

# Research Progress of miRNA Regulating Cell Signaling Pathways Related to Hepatocarcinogenesis

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**Abstract:** Hepatocellular carcinoma (HCC) is one of the most common malignant tumors in clinical practice. The pathogenesis of HCC is still unclear. Currently, the clinical treatment of HCC is poorly targeted and the therapeutic effect is poor. MicroRNAs (miRNAs) are closely related to the occurrence of HCC, and they are mainly involved in the occurrence and development of HCC through binding to target genes or acting on related signaling pathways. In recent years, studies have shown that miRNA can be used as a potential biomarker for diagnosis and prognosis of HCC. In addition, studies have also shown that miRNA plays a tumor-suppressing or tumor-promoting role in the process of HCC by regulating the biological processes of tumor cell proliferation, migration, invasion and metastasis. In this paper, the recent studies on miRNA signaling pathways related to the occurrence and development of HCC were reviewed, with a view to providing ideas for the clinical diagnosis and treatment of HCC.

**Key words:** MicroRNAs; Target gene regulation; Hepatocellular carcinoma; Cell signaling pathway

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As a small single-stranded RNA molecule with a length less than 200nts in non-coding RNA, miRNA can act on target mRNAs based on the principle of sequence complementarity to control gene expression, play a role in gene regulation, and play a non-negligible role in transcription and translation.

From the neglect of miRNAs when they were first discovered to the widespread attention now, miRNAs have attracted much attention in the field of gene regulation and molecular targeted therapy in life science research in just 20 years, and continue to attract the attention of the scientific community. The research on HCC mainly focuses on the levels of cells, signaling pathways and genes. In recent years, the research on cell signaling pathways has become a hot topic. Current studies have shown that Notch signaling pathway, VEGF signaling pathway, Hedgehog signaling pathway, Wnt/ $\beta$ -catenin signaling pathway, PI3K/Akt signaling pathway, Hippo signaling pathway and so on are the major signaling pathways related to HCC occurrence. This paper in recent years were reviewed and the Wnt/ $\beta$ -catenin, PI3K/AKT, Hippo signaling pathways relevant miRNA research, and the function of miRNA and its molecular mechanisms are summarized and expounded the miRNA value in the diagnosis and treatment of HCC, To explore how miRNA plays a role in Wnt/ $\beta$ -catenin, PI3K/Akt and Hippo signaling pathways to regulate the occurrence of HCC, so as to provide help for the diagnosis, treatment and prognosis evaluation of HCC in clinical practice.

## 1 Overview of miRNA function

MiRNA is a class of small non-coding single-stranded RNA molecules existing in organisms. Its main function is to recognize the 3' untranslated regions (3' -UTR) at the 3' end of target mRNA, and its specific binding affects target mRNA expression through the following two mechanisms: one is

to cause target mRNA degradation, the other is translation inhibition<sup>[1]</sup>. Studies have pointed out that miRNA inhibits the expression of target genes and plays an important role in the regulation of a series of life activities, such as proliferation, differentiation and apoptosis in the process of cell development, especially when cancer occurs<sup>[2]</sup>. As an important regulatory factor involved in the occurrence of HCC, miRNA can not only regulate the expression of oncogenes, but also interact with tumor inhibitors and play different feedback roles in the process of promoting and suppressing cancer.

## 2 Different signaling pathways are associated with miRNAs

### 2.1 Wnt/ $\beta$ -catenin signaling pathway-related miRNAs

Wnt/ $\beta$ -catenin signaling pathway is one of the intracellular signal transduction pathways involved in the regulation of tumorigenesis, which is particularly critical in the occurrence of HCC. The Wnt/ $\beta$ -catenin signaling pathway is mainly composed of Wnt proteins, transmembrane receptors, cytoplasmic proteins, nuclear transcription factors, downstream target genes, etc.

