REVIEWS

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JIE XU^{1, B–E}, JI LI^{2, B}, TAI-HAO ZHENG^{3, B}, LIAN BAI^{1, B}, ZUO-JIN LIU^{2, E, F}

MicroRNAs in the Occurrence and Development of Primary Hepatocellular Carcinoma^{*}

¹ Department of Gastrointestinal Surgery, Yongchuan Hospital, Chongqing Medical University, Chongqing, China

² Department of Hepatobiliary Surgery, Second Affiliated Hospital of Chongqing Medical University, Chongqing, China

³ Department of Oncology, Yongchuan Hospital, Chongqing Medical University, Chongqing, China

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Abstract

Hepatocellular carcinoma is one of the deadliest types of cancer. Despite improvements in treatment over the past few decades, patient survival remains poor and there is an urgent need for development of targeted therapies. MicroRNAs represent a class of small RNAs, frequently deregulated in human malignancies. We are reviewing the role of microRNA in the development of primary hepatocellular carcinoma and its use as a biomarker for early diagnosis and clinical treatment. First, we describe the current incidence and possible causes of the incidence of hepatocellular carcinoma, followed by the introduction of microRNA synthesis, maturation and function, and finally we explain the role of microRNA in the development of hepatocellular carcinoma and its clinical value as a biological marker in the diagnosis and treatment of liver cancer. A comprehensive analysis of cellular microRNA is a benefit for early diagnosis of hepatocellular carcinoma and early clinical intervention, and microRNA is considered by some to be a key target of gene therapy to control the occurrence and development of hepatocellular carcinoma. (*Adv Clin Exp Med* 2016, 25, 5, 971–975).

Key words: microRNA, HCC, occurrence, development, biomarkers.

Hepatocellular carcinoma (HCC) is the fifth most common malignant cancer and the second most common cause of cancer-related deaths in the world [1]. Population-based studies show that the incidence rate continues to approximate the death rate, indicating that most of the patients who develop HCC die of it [1]. In recent years, the molecular mechanisms for the development of hepatocellular carcinoma have made tremendous progress. It has been reported that the abnormal expression of cell cycle regulatory proteins such as P53, c-Myc, CyclinD1 and the genes involved in the Wnt/-catenin signaling pathway and a variety of tyrosine kinase growth factor receptors and ligands play a significant role in the pathogenesis of hepatocellular carcinoma [3, 4]. But microRNAs, as the upstream regulatory molecules of this se-

ries of important genes, may play a critical role. The aim of this paper was to summarize the role of microRNAs in the occurrence and development of hepatocellular carcinoma. Meanwhile, we depicted microRNAs as a biomarker for early diagnosis and treatment of hepatocellular carcinoma.

Hepatocellular Carcinoma

Hepatocellular carcinoma is one of the most common cancers in the world. It is the second leading cause of cancer-related death of men and sixth leading cause of cancer-related deaths of women [5]. Hepatocellular carcinoma (HCC), of which 70–85% of patients have primary HCC, rarely can be detected in its early stages, so the survival

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of HCC patients is only a few months [6]. About 90% of patients have varying degrees of liver cirrhosis. The high risk factors of HCC, such as hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, alcohol abuse, hemochromatosis psychosis and so on, may lead to a chronic liver disease, then with progression to cirrhosis, eventually leading to the occurrence of HCC [1, 2, 7].

