Prevention and Treatment of Hepatocellular Carcinoma Using miRNAs

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Abstract
Hepatocellular carcinoma (HCC) is the second leading cause of death due to cancer. Liver transplantation, surgical liver resection, chemotherapy, and radiotherapy are the main options for the treatment of HCC. However, these methods are unable to limit the growth, survival, and metastasis of HCC cells. Several signaling pathways control propagation, metastasis, and recurrence of HCC. Recent studies have established new approaches for the prevention and treatment of HCC using miRNA technology. MicroRNAs are a class of non-coding RNAs with an average of 22 nucleotides that play critical roles in controlling gene expression in a variety of biological processes. miRNAs can induce or suppress HCC proliferation, migration, metastasis, and tumorigenesis. The anticancer effects of molecular agents can be evaluated directly in animal models or indirectly through the injection of HCC cell lines treated with anti-cancer agents. Targeting cancer-specific signaling pathways with miRNAs can be novel therapeutic strategies against HCC. This study provides the latest findings on using miRNAs in the control of HCC in both in vitro and in vivo models.

Keywords: Cancer, Hepatocellular carcinoma, miRNA, Signaling pathways


Introduction
Hepatocellular carcinoma (HCC) is the second leading cause of cancer-related death around the world.1-4 Non-viral (alcohol consumption and non-alcoholic fatty liver)5-7 and viral (hepatitis B/C virus) risk factors8,9 enhance the risk of HCC.10-12 There are three main options, including liver transplantation,13-15 surgical liver resection,16-18 and non-surgical methods (chemotherapy and radiotherapy) for the treatment of HCC.19-21 However, these approaches are unable to limit the progression and metastasis of HCC cells and cause side effects on the surrounding healthy cells.22,23 Several signaling pathways, including Wnt, Notch, EGF, SHH, hippo, and BMPs are associated with cell-division, metastasis, epithelial to mesenchymal transition (EMT), migration, and tumorigenesis of HCC.24-27 Targeting these signaling pathways may promote the treatment of the disease.28-30 Recent studies have established new approaches for the prevention and treatment of HCC using miRNA technology.31-33 microRNAs are a branch of RNA interference (RNAi) technology that contain about 20 nucleotides and target the specific mRNA in the cells.34,35 Evidence from miRNA expression profiles shows that some miRNAs are upregulated in HCC (oncomiR) and enhance the acquisition of metastatic potential.36,37 miRNAs can inhibit the expression of specific proteins (ligand or secondary messenger) in tumor-promoting signaling pathways and enhance HCC treatment efficacy.38,39 Molecularly targeted therapies using miRNAs with a high degree of specificity may be a suitable strategy in cancer treatment.40,41 This study provides the latest findings on using miRNAs in the control of HCC in both in vitro and in vivo models.

The Canonical miRNA Biogenesis
MicroRNAs are a class of non-coding RNAs with an average of 22 nucleotides that play an important role in controlling gene expression.42 miRNAs by microRNA-binding sites in the 3’ UTR of the target mRNAs trigger mRNA degradation to control the rate of translation.43 miRNAs can bind with the 5’ UTR, coding sequences, and gene promoters44 to regulate the expression of target genes or suppress translation by one of two distinct mechanisms.44 Pri-miRNAs or primary miRNAs are produced by the RNase II or III (pol III) in the nucleus.35,46 Subsequently, pri-miRNA with the Drosha/DGCR8 holoenzyme undergoes nuclear cleavage to produce a hairpin structured precursor or the precursor miRNA (pre-miRNA) with ~60-70 nt.47 Exportin-5 (Exp5) and Ran-GTP can transport pre-miRNAs to the cytoplasm.48 Dicer is an RNase III endonuclease that combined with the transactivating response RNA-binding protein (TRBP) cleaves pre-miRNA hairpin to form a mature microRNA duplex (~22 nt).49-51 Finally, miRNA binds with the AGO protein (RNA-induced silencing
Targeting Signaling Pathways in HCC with miRNAs

Several previous studies have provided evidence that miRNA can suppress HCC metastasis\(^\text{54,55}\) (Table 1). miRNAs have been shown to control several signaling pathways, including Wnt, Notch, FGF, SHH, and hippo, and suppress the tumorigenesis of HCC (Figure 1).