Wnt1 is a key upstream molecule of the Wnt/ $\beta$ -catenin signaling pathway. When the Wnt1 protein molecules bind to the Frizzled receptor proteins, the Wnt1 pathway is activated, causing a complex cascade reaction. Wnt1 protein expression was found to be increased in HCC tissues and cell lines, and Wnt1 protein antibody can reduce the proliferation and viability of HCC cell lines HUH7 and Hep40, induce tumor cell apoptosis, inhibit the classical Wnt signaling pathway, but have no effect on the growth of normal liver cells, indicating that Wnt1 is closely related to the occurrence of HCC<sup>[3]</sup>. Studies have shown that miR-122 can target Wnt1, down-regulate Wnt1 expression, affect Wnt/ $\beta$ -catenin pathway signal transduction, and regulate the occurrence of liver cancer<sup>[4]</sup>.

Frizzled is one of the important transmembrane receptor proteins of the Wnt/ $\beta$ -catenin signaling pathway. It mainly combines with Wnt proteins and activates the Wnt/ $\beta$ -catenin signaling pathway. MiR-1324 directly targets FZD5 mRNA to inhibit the activation of Wnt/ $\beta$ -catenin signaling pathway in liver cancer<sup>[5]</sup>.

GSK-3 $\beta$  is an important member of the Wnt /  $\beta$ -catenin signaling pathway, which can degrade  $\beta$ -catenin by phosphorylation at Ser33, Ser37 and Thr41. In vitro experiments have confirmed that miR-1246 and miR-26a-5p can directly bind to GSK-3 $\beta$ mRNA, inhibit its post-transcriptional translation, and thus promote the activation of  $\beta$ -catenin pathway, was negatively correlated with the clinical stage and prognosis of the tumor<sup>[6]</sup>. MiR-500a can directly bind GSK-3 $\beta$  mRNA and SFRp2's 3'-UTR, and at the same time enhance the binding of Wnt proteins to the Frizzled receptor, reduce the degradation of  $\beta$ -catenin, and enhance the activation of downstream targets of the Wnt pathway<sup>[7]</sup>.

By tumor antigen MA1 (PNMA1) is the control factor of the  $\beta$ -catenin, can activate the Wnt/ $\beta$ -catenin signaling pathway, promote the occurrence of HCC cells EMT, compared to normal tissue, PNMA1 expression in HCC, and rise in PNMA1 with HCC Edmondson-Steiner grading and closely related BCLC staging, and poor prognosis in patients with HCC, miR-a-5p33 is the transcription regulatory factors, involved in the upstream of PNMA1 regulation, raise miR-a-5p33 expression, at the same time, down-regulation of PNMA1 expression can be used as a new method of HCC gene therapy<sup>[8]</sup>.

Overexpression of miR-186 can inhibit the accumulation of  $\beta$ -catenin and block the activation of Wnt/ $\beta$ -catenin signal in HCC cells, It plays an important role in regulating the proliferation, migration and invasion of hepatoma cells, MCRS1 is a new target of miR-186, MCRS1 mandatory expression can inhibit the miR-186 regulation role in cell growth and metastasis of liver cancer, and prevent miR-186 for Wnt/ $\beta$ -catenin signal blocking effect, thus miR-186 / MCRS1 may become the new therapeutic targets for HCC<sup>[9]</sup>.

### 2.2 PI3K/AKT signaling pathway-related miRNAs

The PI3K/Akt signaling pathway is mainly composed of phosphatidylinositide 3-kinases (PI3K/Akt) protein kinase B(PKB, Akt). As one of the many mechanisms regulating cell cycle and apoptosis, the PI3K/Akt signaling pathway is malregulated by one component of the pathway, which may cause the occurrence of tumors<sup>[10]</sup>.

Insulin-like growth factor 1 (IGF-1R) receptor is a key upstream molecule, which can promote carcinogenesis of hepatocytes through activation

of PI3K/Akt signaling pathway, and IGF-1R overexpression is closely related to the occurrence, development and metastasis of HCC<sup>[11]</sup>. Restoration of miR-133a expression in HepG2 cells can inhibit cell proliferation, metastasis and invasion. Bioinformatics analysis and luciferase detection proved that IGF-1 was a direct target, and overexpression of miR-133a inhibited the activation of Akt and inhibited the growth of tumor cells<sup>[12]</sup>. DLEU1 can act as an oncogenic lncRNA, regulate IGF-1R and PI3K/Akt pathway by regulating miR-133a expression in HCC, and knock out DLEU1 can inhibit the progression of HCC by regulating the miR-133a/IGF-1R axis<sup>[13]</sup>. MiR-342-3p inhibited the proliferation of HCC cells by directly targeting IGF-1R, thereby inhibiting the PI3K/Akt/GLUT1 axis mediated glycolysis<sup>[14]</sup>. MiR-944 also targets the expression of IGF-1R and the activation of PI3K/Akt pathway, and inhibits the proliferation of liver cancer cells<sup>[15]</sup>.