The Biosynthesis, Maturation and Function of microRNA

MicroRNA is a class of endogenous, small RNA, with a length of about 20–24 nucleotides. In the nucleus, the transcription of microRNA was mainly by polymerase II. The process of transcription was as follows. First, at the beginning of transcription, microRNA evolved into primary microRNA (PRI-microRNA) under the effect of polymerase II. Then PRI-microRNA forms one or more hairpin structures of individual microRNA precursor (pre-microRNA) under the action of the enzyme family of RNase III. Pre-microRNA was firstly cleavage by specific Drosha, Dicer and Argonaute and other endogenous enzymatic and then fully or partially complementary bind to the target gene mRNA 3'-UTR region which then results in negative regulation of target gene expression by degradation of the target mRNA or inhibition of post-transcriptional translation. Next, the microRNA was untied into two strands. One has been binds mRNA 3'-UTR, was selectivity binding silencing complex (RISC) induced by RNA protein and finally becomes mature microRNA under the effect of the regulation gene. The other strand is rapidly degraded, eventually producing one or more functions of microRNAs [8–10]. MicroRNAs do not encode proteins. Though microRNAs are to modulate associated with gene expression at the level of transcription or translation. So microRNAs are involved in a variety of biological processes, such as development, proliferation, differentiation and apoptosis, etc. It has been reported that several microRNAs can regulate the same gene, or by a combination of several microRNAs to regulate the expression of one gene [11, 12]. Lewis et al. [13] reported that microRNAs regulate one-third of the human genes. The regulation of microRNAs may be through the relevant enzymes, promoter hypermethylation and microRNA's absence of a target gene binding site [14].

The Role of microRNA in the Occurrence and Development of HCC

The occurrence and development of HCC through genes down-regulated a variety of molecular pathways, including p53, RAS/MAPK, PI3K/Akt/mTOR, Wnt/ β -catenin β , MET, Myc gene, transforming growth factor- β , resulting in molecular genetic and epigenetic histological changes. Particularly, abnormal expression of microRNAs affects these critical cancer-related pathways [15]. It is reported that sustained microRNA-122 [16–19], microRNA-101 [20, 21]) down-regulated and microRNA-21 [22], microRNA-221 [23] up-regulated in the occurrence of hepatocellular carcinoma seems to be particularly important.

MicroRNAs and Occurrence of HCC

MicroRNAs whose expression is increased in tumors may be considered as oncogenes. Apoptosis is a major obstacle that must be addressed in the process of malignant tumor transformation and progression. These oncogene microRNAs usually promote tumor development by negatively inhibiting tumor suppressor genes and/or genes that control cell differentiation or apoptosis. Many miRNA genes have been found that are significantly over-expressed in different cancers. The evolution of tumor cells is to evade apoptosis so that they stay away from the tumor monitoring system to survive. Many signals induced apoptosis concentrated in the mitochondria, and then release a powerful catalyst for apoptosis – cytochrome C. The Bcl-2 protein family, whose members have apoptosis (BIM, BMF, Bax, Bak, Bid) or anti-apoptotic (Bcl-2, Bcl-W, Bcl XL, Mcl-1) function in the regulation of mitochondrial death signal transduction plays an important role. It has been reported that abnormal microRNAs in tumor cells can help evade the action of the Bcl-2 family to escape apoptosis [24]. It is reported that HBsAg suppresses the expression of MICA and MICB to prevent the NKG2D-mediated elimination of HCC cells. The process is through unregulated microRNA-133 and down-regulated microRNA-9 [25]. So the occurrence of HCC could be the risk factors' abnormal regulation of the expression of certain microRNAs.

MicroRNAs and Metastasis of HCC

Invasion and metastasis are the two most critical signs of cancer and usually are the main causes of death, especially in patients of hepatocellular carcinoma. The high rate of tumor recurrence, mainly due to the intrahepatic metastasis of cancer cells, is the main problem involved in the survival of patients with hepatocellular carcinoma after curative resection. Identification and understanding of the mechanisms associated with transfer are very important for the treatment of HCC metastasis. More and more upstream regulator genes related to tumor metastasis, such as pre-transfer microRNA and anti-metastatic microRNA, are found to have an important role in the invasion and metastasis of HCC. Genome-wide screening has identified that miR-134 acts as a metastasis suppressor by targeting integrin beta 1 in hepatocellular carcinoma [26]. microRNA-21, which can change the focal adhesion kinase (FAK) expression and phosphorylation of MMP2 and MMP9 matrix metalloproteinase, together with the downstream regulator of PTEN, participate in the metastasis and invasion of tumor cells. A recent discovery of PTEN is that microRNA-221 and microRNA-222 directly target genes involved in TRAIL resistance and increase the mobility of HCC [27]. In addition to the PTEN gene, microRNA-221 and microRNA-222 can also directly regulate protein phosphatase 2A subunit B (PPP2R2A) and tumor suppression factor TIMP3 expression, thereby activating the Akt signaling pathway and metal epitope peptide to promote the invasion and metastasis of HCC [28]. A report also shows a new microRNA (microRNA-143) mediated nuclear factor κ B (NF- κ B), promotes hepatitis B virus-related hepatocellular carcinoma metastasis [29]. Hepatitis B virus-related hepatocellular carcinoma invasion and transfer work separately through NF- κ B signaling pathway unregulated microRNA-143 expression and inhibiting the fibronectin III domain containing 3B. In addition, microRNA-17-5p upregulate in HCC to promote p38 mitogen-activated protein kinase activation and heat shock protein 27 phosphorylation increased, which the metastasis of HCC relies on [30].

