Review article

MicroRNAs: Novel Diagnostic and Therapeutic Tools for Hepatocellular Carcinoma

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Abstract Hepatocellular carcinoma (HCC) is the fifth most frequent malignant tumor and the third leading cause of cancer-related mortality in the world. Recently, an emerging class of highly conserved non-coding small RNAs, microRNAs (miRNAs), has been found to be aberrantly expressed in HCC and some of them are functionally involved in HCC carcinogenesis and its progression. Certain miRNAs are related to HCC subtypes, which points to the potential of miRNAs for HCC patient stratification of diagnosis and prognosis. The main objective of this review is to shed light on the diagnostic roles of miRNAs as a group of new biomarkers for HCC. In addition, miRNAs-related function and therapeutic strategies to treat HCC are also discussed.

KeyWords: MicroRNAs (MiRNAs); Hepatocellular carcinoma (HCC); Diagnosis; Therapy

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common neoplasm worldwide, and the third most common cause of cancer-related death, accounting for as many as 500,000 deaths annually [1-2]. The occurance of HCC varies by geographic location from a relatively rare tumor, for instance, those found in North America and Europe, to a very common and highly malignant tumor such as those in sub-Saharan Africa and Southeast Asia [1]. Most patients with HCC also suffer from coexisting cirrhosis, which is the major clinical risk factor for hepatic cancer and is correlated to hepatitis B virus (HBV) or hepatitis C virus (HCV) infection[3]. Chronic HBV infection is prevalent in Asian countries and accounts for most cases of HCC. In contrast, chronic HCV infection is more common in Western countries. However, cirrhosis from non-viral causes such as alcoholism, hemochromatosis and primary biliary cirrhosis are also associated with an elevated risk of HCC. As numerous studies in humans and animal models have shown, concomitant risk factors such as HCV infection, in addition to alcoholism, tobacco use, diabetes or obesity increase the relative risk of HCC development[4-6]. Contemporary available treatments of HCC are largely inadequate. Current treatments for HCC include liver resection, transplantation, various local ablative and trans-arterial therapies. Surgical resection and liver transplantation are the main curative treatments. Unfortunately, only 20% of patients, mostly diagnosed by regular screening, may benefit from these surgical therapies. Most other patients either present late with an advanced tumor or have severe underlying cirrhosis, precluding any surgical or even loco-regional therapies. These patients can only be palliated by chemotherapy or the best possible supportive treatment.

MicroRNAs (miRNAs) are a group of small regulatory small non-coding RNAs of 19-22 nucleotides involved in the control of gene expression at the post-transcriptional level [7]. This control allows for fine-tuning of the cellular phenotype, including regulation of proliferation, cell signaling, and apoptosis [8-11]. Spontaneously, miRNAs contribute to HCC biology. Recent investigations have demonstrated aberrant expression of particular miRNAs in HCC cells. Modulation of miRNA expression in vitro as well as in vivo has revealed an important role for miRNAs in initiation and progression of HCC. In this review, the miRNA processing pathway and recent findings on miRNAs expression and function in HCC will be discussed. In addition, the potential to target miRNAs, which may translate into novel therapeutic strategies for HCC in the future, will also be highlighted.

MiRNAs processing pathway

MiRNAs are endogenous, single-stranded RNA molecules consisting of approximately 22 non-coding nucleotides that regulate target genes[7-8]. There are roughly 500~1000 different mammalian miRNA genes. A complete list and details on the nomenclature of the miRNAs is available at Sanger mirBase 10.1 (http://microrna.sanger.ac.uk/ sequences/) [12]. Up to 600 miRNAs have been identified in humans [7-8] . The miR processing pathway is

