2-3p and -5p)	Tumor suppressor, stress biomarker in brain disorders	Ubiquitous, the levels of -1-3p are highest in the bladder, gallbladder and colon; the levels of -5p are highest in the brain and spinal cord
miR-375	Suppresses core hallmarks of cancer; contributes to pancreatic differentiation and glucose-regulated insulin secretion	Pituitary gland
miR-181d	Tumor suppressor, promotes differentiation of dendritic cells and neurons	Ubiquitous, the levels are highest in the brain
miR-99a	Inhibits inflammation and carcinogenesis	Ubiquitous
miR-197 (-5p and -3p)	Suppressed the proliferation, migration and invasion of some cells, induces epithelial–mesenchymal transition in other cells	Ubiquitous, the levels of -5p are highest in the colon and skin
miR-146b	Regulates the innate immune response in the context of various pathologies	Levels are highest in the lung, thyroid, spleen, epididymis
miR-103a (-2-5p and -3p)	Promotes proliferation and inhibits apoptosis; regulates endothelial function	-2-5p is ubiquitous; the levels of -3p form are highest in the brain
miR-30c (-1-3p, -2-3p and -5p)	Tumor suppressor, also inhibits macrophage-mediated inflammation	-1-3p and -2-3p are ubiquitous; the levels of -5p form are highest in the thyroid, muscle and brain
miR-331-3p	Tumor suppressor for some cells, oncogene for other cells	Levels are highest in the brain, muscle and thyroid
miR-24-2-5p	Tumor suppressor for some cells, oncogene for other cells	Not described in tissue atlas; closely related forms are ubiquitous, with highest levels in the skin, muscle, nerves
mR-29a-3p	Tumor suppressor for some cells, oncogene for other cells	Ubiquitous; the levels are highest in the brain and muscle
miR-885-5p	Tumor suppressor for some cells, oncogene for other cells	Levels are highest in the brain
miR-199a (5p and -3p)	Tumor suppressor; inhibits functioning of brown adipose tissue	Both isoforms are ubiquitous with exception of the brain; the levels are highest in the thyroid, skin, epididymis and bone
miR-505	Tumor suppressor	Ubiquitous

*Related molecules that belong to the miR-15/107 group with a common seed. Tissue expression patterns are shown according to Ludwig *et al.* (65).

particular body compartment according to current needs of the entire organism. The principles of endocrine regulations serve as a foundation for the following surmise: Organs may actively help maintain the homeostasis of the entire body through the cooperative contribution to the collective well-being.

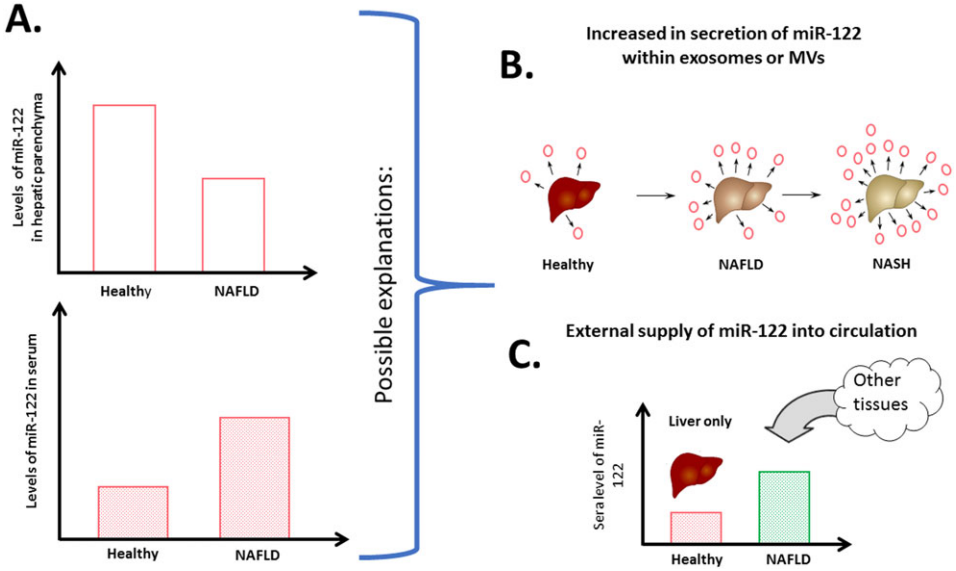

Figure 1 miR-122 expression paradox: observed intracellular–extracellular seesaw and its possible explanations. (A) The levels of miR-122 are elevated in the sera of NAFLD individuals, while intracellular levels of miR-122 in patients with NASH are lower than those in the livers of healthy individuals. (B) Intracellular–extracellular seesaw paradox may be explained by stress-associated excretion of these miRNAs within exosomes or MVs. (C) Relative contributions of these tissue-specific pools of miRNA may change with a progression of a NAFLD to NASH, where the levels of serum miRNAs may be defined by external rather than internal supply of miRNA. [Colour figure can be viewed at wileyonlinelibrary.com]

While observing an increase in the tissue production of a certain molecule proceeding in concert with progressive deterioration of this tissue, we should assume either that this molecule promulgates the pathology or that an increase in its expression is compensatory by its nature. Thorough review of the known physiological functions of miR-122 does not support its pathogenic role. Moreover, many independent lines of evidence point at miR-122 as an endogenous liver-specific tumor suppressor (74,75).