MicroRNAs as a Biomarker in the Diagnosis and Treatment of HCC

The different expression of microRNAs in hepatoma cells and normal liver cells has revealed the potential value of detecting microRNAs in the

diagnosis of HCC. It is reported that liver cancer can be divided into three main clusters based on the abundance of microRNA expression profiles [31]. The calculation procedure of human microRNA microarray in the detection of abnormal microRNAs in liver cancer can reach an overall prediction accuracy of 97.8%. Murakami et al. used a computer program to identify three significant up-regulated and five down-regulated microRNAs in 25 samples comparative of liver cancer and cancerous tissue. Serum levels of microRNA-25, microRNA-375 and let-7f can be clearly distinguished in HCC and non-HCC cases and depict the levels of microRNA-375 alone show the potential for HCC diagnosis [32].

A tumor can inhibit the expression of some microRNAs in normal liver tissue and the inhibited microRNAs will be down-regulated in the occurrence and metastasis of tumors. For clinical treatment, supplementing the down-regulated expression of microRNA in the patients of HCC would be a good strategy. MicroRNA replacement therapy of primary HCC has been applied in a mouse model and the demonstrated systems complementing microRNA-6a, -122 and -124 can reduce tumorigenesis and metastasis of HCC [33, 34]. By contrast, the inhibition of onco-miR-221 can prolong survival and shrink the volume of a tumor [35, 36]. With the progress of targeted gene therapy, microRNAs can be useful as a potential therapeutic target for HCC, because microRNA is involved in the occurrence and development of HCC. However, this hypothesis needs to be tested in clinical patients with hepatocellular carcinoma. Furthermore, chemotherapy will kill normal cells and result in significant toxicity to patients. In a mouse model for the treatment of hepatocellular carcinoma by microRNAs, no toxicity was observed. MicroRNA-21 as a target of antagomirs or microRNA-181b may be effective in improving the efficacy of the treatment. By contrast, down-regulating the expression of multidrug resistant (MDR) proteins to restore the contents of microRNA-122 in hepatoma cells, will make hepatoma cells more sensitive to doxorubicin and vincristine [19].

To sum up, the results of the present studies show that many expressions of microRNAs are varied in HCC. Therefore, controlling the occurrence and development of liver cancer by directly targeting control of a large number of the key genes in hepatoma cells is a great development of potential treatment options for improving clinical outcomes. At the same time, comprehensive analysis of the expression levels of microRNAs in liver cells may have certain significance in the classification and diagnosis of early HCC. For more information from the genome-wide association

studies, through the auxiliary of a high-resolution single nucleotide polymorphism (SNP) research platform and next-generation sequencing technologies, we can find abnormal microRNA expression earlier in patients with hepatocellular carcinoma. Meanwhile, some abnormal expressions of microRNAs associated with tumors may be applied to

the development of chemotherapeutic agents. As a consequence, future laboratory research and clinical research should attach great importance to the aspects of microRNAs for the early diagnosis and treatment of HCC, and the potential for better clinical application in the treatment of patients with hepatocellular carcinoma.