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initiated by transcription of miR-encoding cellular genes by RNA polymerase II (Pol II) to produce hairpin-containing primary miRs (pri-miRs). These pri-miRs may be derived from intronic sequences and may be polycistronic. Within the nucleus, pri-miRs are processed to form precursor miR (pre-miR) hairpins of 60~80 nt in length. This step is catalyzed by the microprocessor complex, which contains Drosha and di George Critical Region 8 (DGCR8) proteins. Drosha functions as an RNAse III enzyme and DGCR8 is its double stranded RNA binding protein collaborator. Pre-miRs are exported from the nucleus to the cytoplasm by the RanGTP-dependent Exportin 5 transporter. Pre-miRs are then processed by Dicer with associated TAR RNA-binding protein (TRBP) to form a staggered RNA duplex of 21-24 bp with 2 nt 3' overhangs. This duplex is handed on to the RNA induced silencing complex (RISC), including several components such as Argonaute 1 (AGO1), Argonaute 2 (AGO2) and Fragile X proteins. One strand of the RNA duplex, which is designated the passenger strand, is cleaved within RISC and is then released from the complex. The remaining intact single stranded guide RNA activates RISC to direct target-specific silencing. Mature cellular miRs are usually not entirely complementary to their targets and bind to the 3' untranslated regions (UTR) of cognate mRNA to induce translational suppression. Hybridization between target and nucleotides 2-8 from the 5' end of the guide strand, termed the seed sequence, is all that is required to cause translational suppression [13]. When base pairing between entire guide and target is perfectly matched, the AGO2 component of RISC exerts silencing through site-specific cleavage ('slicing') of the guide complement [14]. In mammal cells, each mRNA can be regulated by several miRNAs, and one miRNA can identify several targets [15]. Intriguingly, miRNAs may also lead to an up-regulation of a gene expression [16]. However, the exact mechanism is currently unknown, but may be the result of direct effects such as chromatin remodeling, or indirect effects, e.g. suppression of transcriptional repressors. It has been suggested that expression of 30% of human genes may be regulated by miRNAs[17] .

MiRNA expression profiles in HCC

In HCC, different specific miRNAs have been found to be aberrantly expressed. An array-based analysis identified 44 miRNAs that were expressed at lower levels in HCC than in normal livers [18]. Another study comparing HCC to liver cirrhosis demonstrated down-regulation of 34 miRNAs and increased expression of only one miRNA (miR-221) in HCC [19]. The two studies mentioned above both have detected the decreased expression of mir-199, as well as the liver-specific miRNA, mir-122. Other miRNAs demonstrating decreased expression in HCC include family members of lethal-7 (let-7), mir-125, mir-150, mir-195, and mir-200[20-25]. In contrast, there is another group of miRNAs that exhibit increased expression in tumors. Specific examples in HCC include miR-21, the miR-17-92 cluster and miR-221/222 [19, 21, 23, 26-28]. Although the phenotypes can be broadly distinguished histologically or immunologically, HCC can vary widely in their clinical behavior and prognosis. The use of miRNA-based classifications that correlate with etiology, pathogenetic changes, or malignant tendency will enhance molecular diagnosis and enable further definition of these phenotypes [29-30]. Thus, miRNA profiling studies could be used for providing potentially useful molecular diagnostic markers.