In a murine model of increased exposure to dietary triglycerides (TG) – fetal exposure to maternal consumption of high-fat diet in prenatal or postnatal period – the levels of hepatic expression of miR-122 in the offspring are suppressed by high-fat diet proportionally to observed metabolic damages (76). In miR-122 knockout mice, the hepatic metabolism of the lipids is perturbed, leading to the development of microvesicular steatosis and inflammation with eventual progress to NASH and fibrosis (74,77). Even in adult mice, effects of even a small reduction in the whole miR-122 pool are profound and sufficient for both the de-repression of target mRNAs and the developing prominent features of liver toxicity, such as hepatocyte turnover (78).

In an *in vitro* model of NAFLD-cultured HepG2 and Huh7 hepatocytes, an exposure to overwhelming influx of free fatty acids leads to suppression of endogenous expression of miR-122, thus de-repressing its target YY1, a transcription factor of the Polycomb group protein family, which, in turn, increases in its levels and inhibits bile acid–farnesoid X receptor signaling axis, thus promoting further accumulation of TG (79). When the supply of miR-122 mimics comes from an extrinsic source, through tail-vein injection into the bloodstream of NASH model mice, the TG accumulation in their liver decreases and the steatosis scores improve (79). Importantly, these beneficial changes are accompanied by miR-122-driven reversal of the expression trends previously described for YY1 and farnesoid X receptor-encoding genes (79).

The review of the tissue-expression profiles of miR-122 shows that both of its isoforms, miR-122-3p and miR-122-5p, are expressed outside of the liver, in endotheliocytes and adipocytes (65), as well as in intestinal epithelial cells and in fibroblasts (80). In omental fat of patients with obesity, miR-122 is expressed at the levels more than two times higher than those of omental tissue of individuals with healthy weight (81). The same study also reported correlations between expression on various miRNAs in the omental and subcutaneous fat, and concentration of these molecules in serum, showing that circulating miRNA levels reflect expression in omental fat (81).

On the basis of the physiological role of miR-122 and on observed expression patterns of this regulatory molecule in liver parenchyma, in omental fat and in serum, we hypothesize the following. When accumulation of fat and a development of inflammation lead to a suppression of the miR-122 encoding gene in the liver, the miR-122 concentrations within hepatocyte-derived exosomes drop. Somehow, by not-yet-clear mechanism, peripheral tissues sense this drop, possibly through a release from an expression control normally provided by external supply of miRNA. Recent experiments, indeed, show that adipose cells respond to miRNA-carrying exosomes by reproducible change in transcription levels from certain promoters (82). It is also possible that in response to

initial steatosis-induced drop in serum concentrations of miR-122, adipose and other issues chime in by collectively building up serum pool of miR-122, thus supporting its hepatic functions (Fig. 1C). Thus, miR-122 and possibly some other miRNAs form a basis for an additional loop of endocrine regulation connecting the liver and the adipose compartments.

Remarkably, the recent work of Thomou and co-authors showed that exosomal miRNAs found in human and animal sera are predominantly derived from adipose tissue (83). In their elegant series of experiments with white and brown adipose tissue transplants, as well as with the preparations of serum exosomes, these authors showed that secreted miRNAs, indeed, regulate gene expression in distant tissues (83). The loss of adipose compartments is known as 'lipodystrophy', an enigmatic condition that is intimately connected to the functioning of miRNA machinery. In particular, mice with a fat-specific knockout of Dicer-encoding gene lose intra-abdominal and subcutaneous white fat and develop severe insulin resistance within peripheral organs (84). Even more intriguing is the way that humans with lipodystrophy exhibit a substantial decrease in the levels of circulating exosomal miRNAs (83) and a significant propensity for developing highly progressive NAFLD (85,86). These observations point that the likely source of the compensatory miR-122 concentrations observed in sera of patients with NAFLD may be found within the adipose compartment.

A tissue-cooperative homeostatic model of non-alcoholic fatty liver disease

A fine balance in the production of miR-122 from the liver to the adipose and other peripheral tissues forms a basis for tissue-cooperative homeostatic model of NAFLD. In this model, the so-called simple or benign steatosis cases remain non-progressive owing to successful compensation of the drop in hepatic production of miR-122, while the instances of the NASH-related liver carcinogenesis or the rapid fibrotic progression are explained by the failure of adipose compartment with subsequent local (cancerous) or global (cirrhotic) decompensation of liver parenchyma (Fig. 2).

To validate the proposed model, paralleled investigation of miRNA expression levels in the liver parenchyma, in the adipose and in the serum is warranted. We expect that with gradual accumulation of fat in the liver, the measured levels of intrahepatic production of miR-122, and, possibly, other liver-aiding miRNAs, would fall. Observed decreases would be paralleled by the ramping up of the production of miR-122 by adipose, and a notable increase in the concentrations of these molecules in serum, which may allow using the concentration of these molecules for diagnosing NAFLD. At later stages of liver disease, which coincide with the metabolic deterioration of other organs and tissues (15,16,87), the ability of adipose to correct for hepatic miRNA production should become

