Biochemical and Biophysical Research Communications xxx (2016) 1–9

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

The role of microRNAs in liver injury at the crossroad between hepatic cell death and regeneration

Volker M. Lauschke*, Souren Mkrtchian, Magnus Ingelman-Sundberg

Department of Physiology and Pharmacology, Section of Pharmacogenetics, Karolinska Institute, Stockholm, Sweden

ARTICLE INFO

Article history:
Received 18 August 2016
Received in revised form
19 October 2016
Accepted 23 October 2016
Available online xxx

Keywords:
Liver disease
Fatty liver
Apoptosis
miRNA
Plasticity
Dedifferentiation

ABSTRACT

The liver fulfills critical metabolic functions, such as controlling blood sugar and ammonia levels, and is of central importance for lipid metabolism and detoxification of environmental and chemical agents, including drugs. Liver injuries of different etiology can elicit a spectrum of responses. Some hepatocytes initiate molecular programs resulting in cell death, whereas others undergo cellular divisions to regenerate the damaged organ. Interestingly, recent research indicates that microRNAs serve as very rapid as well as long-term regulators in these processes. In this review, we discuss their importance in liver disease etiology and progression as well as for therapy with particular focus on metabolic and inflammatory conditions. Furthermore, we highlight the central role of microRNAs in controlling hepatocyte differentiation and plasticity, which are required for successful regeneration, but under certain conditions, such as chronic liver insults, can result in the formation of hepatocellular carcinoma.

1. Introduction

Liver cell physiology is tightly controlled by a complex maze of regulatory networks acting on transcriptional, posttranscriptional and posttranslational levels and perturbations of these homeostatic mechanisms are often associated with liver diseases of various etiology. The ongoing “RNA revolution” unveiled completely new layers of regulation, accomplished by non-coding RNAs that are transcribed from genomic loci, previously considered as “junk” DNA. One of the most extensively studied classes of non-coding RNAs to date are micro RNAs (miRNA), short non-coding RNAs, which control the expression of >60% of human protein coding genes [1]. Thus, miRNAs are involved in regulation of many cellular and developmental events, such as cell differentiation, proliferation, morphogenesis, metabolism and apoptosis.

miRNA biogenesis begins in the nucleus with the transcription of primary (pri) miRNA by RNA polymerase II. The 5′-capped, polyadenylated stem-loop (hairpin) structure of pri-miRNA is recognized by DGCR8/Drosha microprocessor complex that cleaves it to produce the precursor (pre) miRNA hairpins. These are subsequently exported into the cytoplasm by Exportin 5 (XPO5) and further cleaved by Dicer to yield the mature, double-stranded, 18–25 nt long miRNA molecules. One of the strands, termed the guide strand, associates with proteins from the Argonaut family (Ago) forming the RNA-Induced Silencing Complex (RISC). The successfully loaded RISC targets complementary recognition elements on mRNAs, mostly located in the 3′-UTRs, resulting in degradation or translational repression of the respective transcript. Therefore, the common regulatory effect of miRNA is the suppression of the biological function supported by the targeted mRNA/protein.

miRNA expression signatures are highly tissue specific. Overall, 2861 miRNAs have been identified to date, of which around 1300 miRNAs have been reported to be expressed in human liver [2–4]. Importantly, only 9 miRNA species account for around 90% of all miRNA molecules in the human liver, of which miR-122 is the most abundant [5]. Surprising data shedding more light on the liver-specific roles of miRNAs were provided by studies on tissue-specific Dicer1 knock-out (KO) mice, in which Dicer1 was deleted only in hepatoblast-derived cells [6]. Despite the significant downregulation of liver-specific miRNAs, hepatic basal functionality was maintained, as reflected by normal blood glucose,

Abbreviations: ALF, Acute liver failure; DILI, Drug-induced liver injury; EMT, Epithelial-mesenchymal transition; HCC, Hepatocellular carcinoma; HCV, Hepatitis C virus; NAFLD, Non-alcoholic fatty liver disease; NASH, Non-alcoholic steatohepatitis; RISC, RNA-induced silencing complex.

