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Editorial
Role of ncRNAs in Hepatocellular Carcinoma

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Hepatocellular carcinoma (HCC) is one of the most common malignant tumors worldwide and a major cause of cancer-related mortality in China. Most cases of HCC are diagnosed in the advanced stage. Until now, Sorafenib and Regorafenib, two FDA approved drugs, only can prolong 2-3-month survival for unresectable HCC patients. So the advance on molecular mechanisms under HCC progress might sled on its diagnosis and treatment. Increasing studies figure out that noncoding RNAs (ncRNAs), a class of RNAs with no protein-coding function that are widely expressed in tumor, play an important role in tumor progression including HCC. This special issue provided a platform to introduce and disclose the role of ncRNAs in HCC. At last, five articles, including three review articles and two research articles, were accepted for publication. Here, three guest editors summarily introduced these published articles in this editorial.

J. Yang et al. explored the role of miR-219-5p in tumor growth and metastasis of human HCC. They revealed that elevated expression of miR-219-5p was positively correlated with poor prognostic features including liver cirrhosis, vascular invasion, and poor differentiation. Moreover, miR-219-5p upregulation was recognized as an independent predictor for dismal prognosis of HCC patients. Functionally, miR-219-5p contributed to cell proliferation and invasion of HCC cells in vitro and in vivo by targeting cadherin 1 (CDH1). The regulatory mechanism underlying aberrant expression of miR-219-5p and the novel targets involved in the oncogenic role of miR-219-5p in HCC should be further investigated.

Accumulating studies verify that liver cancer stem cells (CSCs) account for tumor initiation, metastasis, recurrence, and chemoresistance. J. Zhao et al. summarized the recent literatures regarding the diverse regulatory role of two main classes of ncRNAs, miRNAs, and IncRNAs, in CSC maintenance. They reviewed miRNAs and IncRNAs regulation of signaling pathways, stemness-related transcriptional factors and markers, tumor-associated genes, etc. in the maintenance of liver CSCs. The critical roles of miRNAs and IncRNAs in the regulation of liver CSCs will provide new idea to develop therapeutic strategies for liver cancer by targeting ncRNAs.

H. Zhou et al. reported a review on recent knowledges about the ncRNAs in the development of HCC. Notably, abnormally expressed microRNAs in tissues and serum of patients may serve as prognostic or diagnostic biomarkers of HCC. Furthermore, ncRNAs may be involved in a variety of pathological processes such as cell proliferation, apoptosis, angiogenesis, invasion, and metastasis. In addition to IncRNA and miRNA, other ncRNAs, including circRNA and snoRNA, also may have significant influence on the development and progression of HCC.

To identify ncRNAs involved in HCC, T. Falcon et al. analyzed total RNA from 41 pairs of primary solid tumor and adjacent tissue samples from The Cancer Genome Atlas (TCGA) using the function GDCdownload with the option legacy = FALSE. They found that there are 234 miRNAs, 92 pre-miRNAs, and 122 IncRNAs differential expression between tumor and normal samples. Then the authors explored the pathways that differentiate both groups and the regulatory ncRNAs and their putative targets. They found that the most represented pathways in differentially expressed transcripts are involved in bile metabolism, fear behavioral response, and immune-related categories. A set of IncRNAs includes FAM170B-AS1 and TTNAS1 that could be the potential targets of future studies in HCC. These two papers indicated that ncRNAs may be promising prognostic
biomarkers of HCC and therapeutic targeting of ncRNAs may be a potential strategy for preventing progression in patients with HCC.

X. Hu et al. gave a system landscape on the role of LncRNAs in HCC development based on basic research and clinic study, including various tumor suppressor and oncogenic LncRNA, the signal pathway involved in LncRNA deregulation, and function in HCC development. Meanwhile, they also discussed the potential role of LncRNA for HCC diagnosis, recurrence, and outcome prediction and target therapy. Based on the review on LncRNA and HCC, it will help us to expand our current research horizon, to promote the diagnosis and treatment study on HCC in further.

Kangsheng Tu
Qiongzhu Dong
Junyan Tao
Prospects of Noncoding RNAs in Hepatocellular Carcinoma

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Hepatocellular carcinoma (HCC) is a global health problem and one of the most common malignant tumors. Recent studies have shown that noncoding RNAs (ncRNAs) contribute to the pathogenesis of hepatocellular carcinoma (HCC). These RNAs may be involved in a variety of pathological processes such as cell proliferation, apoptosis, angiogenesis, invasion, and metastasis. In addition, abnormal expression of ncRNAs in HCC may provide potential prognostic or diagnostic biomarkers. This review provides an overview of the role and potential applications of ncRNAs, miRNAs, lncRNAs, circRNAs, and snoRNAs in liver cancer.