It has been shown that overexpression of miR-34, miR-200, miR-133, and miR-663a inhibits the activation of the TGF-β ligand.\(^\text{56,57,59}\) miR-122 and miR-3194-3p have been found to suppress the Wnt/β-catenin pathway in HCC.\(^\text{56,58}\)

### Table 1. Effects of miRNA on Signaling Pathways Related to HCC

<table>
<thead>
<tr>
<th>Pathway</th>
<th>miRNA</th>
<th>Target</th>
<th>HCC cell line</th>
<th>Animal model</th>
<th>Result</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGF-B</td>
<td>miR-200</td>
<td>Ligand</td>
<td>MHCC-97 H, SMMC-7721, HepG2, Huh7</td>
<td>-</td>
<td>Inhibit HCC proliferation, EMT, and invasion</td>
<td>56</td>
</tr>
<tr>
<td></td>
<td>miR-663</td>
<td>Ligand</td>
<td>SK-Hep1, Huh7 and other HCC line</td>
<td>-</td>
<td>Inhibit the tumor growth and invasion</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>miR-133</td>
<td>Ligand</td>
<td>Huh7, HepG2, Hep3B</td>
<td>-</td>
<td>Decrease the HCC proliferation</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>miR-298</td>
<td>B-catenin</td>
<td>MHCC-97 H, HCCLM3</td>
<td>MHCC-97H subcutaneously to flank of nude mice</td>
<td>Decrease proliferation, migration, increase apoptosis, decrease tumor growth</td>
<td>59</td>
</tr>
<tr>
<td>Wnt</td>
<td>miR-504</td>
<td>FZD receptor</td>
<td>HepG2, Huh7</td>
<td>-</td>
<td>Decrease the HCC proliferation and metastasis</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>miR-122</td>
<td>Pathway</td>
<td>SMCC7721, Bel-7402</td>
<td>5×10⁵ cells subcutaneously to flank of nude mice</td>
<td>Decrease the HCC proliferation, survival and tumor weight</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>miR-148b</td>
<td>Wnt1</td>
<td>HepG2</td>
<td>5 × 10⁵ HepG2 subcutaneously to flank of nude mice</td>
<td>Induce apoptosis and cell cycle arrest, inhibit tumor growth</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>miR-194-3p</td>
<td>BCL9</td>
<td>MHCC-97 H, Hep3B</td>
<td>5 × 10⁵ MHCC-97H or Hep3B to tail veins</td>
<td>Inhibit migration, invasion, and metastasis</td>
<td>62</td>
</tr>
<tr>
<td>Ssh</td>
<td>miR-138</td>
<td>Smo receptor</td>
<td>HepG2</td>
<td>1×10⁵ MHCC-97H to flank of nude mice then cut and transplant to left liver</td>
<td>Decrease colony formation and invasion, increase apoptosis</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td>miR-3163</td>
<td>NICD</td>
<td>MHCC-97 H, LM-3</td>
<td>MHCC97-H subcutaneously or intraportal of nude mice</td>
<td>Decrease the tumor growth</td>
<td>64</td>
</tr>
<tr>
<td>Notch</td>
<td>miR-206</td>
<td>NICD</td>
<td>HepG2</td>
<td>-</td>
<td>Cell cycle arrest, apoptosis, and inhibit the EMT</td>
<td>65</td>
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<tr>
<td>EGF</td>
<td>miR-874</td>
<td>ERK</td>
<td>SK-Hep1</td>
<td>overexpressed miR-874 SK-hep-1 to BALB/c nude mice</td>
<td>Inhibit proliferation and metastasis, decrease the tumor size</td>
<td>66</td>
</tr>
<tr>
<td>VEGF</td>
<td>miR-181a-5p</td>
<td>c-met</td>
<td>SNU, Mahlavu</td>
<td>-</td>
<td>Inhibit proliferation and metastasis</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>miR-126</td>
<td>VEGF/FGF</td>
<td>BEL-7402</td>
<td>-</td>
<td>Inhibit migration and invasion</td>
<td>67</td>
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<tr>
<td></td>
<td>miR-345</td>
<td>VEGF</td>
<td>HCCLM3, SMMC-7721, MHCC-97H</td>
<td>subcutaneously to flank of nude mice</td>
<td>Inhibit proliferation and tumor growth</td>
<td>67</td>
</tr>
<tr>
<td>Stat3</td>
<td>miR-30e</td>
<td>Jak</td>
<td>HepG2, Huh7</td>
<td>6×10⁵ HCCLM3 cells intravenously into nude mice</td>
<td>Inhibit the EMT and metastasis</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>miR-9-3p</td>
<td>TAZ</td>
<td>Huh1, HLF</td>
<td>-</td>
<td>Inhibit proliferation</td>
<td>68</td>
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<tr>
<td></td>
<td>miR-186</td>
<td>YAP</td>
<td>HepG2, Hep3B, SNU1858</td>
<td>-</td>
<td>Inhibit the migration and proliferation</td>
<td>68</td>
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<tr>
<td></td>
<td>miR-592</td>
<td>HIF-1α</td>
<td>SK-hep1, SMMC-7721</td>
<td>1.5 × 10⁴ SK-Hep1 subcutaneously to flank of SCID mouse</td>
<td>Inhibit proliferation tumor growth, and glycolysis</td>
<td>67</td>
</tr>
<tr>
<td>HIF-1α</td>
<td>miR-144</td>
<td>Cyclin B1</td>
<td>HepG2, SMMC-7721</td>
<td>5×10⁴ MHCC-97H subcutaneously to flanks of nude mouse</td>