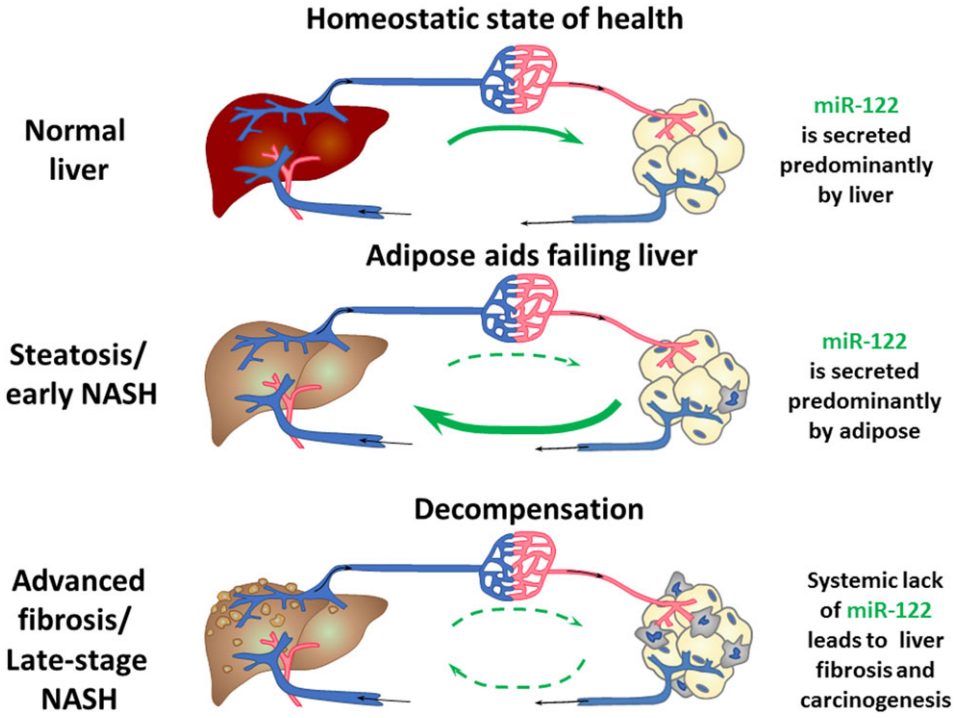

Figure 2 Homeostatic, tissue-cooperative model of NAFLD progression. While the drop in hepatic production of miR-122 remains compensated by adipose, liver steatosis remains non-progressive. With time, progressive changes in adipose histology and/or biochemistry lead to the failure of compensation, and the tapering of external supply of miR-122 to the liver. The instances of the NASH-related liver carcinogenesis or the rapid fibrotic progression are explained by with subsequent local (cancerous) or global (cirrhotic) decompensation of liver parenchyma. [Colour figure can be viewed at wileyonlinelibrary.com]

exhausted, and the resultant serum levels of miR-122 should gradually fall. This decrease in miR-122 serum levels should precede the accumulation of the collagen in hepatic parenchyma and the decompensation of liver miRNA homeostasis. Additionally, as the supply of tumor-suppressing miR-122 peters out, the inflamed milieu of fibrotic liver should become more supportive of malignant growth, thereby contributing to the increased incidence of HCC seen in patients with NAFLD (Fig. 3).

Recent longitudinal studies of miRNA production dynamics in patients with NAFLD support the hypothesis proposed earlier. In particular, the study of serum miR-122 levels in 305 longitudinally assessed Japanese patients with histologically proven NAFLD showed that patients with higher serum levels of miR-122 are less likely to develop HCC (60). These observations are in agreement with the loss of hepatocyte differentiation and the acquisition of invasive properties in hepatic parenchyma deficient for this miRNA (88). It is notable that the specific microenvironment of the HCC stem cell niche is embodied within the so-called fibrous nests (89) that share the signaling environment for both the fibrosis and the carcinogenesis. Common features of these hepatic processes include their dependence on the lack of miR-122, which normally suppresses the Wnt signaling (75,90) and the remodeling of the laminin-containing basement membranes (91).

In HCC-free patients with severe fibrosis stages (especially with fibrosis stage 4), the levels of miR-122 in serum tend to be lower than those in individuals with a lesser degree of liver fibrosis (60). In another longitudinal study of 36 HCC-free patients with NAFLD, the serum levels of miR-122 were quantified at the visits when serial biopsies were collected (92). In cases in which improvements of histopathological scores were detected (steatosis, ballooning and stage), serum miR-122 levels were significantly lower at the second biopsy than noted at the first biopsy, while the levels of miR-122 in patients who showed no improvement at second biopsy were no different than those of the first biopsy (92).

Conclusions and future directions

In the most general sense, the idea about possible participation of miRNAs in a homeostatic signal exchange between various types of cells has been suggested earlier (93–95). Recently presented experimental results (83) provide critical evidence to support this hypothesis and provide additional insights into NAFLD progression. According to tissue-cooperative homeostatic model of NAFLD proposed earlier, during early stages of this disease, the falling levels of the intrahepatic production of miRNA are offset by an increase in production by adipose, which secretes miRNA-containing exosomes into the bloodstream. After delivery to the liver, these molecules are taken up by the liver cells, wherein they regulate their intrinsic physiological targets. Thus, adipose-derived miRNAs augment endogenous production of miRNA by the liver. When the deterioration of other peripheral organs – including adipose – catches up with the failing hepatic parenchyma, the external supply