* Corresponding author. Department of Physiology and Pharmacology, Section of Pharmacogenetics, Karolinska Institutet, SE-171 77, Stockholm, Sweden.
E-mail address: volker.lauschke@ki.se (V.M. Lauschke).

http://dx.doi.org/10.1016/j.bbrc.2016.10.084
0006-291X/

albumin, cholesterol, and bilirubin levels. However, at 2–4 months of age KO mice exhibited significant hepatic steatosis and glycogen depletion, increased apoptosis and proliferation as well as portal inflammation. Interestingly, in a different Dicer1 KO model, in which Dicer1 was conditionally deleted exclusively in mature hepatocytes expressing albumin, fetal genes were found to be persistently expressed and hepatocytes showed increased proliferation resulting in spontaneous development of hepatocellular carcinoma (HCC) [7]. Overall these data demonstrate essential roles of miRNAs in hepatocyte maturation, regeneration, hepatic gene regulation and tumor suppression in the liver.

In this review, we summarize recent research that indicates key roles for miRNAs in liver disease etiology, progression and therapy with particular focus on metabolic and inflammatory conditions as most prevalent liver disorders.

2. The involvement of miRNAs in liver injury

The liver faces a variety of diverse insults that result in different response patterns and outcomes. Acute injuries typically are related to liver resections or are induced by toxins. Upon partial hepatectomy, immediate-early genes are activated already after 3 h [8]. Differential gene expression after surgical injury includes an upregulation of anti-apoptotic genes, such as *BCL2* and the NF-κB activator *TIFA* and downregulation of *CEBPA*, a negative regulator of proliferation [8]. Importantly, differential expression of protein coding genes is paralleled by extensive changes in miRNA signatures with similar kinetics [9–11]. Similarly, expression of a variety of miRNAs, including miR-122, miR-192 and miR-711 is rapidly changed upon acetaminophen-mediated drug induced liver injury (DILI) [12]. Acute hepatic insults are mostly self-limiting and result in regeneration of damaged parts of the organ and recovery of the injury. Yet, if too severe, acute liver damage results in fulminant liver failure, for which orthotopic liver transplantation is the only therapeutic option.

In contrast, chronic liver injury is persistent, often over years. Damage-induced cell death exceeds the proliferative capacity of the liver, resulting in scarring, progressive loss of hepatocytes and eventual organ failure. Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease affecting between 20% and 44% of European adults and 43–70% of patients with type 2 diabetes [13]. It is one prime cause for chronic and end-stage liver disease, such as cirrhosis and primary hepatocellular carcinoma, associates with elevated mortality and increases individual health-care costs by 26%, thus representing an important clinical and economic burden for European countries [14,15]. Prominent risk factors are obesity and insulin resistance, but NAFLD can also develop due to liver injury or be caused by certain medications as one manifestation of DILI. Besides NAFLD, viral hepatitis mostly due to hepatitis B or C virus (HBV and HCV) infections and alcoholic hepatitis are the most common chronic liver diseases with prevalence rates between 0.3% and 2% in the general population [16].

2.1. Hepatic miRNAs in metabolic diseases

The liver is of central importance in lipid and amino acid metabolism and for control of blood glucose levels. The functional networks that ensure metabolic homeostasis are controlled by feedback regulation involving key transcription factors that respond either directly or indirectly to alterations in nutrient or hormone exposure. Importantly, when perturbed, misregulated feedback mechanisms can result in liver disease with a growing body of evidence indicating that many critical factors are under substantial miRNA control.