Mammals target of rapamycin (mTOR), a target molecule of rapamycin, belongs to PI3K related kinase family, and can regulate cell growth, proliferation and cell cycle. MiR-511 was significantly down-regulated in HCC cells, and the overexpressed miR-511 could inhibit the phosphorylation of Akt / mTOR, thus inhibiting the growth of HCC cells<sup>[16]</sup>. MiR-100 can promote autophagy in liver cancer cells by reducing mTOR and its receptor levels<sup>[17]</sup>.

By targeting protein tyrosine phosphatase gene (PTEN), miR-155-5p activates PI3K/Akt pathway and promotes the proliferation, metastasis and invasion of HCC cells, proving that miR-155-5p plays a crucial role in the progression of HCC and may become a new target for the diagnosis or treatment of HCC<sup>[18]</sup>. MiR-494-3p also promotes the metastasis of HCC cells by directly inhibiting the expression of PTEN, providing a new target for HCC gene targeted therapy. Moreover, miR-494-3p is highly expressed in HCC tissues, and may also be used as a biomarker to evaluate the prognosis of HCC patients<sup>[19]</sup>.

MiR-1296 can directly target arginine-serine protein kinase 1 (SRPK1), reduce the expression of SRPK1, affect the activation of PI3K/Akt signaling pathway, inhibit the migration, invasion and EMT process of HCC cells, and the combination of miR-1296 and SRPK1 expression level can be used as a potential indicator for predicting the prognosis of HCC patients<sup>[20]</sup>.

The expression of macrophage colony stimulating factor (MCSF) in liver cancer was determined by enzyme-linked immunosorbent assay, and using cell migration analysis to explore the liver cancer cells of macrophage, the influence of was studied by immunohistochemical miR-26a and MCSF expression, the relationship between the find miR-26a can pass a targeted the expression of PI3K/AKT signal pathway cut MCSF which affects the liver cancer cell autophagy<sup>[21]</sup>.

### 2.3 Hippo signaling pathway-related miRNAs

Hippo signal transduction is a major tumor suppressive pathway discovered in recent years, and plays a key role in inhibiting the proliferation, development and carcinogenesis of HCC<sup>[22]</sup>. Hippo LATS kinase signaling pathways first activated cells, then inhibit YAP transcriptional activity, achieve YAP phosphorylation<sup>[23]</sup>, phosphorylation of the nuclear effect of YAP is the Hippo pathway, it with Src homology phosphoric acid tyrosine phosphatase 2 (SHP2) interaction, which can control cell proliferation and survival, the effect on each other and further activate the Hippo signaling pathways downstream of small molecules, to play a role of tumorigenic together, regulation of cell proliferation, differentiation, inducing liver cells arise<sup>[24]</sup>.

As a core factor of Hippo signaling pathway, LATS1 plays a key role in regulating the expression of downstream oncogene YAP, and lowering the expression of LATS1 can inhibit Hippo signaling<sup>[25]</sup>. MiR-29C-3p plays a tumor suppressor role in HCC, inhibiting the expression of DNMT3b and promoting the demethylation of LATS1, thereby activating the Hippo signaling pathway to inhibit the development of HCC<sup>[26]</sup>. GPC3, the target protein of miR-520c-3p, has a regulatory effect on YAP, and Hoxa-As2 can promote the migration and invasion of HCC cells, and overexpression of miR-520c-3p can reduce the oncogenic effect of Hoxa antisense RNA2 (Hoxa-As2) in HCC cells, and inhibit the proliferation, invasion and migration of HCC cells<sup>[27]</sup>.

As an oncogenic gene, overexpression of miR-665 can promote the proliferation, migration and invasion of HCC, and protein tyrosine phosphatase receptor type B (PTPRB) is the direct target of miRNA-665, miRNA-665 specifically binding with PTPRB activates Hippo signaling pathway, plays a

role in carcinogenesis, and inhibits the expression of miR-665, which may become a potential therapeutic method for HCC<sup>[28]</sup>.