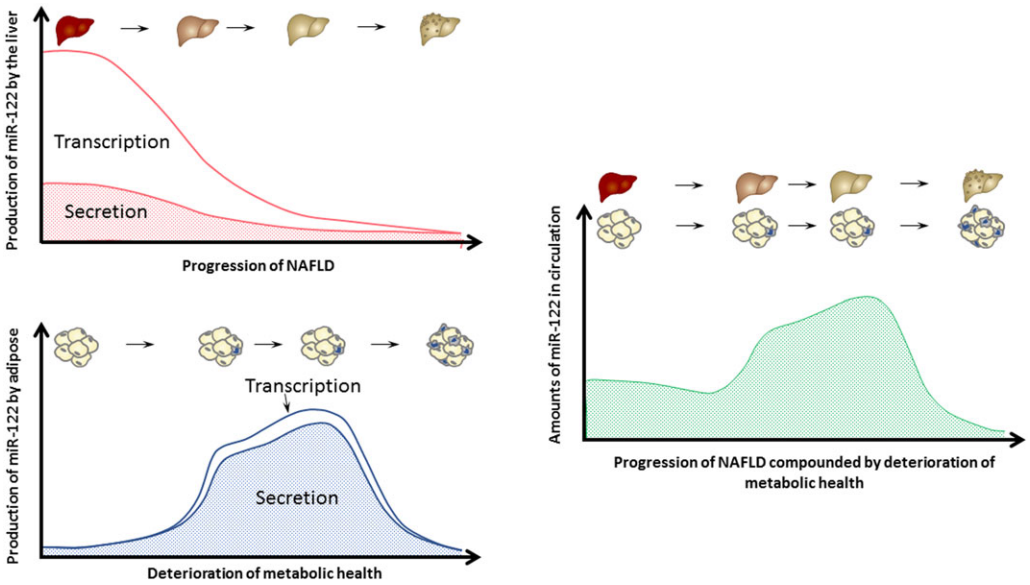

Figure 3 Hepatic-specific and adipose-specific contributions to serum concentrations of miRNA-122 at various stages of NAFLD. According to the model, at early stages of NAFLD, the levels of intrahepatic production of miRNAs gradually drop, while the secretion of liver-aiding miRNAs by adipose becomes ramped up. The superposition of these two process leads to a notable increase in the concentrations of these molecules in serum. In the course of the progression of NAFLD to NASH, the metabolic deterioration of other organs and tissues sets in, and the ability of adipose to correct for the lack of hepatic miRNA production becomes exhausted. As a result, the serum levels of miR-122 fall right before the decompensation of liver miRNA homeostasis. Insufficiency of the control previously provided by internally/externally supplied miR-122 leads to the increase in incidence of hepatocellular carcinoma (HCC) seen in patients with NASH. [Colour figure can be viewed at wileyonlinelibrary.com]

of liver-supporting miRNAs gradually falls, leading to the fibrotic decompensation of liver functions and an increase in hepatic carcinogenesis.

This hypothesis provides an interesting avenue for the development of miRNA-based supportive treatment for patients with late-stage NAFLD. In particular, purified miR-122-containing exosomes could become a potential cell-free therapeutics for preventing NAFLD-associated increase in the incidence of HCCs. For other diseases, miRNA-infusion-based therapies are proven feasible and already are in clinical trials. For example, miR-16-loaded minicells (TargomiRs) are currently being assessed for their safety and optimal dosing in a phase I trial in patients with malignant pleural mesothelioma (96). A similarly designed trial was recently set for a cohort of patients with advanced solid tumors refractory to standard treatment (97).

It should be noted that the preventive applications would require much better safety profiles than those demonstrated in cancer therapy trials described earlier. Therefore, the development of more effective and safe methods of the delivery of synthetic miRNA analogs is clearly warranted. Clinical trials aimed at abatement of recently detected surge in NAFLD-associated HCCs or, speaking generally, at the development of maintenance therapies for patients with NAFLD should be designed to resemble those on artificially reconstituted high-density lipoprotein particles (98) rather on the treatments of already progressed cancers.

On the other hand, as homeostasis-supporting miRNA infusions would be more likely to find their value as a preventive aid rather than an emergency rescue, it is plausible to surmise that lesser, non-toxic doses may be sufficient. Another possible way of circumventing toxicity may be the development of the mature forms of miRNAs, which do not overload endogenous miRNA processing machinery (78), or by co-manufacturing carrier exosomes along with their cargo, in culture of exosome-producing cells (99). The best candidate for exosome-wrapped miR-122 producer is adipose tissue-derived mesenchymal stem cells (MSCs), known for their capacity to shed large amounts of exosomes into the media (100). This therapy is especially promising, as MSC-derived exosomes with no specific loading are already tested in patients with liver fibrosis as safer, not-that-immunogenic alternative to live MSCs (101). These and other approaches may undergo preclinical testing *in vitro*, e.g. using microscale human liver equivalents supported by advanced microfluidic (102,103) or multi-organ chips capable of maintaining 3D tissues derived from primary cells and biopsies (104).

It should be also noted that an inverse directionality of the changes in serum and tissue levels has been reported for many miRNAs, including miR-132, miR-143, miR-192, and miR-375 (56,105). These observations open an avenue to the development of exosome-delivered miRNA cocktails capable of augmenting the functioning of organs suffering from a variety of chronic diseases.

Conflict of interest statement

The authors declare no conflict of interest.

