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MicroRNAs in Liver Disease

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MicroRNAs are small noncoding RNA molecules that regulate gene expression posttranscriptionally through complementary base pairing with thousands of messenger RNAs. They regulate diverse physiological, developmental, and pathophysiological processes. Recent studies have uncovered the contribution of microRNAs to the pathogenesis of many human diseases, including liver diseases. Moreover, microRNAs have been identified as biomarkers that can often be detected in the systemic circulation. We review the role of microRNAs in liver physiology and pathophysiology, focusing on viral hepatitis, liver fibrosis, and cancer. We also discuss microRNAs as diagnostic and prognostic markers and microRNA-based therapeutic approaches for liver disease.

Keywords: Chronic Liver Disease; Liver Fibrosis; Cirrhosis; Hepatocellular Carcinoma.

MicroRNAs (miRNAs) were first described in 1993, in developmental timing experiments in the nematode *Caenorhabditis elegans*.[1] Since then, these small noncoding RNA molecules, about 22 nucleotides long, were found to be posttranscriptional regulators of gene expression in metazoans and plants.[2,3] The human miRNA family comprises 1733 mature miRNAs, encoded by 1424 precursors (some have miRs annotated on both sides of the hairpin) (data from miRBase 17; www.mirbase.org/). A 2002 report that miRNAs were involved in tumorigenesis led to the identification of many other miRNAs and increased our understanding of their biogenesis and roles in oncogenesis.[4] A PubMed search from June 2011 using the keywords "microRNA" or "miRNA" found these terms in the titles or abstracts of 7691 research articles and 1360 reviews or commentaries; 4536 were related to cancer and 482 were specific to liver diseases, including liver cancers (Figure 1). There has been exponential growth in the number of miRNA articles related to cancer since 2002.

The discovery of miRNAs has increased our understanding of the posttranscriptional regulation of genes and how this process contributes to development of cancer. More than 50% of genes that encode miRNAs are located at fragile sites or in cancer-associated regions of the genome, indicating that miRNAs are cancer related and could serve as diagnostic markers or therapeutic targets.[5] Almost every type of human cancer analyzed has been associated with altered activities of miRNAs. These discoveries have accelerated our understanding of the pathogenesis of human cancer and provide tools for diagnosis and treatment of cancer.

miRNA biogenesis has been well characterized and basically consists of 6 steps: transcription, cleavage, export, further cleavage, strand selection, and interaction with target messenger RNAs (mRNAs). Modification of any of these steps could contribute to development of liver or other diseases, including cancer (Figure 2). Briefly, miRNAs are transcribed from genes by RNA polymerase II into initial transcripts that are processed either via a canonical pathway (cleaved by the Drosha-DGCR8 complex) or the mirtrons pathway (processed by the spliceosome) to form hairpin-like miRNA precursors called pre-miRNAs. These precursors are exported from the nucleus to the cytoplasm, which requires Exportin-5 and Ran-GTP. In the cytoplasm, they are processed by Dicer into imperfect duplexes that consist of mature miRNA and complementary fragments called miRNAs.

The processed miRNAs are loaded onto the RNA-induced silencing complex (RISC) and guided to their mRNA targets through interactions with members of the Argonaute family, such as Ago1–4 or Ago2, to form RISCs that are also called miRNP or miRISC. Gene expression can be reduced or increased by miRNAs via several mechanisms, such as mRNA deadenylation, translational repression or activation, or other undiscovered processes. Different base-paring combinations of miRNA-mRNA, through their unique complementarities, could reduce or increase production of gene products. It is not clear how

Abbreviations used in this paper: ALD, alcoholic liver disease; HNF, hepatocyte nuclear factor; LNA, locked nucleic acid; miRNA, microRNA; miRNP, protein-microRNA complex; NAFLD, nonalcoholic fatty liver disease; qRT-PCR, quantitative reverse-transcription polymerase chain reaction; RISC, RNA-induced silencing complex; siRNA, small inhibitory RNA; TNF, tumor necrosis factor.

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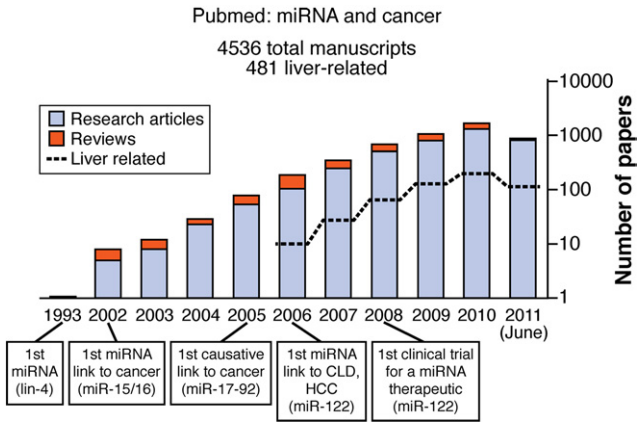

Figure 1. Timeline for studies of miRNAs in cancer. A PubMed search was conducted in June 2011 using the keywords "microRNA" or "miRNA" in titles or abstracts. A subsequent search was then restricted to liver or cancer.