<td>Decrease proliferation, migration, survival, and the tumor size</td>
<td>67</td>
</tr>
<tr>
<td>Cell cycle</td>
<td>miR-214-3p</td>
<td>Serine, threonine kinase</td>
<td>HepG2, Huh7</td>
<td>-</td>
<td>Decrease proliferation, increase the apoptosis</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>miR-300</td>
<td>B-Catenin</td>
<td>HepG2, Huh7</td>
<td>-</td>
<td>Decrease proliferation</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>miR-383</td>
<td>Stat3</td>
<td>HepG2, Huh7</td>
<td>DEN</td>
<td>Increase the apoptosis, decrease proliferation and tumor growth</td>
<td>69</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>miR-644a</td>
<td>Heat shock factor 1</td>
<td>HepG2, SMMC-7721</td>
<td>2 × 10⁵ SMMC-7721 subcutaneously to nude mice</td>
<td>Increase the apoptosis, decrease proliferation and inhibit tumor growth</td>
<td>69</td>
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<tr>
<td></td>
<td>miR-377</td>
<td>Bcl2</td>
<td>HepG2</td>
<td>-</td>
<td>Inhibit proliferation and apoptosis</td>
<td>69</td>
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<tr>
<td></td>
<td>miR-423-5p</td>
<td>ATG7</td>
<td>HepG2</td>
<td>-</td>
<td>Autophagy and cell cycle arrest</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>miR-100</td>
<td>mTOR</td>
<td>HepG2</td>
<td>5×10⁵ HepG2 subcutaneously to flank of BALB/c mice</td>
<td>Autophagy and apoptosis</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>miR-125b-5p</td>
<td>TXNRD</td>
<td>HepG2, SK-hep1</td>
<td>-</td>
<td>Decrease proliferation and migration</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>miR-124</td>
<td>SIRT1</td>
<td>HepG2</td>
<td>5×10⁵ HepG2 subcutaneously to armpit of nude mouse</td>
<td>Increase the apoptosis in combination with Cisplatin</td>
<td>69</td>
</tr>
</tbody>
</table>
Ectopic overexpression of miR-504 in HCC cells leads to blocking the FZD, while the overexpression of miR-298 and miR-148-b inhibits the activation of β-catenin. Overexpression of miR-138 and miR-338-3p was shown to suppress Smo in the SHH pathway. Interestingly, SHH inhibitors accompanied by radiotherapy enhanced the radiosensitivity of HCC. miR-3163 and miR-206 have been reported to suppress the notch 1 intracellular domain (NICD) transcriptional activation in the Notch pathway. It has been confirmed that miR-874 blocks the EGF/ERK pathway in HCC. miR-181a-5p as a selective c-MET inhibitor in the HGF pathway decreases HCC proliferation, migration, and tumor growth. miR-195 inhibits angiogenesis by targeting VEGF and FGF, while miR-126 decreases the expression of VEGF and EGF. miR-30e and miR-345 are able to target the Jak/Stat3 pathway. miR-592 leads to disruption of hypoxia-inducible factor-1α (HIF-1α), suppression of glycolysis and lactate production, and reduction of G6PD mRNA levels in HCC. Anti-proliferative miRNA are significantly downregulated in HCC cell lines. Overexpression of miR-214-3p was reported that reduced HCC progression, by binding to the 3’-UTR of maternal embryonic leucine zipper kinase expression. miR-144 and miR-300 by targeting cyclin B and β-catenin, respectively, could promote cell cycle arrest in HCC. miR-383 by targeting IL-17 can suppress the Stat3 function, and miR-377 represses Bcl-2, thereby increasing apoptosis and decreasing cellular proliferation in HCC. Several studies have shown that miR100 and miR-423-3p induce autophagy. miR-124 interacts with sirtuin 1 (SIRT1) protein to enhance the cytotoxic effects of cisplatin in the CSC subpopulation. Taken together, targeting cancer-specific signaling pathways using miRNAs may be novel therapeutic strategies against HCC. In conclusion, several important signaling pathways are misregulated in HCC compared to the normal hepatocytes. These pathways can trigger EMT, metastasis, migration, and tumorigenesis. Hence, suppression of the critical pathways with miRNAs causes cell cycle arrest, apoptosis, inhibits the tumorigenesis of HCC, and facilitates the sensitivity of HCC cells to drugs. Therefore, miRNAs may be a valuable approach to HCC treatment.

Availability of data and materials
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Statement
Not applicable.

Conflict of Interest Disclosures
The authors declare no conflict of interest.

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References


miRNAs against Hepatocellular Carcinoma