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References

1. Marchesini G, Bugianesi E, Forlani G *et al*. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology* 2003; **37**: 917–923.
2. Kanwar P, Kowdley KV. The metabolic syndrome and its influence on nonalcoholic steatohepatitis. *Clin Liver Dis* 2016; **20**: 225–243.
3. Bellentani S. The epidemiology of non-alcoholic fatty liver disease. *Liver Int* 2017; **37**(Suppl 1): 81–84.
4. Kim CH, Younossi ZM. Nonalcoholic fatty liver disease: a manifestation of the metabolic syndrome. *Cleve Clin J Med* 2008; **75**: 721–728.
5. Sung KC, Ryu S, Lee JY, Kim JY, Wild SH, Byrne CD. Effect of exercise on the development of new fatty liver and the resolution of existing fatty liver. *J Hepatol* 2016; **65**: 791–797.
6. Vilar-Gomez E, Martinez-Perez Y, Calzadilla-Bertot L *et al*. Weight loss through lifestyle modification significantly reduces features of nonalcoholic steatohepatitis. *Gastroenterology* 2015; **149**: 367–378 e5.
7. Bataller R, Rombouts K, Altamirano J, Marra F. Fibrosis in alcoholic and nonalcoholic steatohepatitis. *Best Pract Res Clin Gastroenterol* 2011; **25**: 231–244.
8. McCullough AJ. The clinical features, diagnosis and natural history of nonalcoholic fatty liver disease. *Clin Liver Dis* 2004; **8**: 521–533.
9. Pais R, Charlotte F, Fedchuk L *et al*. A systematic review of follow-up biopsies reveals disease progression in patients with non-alcoholic fatty liver. *J Hepatol* 2013; **59**: 550–556.
10. Singh S, Allen AM, Wang Z, Prokop LJ, Murad MH, Loomba R. Fibrosis progression in nonalcoholic fatty liver vs nonalcoholic steatohepatitis: a systematic review and meta-analysis of paired-biopsy studies. *Clin Gastroenterol Hepatol* 2015; **13**: 643–654.
11. Ma C, Kesarwala AH, Eggert T *et al*. NAFLD causes selective CD4(+) T lymphocyte loss and promotes hepatocarcinogenesis. *Nature* 2016; **531**: 253–257.
12. Zoller H, Tilg H. Nonalcoholic fatty liver disease and hepatocellular carcinoma. *Metabolism* 2016; **65**: 1151–1160.
13. Lou Y, Tian GY, Song Y *et al*. Characterization of transcriptional modules related to fibrosing-NAFLD progression. *Sci Rep* 2017; **7**: 4748.
14. Jozefczuk J, Kashofer K, Ummanni R *et al*. A systems biology approach to deciphering the etiology of steatosis employing patient-derived dermal fibroblasts and iPS cells. *Front Physiol* 2012; **3**: 339.
15. Baranova A, Schlauch K, Elariny H *et al*. Gene expression patterns in hepatic tissue and visceral adipose tissue of patients with non-alcoholic fatty liver disease. *Obes Surg* 2007; **17**: 1111–1118.
16. Shubham K, Vinay L, Vinod PK. Systems-level organization of non-alcoholic fatty liver disease progression network. *Mol BioSyst* 2017; **13**: 1898–1911.
17. Estep JM, Baranova A, Hossain N *et al*. Expression of cytokine signaling genes in morbidly obese patients with non-alcoholic steatohepatitis and hepatic fibrosis. *Obes Surg* 2009; **19**: 617–624.