References

- [1] **Levrero M**: Viral hepatitis and liver cancer: The case of hepatitis C. *Oncogene* 2006, 25, 3834–3847.
- [2] **Lee JS, Chu IS, Heo J, Calvisi DF, Sun Z, Roskams T, Durnez A, Demetris AJ, Thorgerirsson SS**: Classification and prediction of survival in hepatocellular carcinoma by gene expression profiling. *Hepatology* 2004, 40, 667–676.
- [3] **Jiang J, Gusev Y, Aderca I, Mettler TA, Nagorney DM, Brackett DJ, Roberts LR, Schmitggen TD**: Association of MicroRNA expression in hepatocellular carcinomas with hepatitis infection, cirrhosis, and patient survival. *Clin Cancer Res* 2008, 14, 419–427.
- [4] **Roberts LR, Gores GJ**: Hepatocellular carcinoma: Molecular pathways and new therapeutic targets. *Semin Liver Dis* 2005, 25, 212–225.
- [5] **Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D**: Global cancer statistics. *CA Cancer J Clin* 2011, 61, 69–90.
- [6] **Perz JF, Armstrong GL, Farrington LA, Hutin YJ, Bell BP**: The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. *Hepatology* 2006, 45, 529–538.
- [7] **Sanyal AJ, Yoon SK, Lencioni R**: The etiology of hepatocellular carcinoma and consequences for treatment. *Oncologist* 2010, 15, 14–22.
- [8] **Kim VN, Han J, Siomi MC**: Biogenesis of small RNAs in animals. *Nat Rev Mol Cell Biol* 2009, 10, 126–139.
- [9] **Kim VN**: MicroRNA biogenesis: Coordinated cropping and dicing. *Nat Rev Mol Cell Biol* 2005, 6, 376–385.
- [10] **Kim Y, Kim VN**: MicroRNA factory: RISC assembly from precursor microRNAs. *Mol Cell* 2012, 25, 46, 384–386.
- [11] **Lagos-Quintana M, Rauhut R, Lendeckel W, Tuschl T**: Identification of novel genes coding for small expressed RNAs. *Science* 2001, 294, 853–858.
- [12] **Bartel DP**: MicroRNAs: Genomics, biogenesis, mechanism, and function. *Cell* 2004, 116, 281–297.
- [13] **Lewis BP, Burge CB, Bartel DP**: Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. *Cell* 2005, 120, 15–20.
- [14] **Lee YS, Kim HK, Chung S, Kim KS, Dutta A**: Depletion of human micro-RNA miR-125b reveals that it is critical for the proliferation of differentiated cells but not for the down-regulation of putative targets during differentiation. *J Biol Chem* 2005, 280, 16635–16641.
- [15] **Negrini M, Gramantieri L, Sabbioni S, Croce CM**: microRNA involvement in hepatocellular carcinoma. *Anticancer Agents Med Chem* 2011, 11, 500–521.
- [16] **Gramantieri L, Ferracin M, Fornari F, Veronese A, Sabbioni S, Liu CG**: Cyclin G1 is a target of miR-122a, a microRNA frequently down-regulated in human hepatocellular carcinoma. *Cancer Res* 2007, 67, 6092–6099.
- [17] **Kutay H, Bai S, Datta J, Motiwala T, Pogribny I, Frankel W**: Downregulation of miR-122 in the rodent and human hepatocellular carcinomas. *J Cell Biochem* 2006, 99, 671–678.
- [18] **Nassirpour R, Mehta PP, Yin MJ**: miR-122 regulates tumorigenesis in hepatocellular carcinoma by targeting AKT3. *PLoS One* 2013, 8.
- [19] **Bai S, Nasser MW, Wang B, Hsu SH, Datta J, Kutay H**: MicroRNA-122 inhibits tumorigenic properties of hepatocellular carcinoma cells and sensitizes these cells to sorafenib. *J Biol Chem* 2009, 284, 32015–32027.
- [20] **Hou J, Lin L, Zhou W**: 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.
- [21] **Su H, Yang JR, Xu T, Huang J, Xu L, Yuan Y**: MicroRNA-101 down-regulated in hepatocellular carcinoma, promotes apoptosis and suppresses tumorigenicity. *Cancer Res* 2009, 69, 1135–1142.
- [22] **Pineau P, Volinia S, McJunkin K, Marchio A, Battiston C, Terris B**: miR-221 overexpression contributes to liver tumorigenesis. *Proc Natl Acad Sci USA* 2010, 107, 264–269.
- [23] **Zhang Z, Li Z, Gao C, Chen P, Chen J, Liu W**: miR-21 plays a pivotal role in gastric cancer pathogenesis and progression. *Lab Invest* 2008, 88, 1358–1366.
- [24] **Huang S, He X**: The role of microRNAs in liver cancer progression. *Br J Cancer* 2011, 104, 235–240.
- [25] **Wu JI, Zhang XJ, Shi KQ, Chen YP, Ren YF, Song YJ, Li G, Xue YF, Fang YX, Deng ZJ, Xu X, Gao J, Tang KF**: Hepatitis B surface antigen inhibits MICA and MICB expression *via* induction of cellular miRNAs in hepatocellular carcinoma cells. *Carcinogenesis* 2014, 35, 155–163.
- [26] **Zha R, Guo W, Zhang Z, Qiu Z, Wang Q**: Genome-wide screening identified that mir-134 acts as a metastasis suppressor by targeting integrin beta 1 in hepatocellular carcinoma. *PLoS One* 2014, 9, e87665.
- [27] **Garofalo M, Di Leva G, Romano G, Nuovo G, Suh SS, Nanganke A, Taccioli C, Pichiorri F, Alder H, Secchiero P, Gasparini P, Gonelli A, Costinean S, Acunzo M, Condorelli G, Croce CM**: miR-221&222 regulate TRAIL resistance and enhance tumorigenicity through PTEN and TIMP3 downregulation. *Cancer Cell* 2009, 16, 498–509.
- [28] **Wong QW, Ching AK, Chan AW, Choy KW, To KF, Lai PB, Wong N**: MiR-222 overexpression confers cell migratory advantages in hepatocellular carcinoma through enhancing AKT signaling. *Clin Cancer Res* 2010, 16, 867–875.