miRNAs incorporate into RISCs and form complexes with different argonautes. However, miRNAs and their biogenesis pathways are highly conserved evolutionally, from plants to mammals, indicating their importance in cellular processes and development. Recent results indicate that miRNA from plants used as foodstuffs (eg, rice) can regulate gene expression in mammals.[6]

miRNAs regulate diverse physiological and developmental processes by controlling levels of specific mRNAs, so their own expression and processing must be tightly regulated for normal cell function.[7,8] Each miRNA could be transcribed and regulated independently, at the transcriptional levels by activators and repressors, or at the epigenetic level through DNA methylation.[9–11] The expression levels of processing components are also tightly controlled to regulate the abundance of mature miRNAs. Alterations in any of these processes could lead to tumorigenesis or development of other diseases.[12] Single nucleotide polymorphisms in genes that encode miRNAs can affect their processing and target binding, along with cancer risk, response to treatment, and disease progression.[13] The complexity of miRNA regulation and the changes that contribute to tumorigenesis make it difficult to correlate specific miRNAs or features of their processing with particular tumor types. In fact, levels of miRNAs and miRNA processing are likely to vary throughout a tumor; tumor heterogeneity is a barrier to effective diagnosis and treatment.

Chronic liver diseases such as viral hepatitis, which can be caused by infection with hepatitis B or C viruses (HBV or HCV), alcohol consumption, or obesity, are major global health burdens that can increase the risk of hepatocellular carcinoma (HCC). Although vaccination can prevent HBV infection,[14] strategies to eliminate HBV from chronic carriers are ineffective and there are no vaccines for HCV. With 2 recently approved drugs and dozens more in the pipeline, HCV treatment strategies are likely to improve.[15] HCC is a common form of primary liver cancer; it is the third most deadly and fifth most common cancer in the world.[16–18] Despite many years of research into treatment and causes of HCC, it remains one of the most difficult-to-treat malignancies, with a 5-year survival rate of less than 12% in the United States.[18]

miRNAs in Liver Disease

The roles of miRNAs in regulation of gene transcription in animal development have been well documented.[19] Organogenesis and development of the liver have also been well studied.[20] Although we have limited understanding of the role of miRNAs in liver development, these molecules are likely to regulate cell lineages and differentiation. Expression of miR-122 is liver specific and inhibition of miR-122 expression in mice leads to a down-regulation of cholesterol and lipid-metabolizing en-

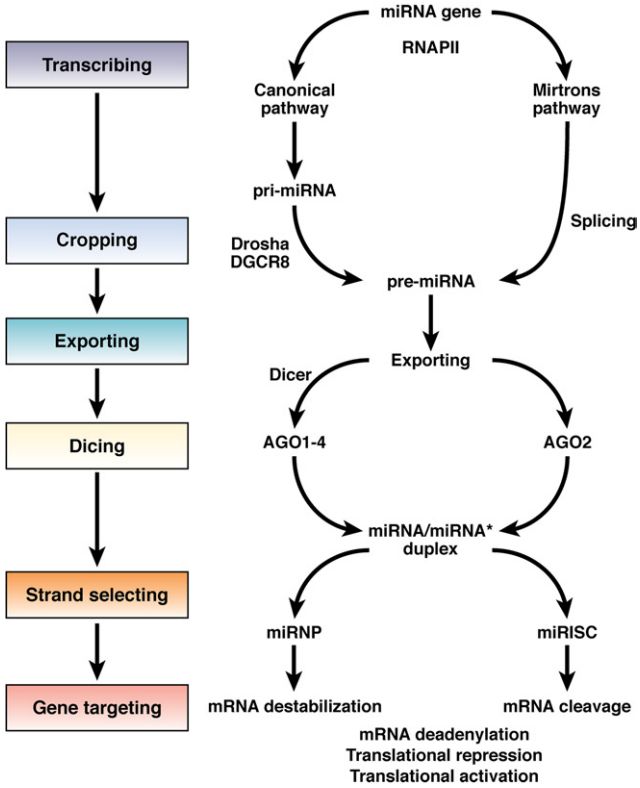

Figure 2. Steps in miRNA biogenesis. miRNAs are mainly transcribed by RNA polymerase II (RNAPII) into initial transcripts know as pre-miRNA, which are processed either via a canonical pathway, in which they are cropped by the Drosha-DGCR8 complex, or via the Mirtrons pathway, in which they are spliced to form hairpin-like miRNA precursors called pre-miRNAs. These precursors are exported from the nucleus to the cytoplasm in an Exportin-5–RanGTP— dependent manner. In the cytoplasm, they are processed by Dicer into an imperfect duplex comprising mature miRNAs and a complementary fragment called miRNA. The processed miRNAs are then loaded onto the RISC and are guided to their mRNA targets through interacting with various members of the Argonaute family such as Ago1-4 or Ago2 to form RISCs that are known as miRNP or miRISC. miRNA-mediated gene silencing or activation could be achieved through several identified mechanisms such as mRNA deadenylation, translational repression or translational activation, and possibly many more mechanisms yet to be discovered. Different base-paring combinations of miRNA-mRNA through their unique complementarities could result in gene silencing or gene activation.