18. Musso G, Gambino R, Cassader M. Gut microbiota as a regulator of energy homeostasis and ectopic fat deposition: mechanisms and implications for metabolic disorders. *Curr Opin Lipidol* 2010; **21**: 76–83.
19. Cheng DD, He C, Ai HH, Huang Y, Lu NH. The possible role of *Helicobacter pylori* infection in non-alcoholic fatty liver disease. *Front Microbiol* 2017; **8**: 743.
20. Meng G, Wu H, Fang L *et al*. Relationship between grip strength and newly diagnosed nonalcoholic fatty liver disease in a large-scale adult population. *Sci Rep* 2016; **6**: 33255.
21. Bhanji RA, Narayanan P, Allen AM, Malhi H, Watt KD. Sarcopenia in hiding: the risk and consequence of underestimating muscle dysfunction in nonalcoholic steatohepatitis. *Hepatology* 2017; **66**: 2055–2065.
22. Huang YY, Gusdon AM, Qu S. Cross-talk between the thyroid and liver: a new target for nonalcoholic fatty liver disease treatment. *World J Gastroenterol* 2013; **19**: 8238–8246.
23. Horwath JA, Hurr C, Butler SD *et al*. Obesity-induced hepatic steatosis is mediated by endoplasmic reticulum stress in the subfornical organ of the brain. *JCI Insight* 2017; **2** pii:90170.
24. Vergani L. Lipid lowering effects of iodothyronines: in vivo and in vitro studies on rat liver. *World J Hepatol* 2014; **6**: 169–177.
25. Coppola M, Glinni D, Moreno M, Cioffi F, Silvestri E, Goglia F. Thyroid hormone analogues and derivatives: actions in fatty liver. *World J Hepatol* 2014; **6**: 114–129.
26. Targher G, Bertolini L, Rodella S, Zoppini G, Zenari L, Falezza G. Associations between liver histology and cortisol secretion in subjects with nonalcoholic fatty liver disease. *Clin Endocrinol* 2006; **64**: 337–341.
27. Tarantino G, Finelli C. Pathogenesis of hepatic steatosis: the link between hypercortisolism and non-alcoholic fatty liver disease. *World J Gastroenterol* 2013; **19**: 6735–6743.
28. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. *Nat Rev Immunol* 2011; **11**: 85–97.
29. Adolph TE, Grander C, Grabherr F, Tilg H. Adipokines and non-alcoholic fatty liver disease: multiple interactions. *Int J Mol Sci* 2017; **18** pii: E1649.
30. Baranova AV. Adipokine genetics: unbalanced protein secretion by human adipose tissue as a cause of the metabolic syndrome. *Genetika* 2008; **44**: 1338–1355.
31. Jarrar MH, Baranova A, Collantes R *et al*. Adipokines and cytokines in non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2008; **27**: 412–421.
32. Whitham M, Febbraio MA. The ever-expanding myokinome: discovery challenges and therapeutic implications. *Nat Rev Drug Discov* 2016; **15**: 719–729.
33. Belizário JE, Fontes-Oliveira CC, Borges JP, Kashiabara JA, Vannier E. Skeletal muscle wasting and renewal: a pivotal role of myokine IL-6. *Springerplus* 2016; **5**: 619.
34. Zarfeshani A, Ngo S, Sheppard AM. Leucine alters hepatic glucose/lipid homeostasis via the myostatin-AMP-activated protein kinase pathway – potential implications for nonalcoholic fatty liver disease. *Clin Epigenetics* 2014; **6**: 27.
35. Gamas L, Matafome P, Seiça R. Irisin and myonectin regulation in the insulin resistant muscle: implications to adipose tissue: muscle crosstalk. *J Diabetes Res* 2015; **2015**: 359159.
36. Menheniott TR, Kurklu B, Giraud AS. Gastrokines: stomach-specific proteins with putative homeostatic and tumor suppressor roles. *Am J Physiol Gastrointest Liver Physiol* 2013; **304**: G109–G121.
37. Estep M, Abawi M, Jarrar M *et al*. Association of obestatin, ghrelin, and inflammatory cytokines in obese patients with non-alcoholic fatty liver disease. *Obes Surg* 2011; **21**: 1750–1757.
38. Leung C, Rivera L, Furness JB, Angus PW. The role of the gut microbiota in NAFLD. *Nat Rev Gastroenterol Hepatol* 2016; **13**: 412–425.
39. Baynes KC, Dhillo WS, Bloom SR. Regulation of food intake by gastrointestinal hormones. *Curr Opin Gastroenterol* 2006; **22**: 626–631.
40. Drucker DJ, Nauck MA. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet* 2006; **368**: 1696–1705.
41. Younossi ZM, Page S, Rafiq N *et al*. A biomarker panel for non-alcoholic steatohepatitis (NASH) and NASH-related fibrosis. *Obes Surg* 2011; **21**: 431–439.
42. Siddiqui MS, Patidar KR, Boyett S, Luketic VA, Puri P, Sanyal AJ. Performance of non-invasive models of fibrosis in predicting mild to moderate fibrosis in patients with non-alcoholic fatty liver disease. *Liver Int* 2016; **36**: 572–579.
43. Chen YM, Liu Y, Zhou RF *et al*. Associations of gut-flora-dependent metabolite trimethylamine-*N*-oxide, betaine and choline with non-alcoholic fatty liver disease in adults. *Sci Rep* 2016; **6**: 19076.
44. Loomba R, Quehenberger O, Armando A, Dennis EA. Polyunsaturated fatty acid metabolites as novel lipidomic biomarkers for noninvasive diagnosis of nonalcoholic steatohepatitis. *J Lipid Res* 2015; **56**: 185–192.
45. Barr J, Caballería J, Martínez-Arranz I, Domínguez-Díez A *et al*. Obesity-dependent metabolic signatures associated with non-alcoholic fatty liver disease progression. *J Proteome Res* 2012; **11**: 2521–2532.
46. Otsuka M, Kishikawa T, Yoshikawa T *et al*. MicroRNAs and liver disease. *J Hum Genet* 2017; **62**: 75–80.
47. DiStefano JK, Gerhard GS. Circulating microRNAs in nonalcoholic fatty liver disease. *Expert Rev Gastroenterol Hepatol* 2016; **10**: 161–163.
48. Wang K, Zhang S, Marzolf B *et al*. Circulating microRNAs, potential biomarkers for drug-induced liver injury. *Proc Natl Acad Sci U S A* 2009; **106**: 4402–4407.
49. Laterza OF, Lim L, Garrett-Engele PW, Vlasakova K *et al*. Plasma microRNAs as sensitive and specific biomarkers of tissue injury. *Clin Chem* 2009; **55**: 1977–1983.
50. Zhang Y, Cheng X, Lu Z *et al*. 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.
51. Xu J, Wu C, Che X *et al*. Circulating microRNAs, miR-21, miR-122, and miR-223, in patients with hepatocellular carcinoma or chronic hepatitis. *Mol Carcinog* 2011; **50**: 136–142.
52. Zhou J, Yu L, Gao X, Hu J *et al*. Plasma microRNA panel to diagnose hepatitis B virus-related hepatocellular carcinoma. *J Clin Oncol* 2011; **29**: 4781–4788.
53. 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.
54. Yamada H, Suzuki K, Ichino N *et al*. 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.
55. Miyaaki H, Ichikawa T, Kamo Y *et al*. Significance of serum and hepatic microRNA-122 levels in patients with non-alcoholic fatty liver disease. *Liver Int* 2014; **34**: e302–e307.
56. 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**: 800–812.
57. Tan Y, Ge G, Pan T, Wen D, Gan J. A pilot study of serum microRNAs panel as potential biomarkers for diagnosis of nonalcoholic fatty liver disease. *PLoS One* 2014; **9**: e105192.
58. Salvoza NC, Klinzing DC, Gopez-Cervantes J, Baclig MO. Association of circulating serum miR-34a and miR-122 with dyslipidemia among patients with non-alcoholic fatty liver disease. *PLoS One* 2016; **11**: e0153497.