### 3 Summary and outlook

MiRNA acts on Wnt/ $\beta$ -catenin, PI3K/Akt, Hippo and other cell signaling pathways through various ways, and is involved in the regulation of the occurrence, metastasis and invasion of HCC, providing a strong theoretical basis for the clinical promotion of effective treatment of HCC, and providing a new reference for improving the efficiency of chemotherapy drug therapy and improving the prognosis of HCC patients. With the further improvement of subsequent studies and the further promotion of miRNA detection, it is of unpredictable value to establish a complete HCC gene database and determine the clinicopathological classification of HCC, so as to provide personalized treatment plans for HCC patients and carry out reasonable prognosis assessment. According to the current studies, detection of the expression of related miRNA in serum can be used as a new method for clinical diagnosis of HCC in the future, and miRNA modulator combined with chemotherapy drugs can be used as the most potential regimen for the treatment of HCC.

### References

- [1] Bartel D P. MicroRNAs: genomics, biogenesis, mechanism, and function [J]. *Cell*, 2004, 116(2): 281-297.
- [2] Chen B, Li H, Zeng X, *et al.* Roles of microRNA on cancer cell metabolism [J]. *J Transl Med*, 2012, 10: 228.
- [3] Wei W, Mei-Sze C, Susan G, *et al.* Blockade of Wnt-1 signaling leads to anti-tumor effects in hepatocellular carcinoma cells [J]. *Wei Wei; Mei-Sze Chua; Susan Grepper; Samuel K So*, 2009, 8(1).
- [4] Ahsani Z, Mohammadi-Yeganeh S, Kia V, *et al.* WNT1 gene from WNT signaling pathway is a direct target of miR-122 in hepatocellular carcinoma [J]. *Appl Biochem Biotechnol*, 2017, 181(3): 884-897.
- [5] Zhu Q, Lu G, Luo Z, *et al.* CircRNA circ\_0067934 promotes tumor growth and metastasis in hepatocellular carcinoma through regulation of miR-1324/FZD5/Wnt/ $\beta$ -catenin axis [J]. *Biochemical and Biophysical Research Communications*, 2018, 497(2).
- [6] Yarui L, Dan G, Yan Z, *et al.* Long non-coding RNA SNHG5 promotes human hepatocellular carcinoma progression by regulating miR-26a-5p/GSK3 $\beta$  signal pathway [J]. *Cell Death & Disease*, 2018,9(Database issue).
- [7] Guo Y, Chen L, Sun C, *et al.* MicroRNA-500a promotes migration and invasion in hepatocellular carcinoma by activating the Wnt/ $\beta$ -catenin signaling pathway [J]. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie*, 2017,91.
- [8] Liu P, Chen B, Gu Y, *et al.* PNMA1, regulated by miR-33a-5p, promotes proliferation and EMT in hepatocellular carcinoma by activating the Wnt/beta-catenin pathway [J]. *Biomed Pharmacother*, 2018, 108: 492-499.
- [9] Wang H, Ou J, Jian Z, *et al.* miR-186 modulates hepatocellular carcinoma cell proliferation and mobility via targeting MCRS1-mediated Wnt/beta-catenin signaling [J]. *J Cell Physiol*, 2019, 234(12): 23135-23145.
- [10] Paul A H, Colleen L M, Ramachandran E, *et al.* Allosteric modulation of Ras and the PI3K/AKT/mTOR pathway: Emerging Therapeutic Opportunities [J]. *Frontiers in Physiology*, 2014, 5.
- [11] Yan X D, Yao M, Wang L, *et al.* Overexpression of insulin-like growth factor-I receptor as a pertinent biomarker for hepatocytes malignant transformation[J]. *World J Gastroenterol*, 2013, 19(36): 6084-6092.
- [12] Wei Z, Kai L, Songyang L, *et al.* MicroRNA-133a functions as a tumor suppressor by targeting IGF-1R in hepatocellular carcinoma [J]. *Tumor Biology*, 2015, 36(12).
- [13] Zhang W, Liu S, Liu K, *et al.* Long non-coding RNA deleted in lymphocytic leukaemia 1 promotes hepatocellular carcinoma progression by sponging miR-133a to regulate IGF-1R expression [J]. *J Cell Mol Med*, 2019, 23(8): 5154-5164.
- [14] Liu W, Kang L, Han J, *et al.* miR-342-3p suppresses hepatocellular carcinoma proliferation through inhibition of IGF-1R-mediated Warburg effect [J]. *Onco Targets Ther*, 2018, 11: 1643-1653.
- [15] Lv L, Wang X, Ma T. microRNA-944 inhibits the malignancy of hepatocellular carcinoma by directly targeting IGF-1R and deactivating the PI3K/Akt signaling pathway[J]. *Cancer Manag Res*, 2019, 11: 2531-2543.
- [16] Cao G, Dong W, Meng X, *et al.* MiR-511 inhibits growth and metastasis of human hepatocellular carcinoma cells by targeting PIK3R3 [J]. *Tumour Biology : The Journal of the International Society for Oncodevelopmental Biology and Medicine*, 2015, 36(6).
- [17] Ge Y, Shi Q, Zheng Z, *et al.* MicroRNA-100 promotes the autophagy of hepatocellular carcinoma cells by inhibiting the expression of mTOR and IGF-1R [J]. *Oncotarget*, 2014, 5(15).
- [18] Fu X, Wen H, Jing L, *et al.* MicroRNA-155-5p promotes hepatocellular carcinoma progression by suppressing PTEN

