CircularRNA as novel biomarkers in liver diseases

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Abstract: The liver is an essential organ that is primarily responsible for digestion and eliminating toxic substances from the body. After the industrial revolution, Western diet and lifestyle changes have increased the incidence of several liver diseases, including non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH), liver cirrhosis, and hepatocellular carcinoma (HCC). NAFLD and NASH are mostly asymptomatic at early stages, and the disease progression from NAFLD to life-threatening HCC remains not fully understood. Circular RNA (circRNA) is consist of a circular structure, and the circRNA-microRNA(miRNA)-mRNA axes have been shown to be involved in several cellular events, including apoptosis, vascularization, metastasis, etc. The highly stable structure of circRNAs has enabled themselves to be used as putative biomarkers of several diseases. Here, we conducted a literature review and discussed the identified roles of circRNAs in NAFLD, NASH, liver cirrhosis, and HCC. For example, deficiency of circRNA_0046366 and circRNA_0046367 has been shown as the characteristics of NAFLD, and restoration of these circRNAs ameliorates the oxidative stress, lipotoxicity, and disease severity in NAFLD. Silencing of circ_0071410 was shown to alleviate hepatic stellate activation, the key step of liver cirrhosis. CDR1 and circ_0067934 can facilitate the invasion and metastasis of HCC, while circMTO1 negatively regulates the progression of HCC. Although several research works have been conducted, the whole picture of circRNA-related underlying mechanisms is unclear. Future works using high-throughput bioinformatic approaches will be needed to delineate the role of circRNAs in liver diseases and to further develop novel diagnostics and therapeutics.

Keywords: Circular RNA; Hepatocellular carcinoma; Liver cirrhosis; Liver diseases; Non-alcoholic steatohepatitis

1. INTRODUCTION

The liver is an essential organ for the digestion and elimination of toxic substances from the body. Western diet and lifestyle changes after the industrial revolution have increased the incidence of diseases of civilization. Remarkably, the liver is one of the important organs that is affected by the revolution of lifestyle and modern diet. Liver disease can be caused by genetic factors or a variety of factors, including viruses, the use of alcohol, and over-nutrition.[1] Without appropriate intervention, liver diseases can progress to hepatitis, liver cirrhosis, or even the rapidly lethal cancer hepatocellular carcinoma (HCC). The treatment outcome of end-stage liver diseases is usually dismal, indicating that early intervention and the development of novel treatment strategies are urgently needed. Circular RNA (circRNA) is a unique type of single-stranded RNA that consist of a circular loop, and has been demonstrated to be involved in many important biological processes.[2] The circular structure allows circRNA itself to be highly stable and can be used as putative biomarkers of various diseases.[2] In this article, we conduct a literature review and discuss the identified roles of circRNA in various liver diseases, including non-alcoholic steatohepatitis (NASH), liver cirrhosis, and HCC (Fig. 1).

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2. IDENTIFICATION OF CIRCULAR RNA

The structure of CircRNA is predominantly consists of a circular loop and multiple microRNA binding sites. The closed structure enables circRNA to be resistant to RNA degradation pathways. Besides, circRNAs function as miRNA sponges to regulate gene expression.[2] Many circRNAs have been shown to exist in cell nuclei, and the circRNA-miRNA-mRNA axes are involved in various signaling cascades, including those related to apoptosis, invasion, vascularization, and metastasis. Importantly, several lines of evidence indicated that circRNAs can regulate

pathogenicity-related gene expression at the transcriptional or posttranscriptional level.[3,4]

3. NONALCOHOLIC STEATOHEPATITIS, LIVER CIRRHOSIS, AND HEPATOCELLULAR CARCINOMA

Nonalcoholic fatty liver disease (NAFLD) is the abnormal fat accumulation in the liver. Without appropriate control, NAFLD may progress to NASH, a more severe condition than NAFLD.[5] Particularly in the Western world, the incidence of NAFLD and NASH are generally increased due to the changes in lifestyle and modern diets. Besides, these liver manifestations are highly associated with liver cirrhosis and HCC.[6] Liver cirrhosis is the stage of severe scarring of the liver that can be induced by different liver diseases and factors, that is, hepatitis, chronic alcoholism, and overnutrition. As more and more scar tissue forms, liver cirrhosis makes the liver difficult to exert regular functions. The damage induced by severe liver cirrhosis is generally believed to be irreversible. Early diagnosis and intervention that may limit or reverse the further damage caused by liver cirrhosis is therefore required.[5,6] HCC is a widespread primary liver malignant tumor with high mortality and predominantly occurs in patients suffering from chronic hepatitis or cirrhosis. HCC is usually highly aggressive, can expand locally, and metastasize to various sites. NASH and NASH-related cirrhosis both carry high risks for the development of HCC. Unfortunately, the prognosis of HCC after treatment is usually poor and the treatment options for HCC treatment is generally limited. Therefore, it is urgently needed to delineate the mechanisms by which NASH develops and progresses to cancer.[7,8]