SREBP1 and 2 are transcription factors controlling cholesterol, fatty acids and phospholipids biosynthesis. In the absence of insulin signaling, these proteins are anchored to the endoplasmic reticulum [17]. Upon insulin binding though, a cascade of events is initiated that results in proteolytic cleavage of SREBP1 and 2, yielding the active transcription factors, which translocate to the nucleus and activate expression of its target genes involved in fatty acid and cholesterol metabolism [17,18]. SREBPs constitute intriguing examples of miRNA regulation as their corresponding genes (*SREBF1* and *SREBF2*) harbor the miRNAs miR-33a and miR-33b within their introns. These co-transcribed miRNAs synergize with their host genes by negatively regulating genes involved in fatty acid degradation, including *CPT1A*, or cholesterol efflux, such as *ABCA1* (reviewed in Ref. [19]). SREBP1 is integrated in a positive feedback loop to upregulate its own expression as well as expression of the co-regulated accompanying miR-33a [20]. Furthermore, SREBP1/miR-33a levels are activated by metabolic cholesterol derivatives via LXRα [21]. Moreover, miR-33a/b target *IRS2*, a critical adapter protein between the insulin receptor and its downstream effectors PI3K/AKT and Ras, thereby reducing insulin signaling and contributing to insulin resistance, as seen in metabolic syndrome [22]. Importantly, inhibition of miR-33 by antisense oligonucleotides or genetic deletion resulted in elevated ABCA1 protein levels and increased high-density lipoprotein (HDL) levels in serum and reduced atherosclerotic lesions in mice and non-human primates, thus providing an appealing strategy for treatment of cardiovascular disease [23–26]. SREBP2 furthermore activates a polycistronic miRNA locus encoding miR-96, miR-182 and miR-183, which target the mRNAs of FBXW7 and INSIG2, two negative regulators of SREBP nuclear translocation [27], thus further promoting cholesterol synthesis. In addition, miR-33b has been shown to impact glucose metabolism by targeting PCK1 and G6PC, two key enzymes of hepatic gluconeogenesis [28].

As indicated above, miR-122 is the most abundant miRNA in human liver and was the first identified to affect hepatic metabolism [29]. This miRNA has been implicated in the activation of SREBP1 in hepatic cells, thus positively impacting on lipogenesis [30], which renders it an interesting target for therapeutic interventions. Indeed, short-term inhibition of miR-122 resulted in increased fatty acid β-oxidation and AMPK activation and reduced hepatic steatosis in a high-fat diet mouse model of obesity, thus resulting in an amelioration of NAFLD [29,30]. However, in miR-122 knock-out mice fed a normal diet detrimental long-term effects were observed that manifest in steatosis with progression to non-alcoholic steatohepatitis (NASH) and hepatocellular carcinoma (HCC) [31,32].

A plethora of other miRNAs, have been shown to be involved in human hepatic lipid metabolism and metabolic disease. Among these, miR-106b, miR-148a and miR-758 also target *ABCA1*, suggesting a key role of miRNA regulation for cholesterol metabolism and secretion [33–35]. miR-103 and miR-107 play pivotal roles in glucose homeostasis and insulin sensitivity by targeting *CAV1*, which negatively regulates insulin receptor stability [36]. Moreover, miR-107 regulates levels of FASN, thereby impacting lipogenesis and intracellular lipid levels [37]. As a consequence, inhibition of miR-103/107 increases insulin sensitivity and insulin-induced glucose uptake. Interestingly, miR-370 has been shown to positively regulate miR-122 in HepG2 cells and drive the stress-, but not the diet-mediated hepatic accumulation of lipids [38]. Levels of miR-34a increased with NAFLD progression being upregulated 2- to 3-fold in NASH patients compared to patients with early stage steatosis [39]. Furthermore, miR-34a increased 4-fold in alcoholic hepatitis [40]. In a systematic screen by Cheung et al. the authors identified 46 miRNAs to be differentially expressed in NASH patients, including miR-122, suggesting that perturbed miRNA signatures and disturbed liver metabolism are also

Please cite this article in press as: V.M. Lauschke, et al., The role of microRNAs in liver injury at the crossroad between hepatic cell death and regeneration, Biochemical and Biophysical Research Communications (2016), http://dx.doi.org/10.1016/j.bbrc.2016.10.084