HCC−related miRNA function

The incidence of HCC is highly correlated with the expression and function of oncogenes and tumor suppressor genes. A large number of HCC-related genes have been identified, including c-myc, N-ras, IGF-II, C-erbB2, C-fos, Bcl-2,C-ets2 . Similarly, a variety of HCC-related tumor suppressor genes have been reported, including p53, Rb, p16,INK4, DCC, MCC,APC, PTEN. Activation of the HCC-related gene or inactivation of the tumor suppressor genes is a key step in the occurrence of HCC. Firstly, activation of c-myc is found to be directly correlated with the occurrence of HCC [31]. Several miRNAs were related with upregulation of c-myc. C-myc can induce gene transcription of E2F1 and miR-17-92 cluster, and sequentially these miRNAs have the function to inhibit the translation of E2F1. As a result, in the presence of the c-myc, miR-17-92 gene cluster can inhibit the activity of E2F1. The effect of c-myc on cell proliferation is weakened by blocking the positive feedback between C-myc and E2F1. In this model, miR-17-92 cluster genes play a certain role as a tumor suppressor gene [32]. However, this result seems to contradict the observation of He et al [33], who found one cluster of miRNAs, the miR-17-92 polycistron, as a potential human oncogene, which is located in a region of DNA that is amplified in human B-cell lymphomas. Therefore, even though E2F1 can promote cell proliferation, it can also cause apoptosis when E2F1 expression level exceeds a certain threshold. In this case, negative regulation of E2F1 by miRNAs may block the apoptosis activity induced by E2F1, promoting c-mycmediated cell proliferation, which supports the model proposed by Horie[34]. The above discoveries can partially explain the role of c-myc, miR-199a, miR-122 and miR-17-92 gene cluster in the chronic viral hepatitis, liver fibrosis, and HCC. Secondly, it is well-known that PTEN is a tumor suppressor gene, and inhibition of PTEN function will lead to the occurrence of HCC. At present, PTEN has been considered one of the prognostic characteristics of tumor patient outcome [32]. Meng et al [35] discovered that human miR-21 can promote the occurrence and invasion of HCC through the degradation of PTEN gene and inhibition of its function. With gene chips to analyze 197 miRNAs expression in 3 normal liver cells and HCC cell lines, abundance of miR-21 in liver cells was found to be 9 times higher than that of the normal liver cells. As a result, HCC cell growth, migration and invasion were strengthened. In addition, miR-21 precursor can also enhance migration capacity of the normal liver cells. In various studies,

Meng et al [18] used bioinformatics tools which lead to the discovery that one of the potential target genes of miR-21 was PTEN. At the same time, they found that over-expression of miR-21 in normal liver cells could lead to down regulation of PTEN. From this, they concluded that over expression of miR-21 in HCC could inhibit tumor suppressor gene PTEN expression and promote the occurrence of HCC. This discovery confirms that miR-21 plays an important role in the occurrence of HCC through PTEN, and also provides a point of reference for further research on miR-21 over-expression in cancer.

MiRNAs as Therapeutic targets for HCC

MiRNA/RNAi-based therapeutic strategies for gene therapy will provide clinicians with an innovative and large repertoire. For example, chemically engineered oligonucleotides, termed 'antagomirs' have recently been developed and proven to be efficient and specific silencers of endogenous miRNAs in mice[36]. The silencing effect was considerably sustained over time probably because of the long half-life of endogenous miRNAs[37]. Additionally, induction of stable loss-of-function phenotypes for specific miRNAs by lentiviral-mediated antagomir expression has recently been described [38]. More excitingly, strategies based on targeting HBV, and to a lesser extent HCV, by both synthetic and expressed activators of the RNAi pathway have proved efficient to inhibit viral replication both in vitro and in vivo [39-40]. The study by Pedersen et al [41] provided great insights into validating sequence predicted targets of cellular miRNAs within the HCV genome. MiRNA-122 antagomir can down-regulate expression of several adult-liver genes [36], providing the potential to generate a new attractive expandable cell source for hepatocyte transplantation that would feature stem/progenitor cell phenotype. Moreover, the effect of miR-122 antagomir in high-fat fed mice may be of therapeutic potential to reduce hepatic steatosis [36] . Finally, Pineau P et al. [28] showed that an antagomiR specific for miR-221 can efficiently inhibit cell growth in vitro. Thus, the use of synthetic inhibitors of HCC specific miRNAs may prove to be a promising approach to HCC treatment.

Conclusions

Taken together, a number of miRNAs have been found to be aberrantly expressed in HCC. Some of them are functionally involved in HCC carcinogenesis and its progression, which suggests the potential of miRNAs for HCC patient diagnosis and treatment. However, further investigation is needed to elucidate the function and mechanism of these HCC specific miRNAs. At the same time, delivery of miRNAs to the tumor remains a major challenge, but may not be prohibitive because of the feature of being endogenously expressed.

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