4. CIRCRNA IN NON-ALCOHOLIC STEATOHEPATITIS

In general, the "two-hit" hypothesis proposed by Day and James[9] is extensively accepted as the major pathogenesis of NAFLD/NASH. The first hit causes fat accumulation in the liver and the second hit induces inflammation and fibrosis. Metabolic syndrome is a cluster of risk factors including insulin resistance, hyperglycemia, hypertension, central obesity, and dyslipidemia. Actually, NAFLD/NASH is regarded as the hepatic manifestation of metabolic syndrome. Once the steatosis is developed, the liver becomes sensitive to a variety of stimuli, including oxidative stress, pro-inflammatory cytokines, free fatty acids, etc. All of these factors are thought to be candidate second hit that causes NASH.[9] Steatosis could be induced by the dysregulation in miRNA expression, triglyceride accumulation, and lipid peroxidation, and recent studies have demonstrated that circRNA may participate in the pathogenesis of NAFLD. First, miR-34a is a miRNA that contributes to the pathogenesis of steatosis, and circRNA_0046367 was demonstrated to endogenously regulate miR-34a.[10] Hepatic steatosis was accompanied with the loss of the expression of circRNA_0046367 in vitro and in vivo. The normalization of circRNA_0046367 levels abolishes the lipid peroxidation, apoptosis, and mitochondrial dysfunction, in steatosis, and simultaneously removed the inhibitory effect of miR-34a on peroxisome proliferator-activated receptor α and further transcriptionally activates lipid metabolism-associated genes. Taken together, these data indicated that circRNA_0046367 serves a crucial role in ameliorating the lipotoxicity and oxidative stress in NAFLD.[10] The same research team also reported that circ_0046366 as another circRNA that may be involved in the pathogenesis of NAFLD. Like circRNA_0046367, circ_0046366 also functions as the antagonist of miR-34a. Circ_0046366 deficiency was found to be the characteristics of high fat-induced hepatocellular steatosis. In addition, restoration of circ_0046366 inhibits hepatocellular steatosis, inactivates miR-34a, and rescues proliferator-activated receptorα α expression with transcriptional activation of lipometabolic genes.[11] Metformin is recommended as an effective drug for NAFLD treatment. Another study examined the effect of metformin intervention and analyzed the coding transcriptome and non-coding RNAs in the liver from mice with NAFLD. The transcriptome alterations in NAFLD are largely alleviated by metformin intervention. Among all of the altered transcriptomes, seven miRNAs that interacts with multiple differentially expressed circRNAs are suggested to be associated with metabolic or liver diseases.[12] However, any direct association between circRNAs and the metabolism in liver diseases was not observed in the study.

5. CIRCRNA IN LIVER CIRRHOSIS

Liver fibrosis as the abnormal formation of large amount of scar (fibrosis) in the liver is an end-stage hepatopathy. Liver fibrosis is generally caused by chronic liver injury and can progress to irreversible cirrhosis with poor survival time and high