59. Liu XL, Pan Q, Zhang RN *et al*. Disease-specific miR-34a as diagnostic marker of non-alcoholic steatohepatitis in a Chinese population. *World J Gastroenterol* 2016; **22**(44): 9844–9852.
60. Akuta N, Kawamura Y, Suzuki F *et al*. Analysis of association between circulating miR-122 and histopathological features of non-alcoholic fatty liver disease in patients free of hepatocellular carcinoma. *BMC Gastroenterol* 2016; **16**: 141.
61. López-Riera M, Conde I, Tolosa L *et al*. New microRNA biomarkers for drug-induced steatosis and their potential to predict the contribution of drugs to non-alcoholic fatty liver disease. *Front Pharmacol* 2017; **8**: 3.
62. Thompson MD, Cismowski MJ, Serpico M, Pusateri A, Brigstock DR. Elevation of circulating microRNA levels in obese children compared to healthy controls. *Clin Obes* 2017; **7**: 216–221.
63. 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**(11): e0142661.
64. Wang S, Wang JQ, Lv XW. Exosomal miRNAs as biomarkers in the diagnosis of liver disease. *Biomark Med* 2017; **11**: 491–501.
65. Ludwig N, Leidinger P, Becker K *et al*. Distribution of miRNA expression across human tissues. *Nucleic Acids Res* 2016; **44**: 3865–3877.
66. Hou J, Lin L, Zhou W *et al*. Identification of miRNomes in human liver and hepatocellular carcinoma reveals miR-199a/b-3p as therapeutic target for hepatocellular carcinoma. *Cancer Cell* 2011; **19**: 232–243.
67. Cheung O, Puri P, Eicken C *et al*. Nonalcoholic steatohepatitis is associated with altered hepatic microRNA expression. *Hepatology* 2008; **48**(6): 1810–1820.
68. Latorre J, Moreno-Navarrete JM, Mercader JM *et al*. Decreased lipid metabolism but increased FA biosynthesis are coupled with changes in liver microRNAs in obese subjects with NAFLD. *Int J Obes* 2017; **41**: 620–630.
69. Diehl P, Fricke A, Sander L *et al*. Microparticles: major transport vehicles for distinct microRNAs in circulation. *Cardiovasc Res* 2012; **93**: 633–644.
70. Lemoinne S, Thabut D, Housset C *et al*. The emerging roles of microvesicles in liver diseases. *Nat Rev Gastroenterol Hepatol* 2014; **11**: 350–361.
71. Rautou PE, Mackman N. Del-etion of microvesicles from the circulation. *Circulation* 2012; **125**: 1601–1604.
72. Camussi G, Deregibus MC, Cantaluppi V. Role of stem-cell-derived microvesicles in the paracrine action of stem cells. *Biochem Soc Trans* 2013; **41**(1): 283–287.
73. Panagiotou N, Wayne Davies R, Selman C, Shiels PG. Microvesicles as vehicles for tissue regeneration: changing of the guards. *Curr Pathobiol Rep* 2016; **4**(4): 181–187.
74. Hsu SH, Wang B, Kota J *et al*. Essential metabolic, anti-inflammatory, and anti-tumorigenic functions of miR-122 in liver. *J Clin Invest* 2012; **122**: 2871–2883.
75. Jin Y, Wang J, Han J, Luo D, Sun Z. miR-122 inhibits epithelial–mesenchymal transition in hepatocellular carcinoma by targeting Snail1 and Snail2 and suppressing WNT/β-cadherin signaling pathway. *Exp Cell Res* 2017; **360**: 210–217.
76. de Paula Simino LA, de Fante T, Figueiredo Fontana M *et al*. Lipid overload during gestation and lactation can independently alter lipid homeostasis in offspring and promote metabolic impairment after new challenge to high-fat diet. *Nutr Metab (Lond)* 2017; **14**: 16.
77. Tsai WC, Hsu SD, Hsu CS *et al*. MicroRNA-122 plays a critical role in liver homeostasis and hepatocarcinogenesis. *J Clin Invest* 2012; **122**: 2884–2897.
78. Valdmanis PN, Gu S, Chu K *et al*. RNA interference-induced hepatotoxicity results from loss of the first synthesized isoform of microRNA-122 in mice. *Nat Med* 2016; **22**: 557–562.
79. Wu GY, Rui C, Chen JQ *et al*. MicroRNA-122 inhibits lipid droplet formation and hepatic triglyceride accumulation via Yin Yang 1. *Cell Physiol Biochem* 2017; **44**: 1651–1664.
80. de Rie D, Abugessaisa I, Alam T *et al*. An integrated expression atlas of miRNAs and their promoters in human and mouse. *Nat Biotechnol* 2017; **35**: 872–878.
81. Heneghan HM, Miller N, McAnena OJ, O'Brien T, Kerin MJ. Differential miRNA expression in omental adipose tissue and in the circulation of obese patients identifies novel metabolic biomarkers. *J Clin Endocrinol Metab* 2011; **96**: E846–E850.
82. De Silva N, Samblas M, Martínez JA, Milagro FI. Effects of exosomes from LPS-activated macrophages on adipocyte gene expression, differentiation, and insulin-dependent glucose uptake. *J Physiol Biochem* 2018: 1–10.
83. Thomou T, Mori MA, Dreyfuss JM *et al*. Adipose-derived circulating miRNAs regulate gene expression in other tissues. *Nature* 2017; **542**(7642): 450–455.
84. Mori MA, Thomou T, Boucher J *et al*. Altered miRNA processing disrupts brown/white adipocyte determination and associates with lipodystrophy. *J Clin Invest* 2014; **124**: 339–351.
85. Ajluni N, Meral R, Neidert AH *et al*. Spectrum of disease associated with partial lipodystrophy: lessons from a trial cohort. *Clin Endocrinol* 2017; **86**: 698–707.
86. Safar Zadeh E, Lungu AO, Cochran EK *et al*. The liver diseases of lipodystrophy: the long-term effect of leptin treatment. *J Hepatol* 2013; **59**: 131–137.
87. du Plessis J, van Pelt J, Korf H *et al*. Association of adipose tissue inflammation with histologic severity of nonalcoholic fatty liver disease. *Gastroenterology* 2015; **149**: 635–648.
88. Coulouarn C, Factor VM, Andersen JB, Durkin ME, Thorgeirsson SS. Loss of miR-122 expression in liver cancer correlates with suppression of the hepatic phenotype and gain of metastatic properties. *Oncogene* 2009; **28**: 3526–3536.
89. Désert R, Mebarki S, Desille M *et al*. "Fibrous nests" in human hepatocellular carcinoma express a Wnt-induced gene signature associated with poor clinical outcome. *Int J Biochem Cell Biol* 2016; **81**: 195–207.
90. Xu J, Zhu X, Wu L *et al*. MicroRNA-122 suppresses cell proliferation and induces cell apoptosis in hepatocellular carcinoma by directly targeting Wnt/β-catenin pathway. *Liver Int* 2012; **32**: 752–760.
91. Alisi A, Arciello M, Petrini S, Conti B, Missale G, Balsano C. Focal adhesion kinase (FAK) mediates the induction of prooncogenic and fibrogenic phenotypes in hepatitis C virus (HCV)-infected cells. *PLoS One* 2012; **7**: e44147.
92. Akuta N, Kawamura Y, Suzuki F *et al*. Impact of circulating miR-122 for histological features and hepatocellular carcinoma of nonalcoholic fatty liver disease in Japan. *Hepatol Int* 2016; **10**: 647–656.
93. Hunter MP, Ismail N, Zhang X *et al*. Detection of microRNA expression in human peripheral blood microvesicles. *PLoS One* 2008; **3**: e3694.
94. Turchinovich A, Tonevitsky AG, Burwinkel B. Extracellular miRNA: a collision of two paradigms. *Trends Biochem Sci* 2016; (10): 883–892.
95. Makarova JA, Shkurnikov MU, Wicklein D *et al*. Intracellular and extracellular microRNA: an update on localization and biological role. *Prog Histochem Cytochem* 2016; **51**(3–4): 33–49.
96. van Zandwijk N, Pavlakis N, Kao SC *et al*. Safety and activity of microRNA-loaded minicells in patients with recurrent malignant pleural mesothelioma: a first-in-man, phase 1, open-label, dose-escalation study. *Lancet Oncol* 2017; **18**: 1386–1396.