- [29] **Zhang X, Liu S, Hu T, Liu S, He Y, Sun S:** Up-regulated microRNA-143 transcribed by nuclear factor kappa B enhances hepatocarcinoma metastasis by repressing fibronectin expression. *Hepatology* 2009, 50, 490–499.
- [30] **Yang F, Yin Y, Wang F, Wang Y, Zhang L, Tang Y, Sun S:** miR-17-5p promotes migration of human hepatocellular carcinoma cells through the p38 mitogen-activated protein kinase-heat shock protein 27 pathway. *Hepatology* 2010, 51, 1614–1623.
- [31] **Toffanin S, Hoshida Y, Lachenmayer A:** MicroRNA based classification of hepatocellular carcinoma and oncogenic role of miR-517a. *Gastroenterology* 2011, 140, 1618–1628.
- [32] **Li LM, Hu ZB, Zhou ZX, Chen X, Liu FY, Zhang JF, Shen HB, Zhang CY, Zen K:** Serum microRNA profiles serve as novel biomarkers for HBV infection and diagnosis of HBV-positive hepatocarcinoma. *Cancer Res* 2010, 70, 9798–9807.
- [33] **Tsai WC, Hsu PW, Lai TC, Chau GY, Lin CW:** MicroRNA-122, a tumor suppressor microRNA that regulates intrahepatic metastasis of hepatocellular carcinoma. *Hepatology* 2009, 49, 1571–1582.
- [34] **Lang Q, Ling C:** MiR-124 suppresses cell proliferation in hepatocellular carcinoma by targeting PIK3CA. *Biochem Biophys Res Commun* 2012, 426, 247–252.
- [35] **Park JK, Kogure T, Nuovo GJ, Jiang J, He L:** miR-221 silencing blocks hepatocellular carcinoma and promotes survival. *Cancer Res* 2011, 71, 7608–7616.
- [36] **Callegari E, Elamin BK, Giannone F, Milazzo M, Altavilla G:** Liver tumorigenicity promoted by microRNA-221 in a mouse transgenic model. *Hepatology* 2012, 56, 1025–1033.

Address for correspondence:

Zuo-jin Liu
Department of Hepatobiliary Surgery
Second Affiliated Hospital of Chongqing Medical University
Cho, 400010 Chongqing
China
E-mail: liuzuojin197164@126.com

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