- through the PI3K/Akt pathway[J]. *Cancer Sci*, 2017, 108(4): 620-631.
- [19] Lin H, Huang Z P, Liu J, *et al.* MiR-494-3p promotes PI3K/AKT pathway hyperactivation and human hepatocellular carcinoma progression by targeting PTEN [J]. *Sci Rep*, 2018, 8(1): 10461.
- [20] Xu Q, Liu X, Liu Z, *et al.* MicroRNA-1296 inhibits metastasis and epithelial-mesenchymal transition of hepatocellular carcinoma by targeting SRPK1-mediated PI3K/AKT pathway [J]. *Mol Cancer*, 2017, 16(1): 103.
- [21] Chai Z, Zhu X, Ao J, *et al.* microRNA-26a suppresses recruitment of macrophages by down-regulating macrophage colony-stimulating factor expression through the PI3K/Akt pathway in hepatocellular carcinoma [J]. *Journal of Hematology & Oncology*, 2015,8.
- [22] Kim W, Khan S K, Liu Y, *et al.* Hepatic Hippo signaling inhibits protumoural microenvironment to suppress hepatocellular carcinoma [J]. *Gut*, 2018, 67(9): 1692-1703.
- [23] Tang C, Takahashi-Kanemitsu A, Kikuchi I, *et al.* TRANSCRIPTIONAL co-activator functions of YAP and TAZ are inversely regulated by tyrosine phosphorylation status of parafibromin [J]. *iScience*, 2018, 1: 1-15.
- [24] Kim M K, Park J Y, Kang Y N. Tumorigenic role of YAP in hepatocellular carcinogenesis is involved in SHP2 whose function is different in vitro and in vivo [J]. *Pathol Res Pract*, 2018, 214(7): 1031-1039.
- [25] Li L, Dong L, Wang Y, *et al.* Lats1/2-mediated alteration of hippo signaling pathway regulates the fate of bone marrow-derived mesenchymal stem cells [J]. *Biomed Res Int*, 2018, 2018: 4387932.
- [26] Wu H, Zhang W, Wu Z, *et al.* miR-29c-3p regulates DNMT3B and LATS1 methylation to inhibit tumor progression in hepatocellular carcinoma [J]. *Cell Death Dis*, 2019, 10(2): 48.
- [27] Zhang Y, Xu J, Zhang S, *et al.* HOXA-AS2 promotes proliferation and induces epithelial-mesenchymal transition via the mir-520c-3p/gpc3 axis in hepatocellular carcinoma [J]. *Cell Physiol Biochem*, 2018, 50(6): 2124-2138.
- [28] Hu Y, Yang C, Yang S, *et al.* miR-665 promotes hepatocellular carcinoma cell migration, invasion, and proliferation by decreasing hippo signaling through targeting PTPRB[J]. *Cell Death & Disease*, 2018, 9(10).