97. 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. *Investig New Drugs* 2017; **35**: 180–188.
98. Tardif JC. Emerging high-density lipoprotein infusion therapies: fulfilling the promise of epidemiology? *J Clin Lipidol* 2010; **4**: 399–404.
99. Katakowski M, Buller B, Zheng X *et al*. Exosomes from marrow stromal cells expressing miR-146b inhibit glioma growth. *Cancer Lett* 2013; **335**: 201–204.
100. Yeo RW, Lai RC, Zhang B *et al*. Mesenchymal stem cell: an efficient mass producer of exosomes for drug delivery. *Adv Drug Deliv Rev* 2013; **65**: 336–341.
101. Lou G, Chen Z, Zheng M, Liu Y. Mesenchymal stem cell-derived exosomes as a new therapeutic strategy for liver diseases. *Exp Mol Med* 2017; **49**(6): e346.
102. Materne EM, Tonevitsky AG, Marx U. Chip-based liver equivalents for toxicity testing – organotypicalness versus cost-efficient high throughput. *Lab Chip* 2013; **13**: 3481–3495.
103. Prodanov L, Jindal R, Bale SS *et al*. Long-term maintenance of a microfluidic 3D human liver sinusoid. *Biotechnol Bioeng* 2016; **113**: 241–246.
104. Poloznikov A, Gazaryan I, Shkurnikov M *et al*. In vitro and in silico liver models: current trends, challenges and opportunities. *ALTEX* 2018; **35**: 397–412.
105. Mehta R, Otgonsuren M, Younoszai Z, Allawi H, Raybuck B, Younossi Z. Circulating miRNA in patients with non-alcoholic fatty liver disease and coronary artery disease. *BMJ Open Gastroenterol* 2016; **3**: e000096.