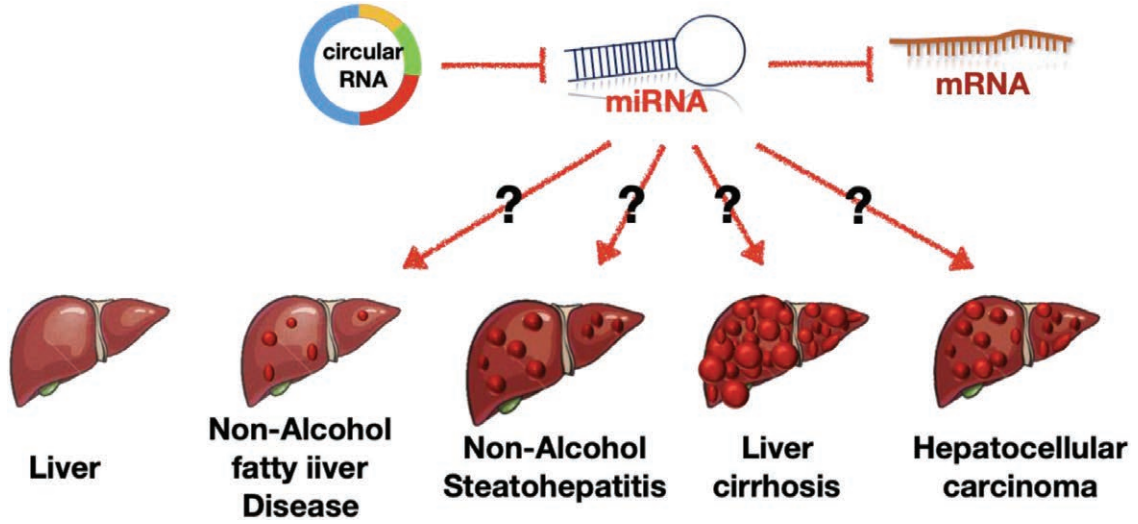

Fig. 1. Scheme illustrating the roles of circRNA/miRNA/mRNA networks in the progression from non-alcoholic steatohepatitis to hepatocellular carcinoma.

mortality. During the progression of liver fibrosis, the accumulation of extracellular matrix modifies the liver structure and largely impairs its physiological function. The activation of hepatic stellate cells and Kupffer cells, and the loss of sinusoidal endothelial cells are believed to contribute to the extracellular matrix synthesis in liver fibrosis.[13] As hepatic stellate cells activate, these cells can transdifferentiate from quiescent cells with perinuclear retinoid droplets to myofibroblasts. These myofibroblasts subsequently acquire the ability for proliferation, contractility, and fibrogenesis, alter the degradation of matrix. Various pathways and mediators, such as autophagy, the stress of endoplasmic reticulum, oxidative stress, receptor-mediated signals, have unraveled the complexity of hepatic stellate cell activation. Hepatic stellate cell activation is also modulated by extracellular signals from hepatocytes, liver sinusoidal endothelial cells, and immune cells.[14] So far, it is extensively elucidating that the regulation of hepatic stellate activation may help the development of novel therapeutics for the treatment of liver fibrosis. Noncoding RNAs such as microRNA and long noncoding RNAs have been reported as important regulators of hepatic stellate cell activation and are involved in the process of liver fibrosis/cirrhosis.[15] Like microRNAs and long non-coding RNAs, circRNAs have also been reported to be associated with hepatic stellate cell activation. In hepatic stellate cells activated by irradiation, 179 and 630 circRNAs are upregulated and downregulated, respectively, when compared with quiescent hepatic stellate cells. Bioinformatics analysis indicated the dysregulated circRNA expression may contribute to the biological process of liver fibrosis and the cellular response to irradiation. Importantly, silencing of hsa_circ_0071410 increases miR-9-5p expression, and further alleviates hepatic stellate activation.[15] These data unraveled the involvement of circRNA in hepatic stellate cell activation induced by irradiation. Further investigations are required to elucidate the involvement of circRNAs in NAFLD/NASH-associated liver fibrosis/cirrhosis.

6. CIRCULAR RNAS IN HEPATOCELLULAR CARCINOMA

Considering HCC as malignant liver tumor with poor prognosis and high mortality, the treatment and management of this liver disease are therefore highly critical. Different genes that directly or indirectly regulates cell cycle, apoptosis, and cell survival, have been demonstrated to play a role in the progression of HCC.[16,17] For example, CDR1 that functions as miR-7 sponge is a intensively investigated circRNA in the research field of HCC. In HCC tissue, CDR1 is upregulated with concomitant downregulation of miR-7. CDR1 is able to facilitate the invasion/proliferation of HCC cells via decreasing miR-7 expression.[18,19] The expression of circ_0067934 is also increased in HCC tissue; circ_0067934 acts as the sponge of miR-1234, and inhibition of circ_0067934 suppresses the invasion and metastasis of HCC.[20] Unlike CDR1 and circ_0067934, circular RNA circMTO1 as another circRNA was found to negatively regulate the progression of HCC through acting as miR-9 sponge.[21]

7. FURTHER CHALLENGE OF CIRCRNA RESEARCHES IN LIVER DISEASES

Although substantial research works have been conducted to investigate the role of circRNAs in the liver physiology and various liver diseases, a whole picture of the source of circRNAs and underlying mechanisms are still lack. Multiple cell types within the liver (e.g., hepatocytes, hepatic stellate cells, Kupffer cells, and immune cells) also increases the difficulty of circRNA researches in liver diseases. Meanwhile, one of well-known features of circRNAs is species-specific or tissue-specific, therefore reducing their translational value in clinical application. Following the development of high-throughput bioinformatic approach, it is still expectable to delineate the involvement of circRNAs in liver diseases and to further develop novel diagnostics and therapeutics. However, various problems of circRNA researches are still needed to be solved and required a massive amount of experiment data, research works, and efforts.

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