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors and a global health problem [1]. Like many other cancers, HCC is characterized by the involvement of multiple gene networks and the imbalance of signaling pathways [2, 3]. These genetic dysregulations involve protein-coding genes and noncoding RNA (ncRNA) genes [4]. Although the former has been the focus of research, the latter has only recently been recognized as playing a role in the pathological processes implicated in HCC [5]. Interestingly, the vast majority of the human genome is transcribed into ncRNA, while less than 2% of the genome directly encodes for proteins [6]. Noncoding RNA is a functional RNA that is not translated into protein [7].

NcRNAs include ribosomal RNA (rRNA), transfer RNA (tRNA), and small nuclear ribonucleic acids (snRNA) that process pre-mRNA, small nucleolar RNA (snoRNA), piwi-interacting RNA (piRNA), microRNA (miRNA), long non-coding RNA (lncRNA), etc. [7]. The common characteristic of these RNA is their ability to exercise their biological functions at the level [8]. Prior work has generally focused on protein-coding genes, with little focus on ncRNA function, often dismissed as nothing more than transcriptional noise [9]. However, recent studies have discovered that ncRNAs play an important role in many biological processes. Research in the field of ncRNA has shown an explosive growth in recent years since their functional role has been recognized [10–12].

With the development of high-throughput sequencing technology, many ncRNAs have been characterized as functional molecules that play an important role in various biological processes and pathological states [13–15]. In the field of hepatocellular carcinoma, some key ncRNAs have been identified as participants in the pathophysiology of the
Table 1: The function of abnormal expression miRNAs in HCC and in the serum of HCC patients.

<table>
<thead>
<tr>
<th>miRNAs</th>
<th>Target gene</th>
<th>Function</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-224</td>
<td>HOXD10</td>
<td>Promoting the invasion and metastasis of cancer cells</td>
<td>[43]</td>
</tr>
<tr>
<td>miR-224</td>
<td>SMAD4</td>
<td>Promoting cell proliferation</td>
<td>[44]</td>
</tr>
<tr>
<td>miR-224</td>
<td>ppp2r1b</td>
<td>Increasing the risk of liver cancer</td>
<td>[45]</td>
</tr>
<tr>
<td>miR-224</td>
<td>p27 and p57</td>
<td>Promoting the progression of hepatocellular carcinoma cell cycle</td>
<td>[46, 47]</td>
</tr>
<tr>
<td>miR-221</td>
<td>DDIT4</td>
<td>Promoting tumor development</td>
<td>[48]</td>
</tr>
<tr>
<td>miR-221</td>
<td>SOCS1 and SOCS3</td>
<td>Enhancing the effectiveness of interferon against HCV</td>
<td>[50]</td>
</tr>
<tr>
<td>miR-21</td>
<td>map2k3</td>
<td>Promoting the proliferation of cancer cells</td>
<td>[51]</td>
</tr>
<tr>
<td>miR-21</td>
<td>PDCD4</td>
<td>Increased the risk of liver cancer invasion and metastasis</td>
<td>[52]</td>
</tr>
</tbody>
</table>

Disease [16, 17]. Thousands of universally transcribed ncRNAs have been identified, and these transcripts greatly outnumber those of protein-coding mRNAs [18]. In addition, some ncRNAs show significant evolutionary conservation, indirectly supporting their functional roles [19, 20]. For example, miRNA and IncRNAs regulate different biological and pathological processes, such as tumor occurrence [21–24].

2. Abnormal Expression miRNAs in HCCs and Serum of Patients with Hepatocellular Carcinoma

MicroRNA (miRNA) is a type of 20-24nt long biologically functional, small molecule that is highly conserved and provides negative regulation [25]. Since their discovery in 1993 in Caenorhabditis elegans, their important roles continue to be described [26, 27]. Their main functions are on the transcription of regulatory proteins encoded by gene expression [28–30]. Liver cancer is both common and lethal, mainly due to ineffective treatment options [31, 32]. MiRNAs regulate protein synthesis and could either be therapeutic agents or targets for intervention [33–35]. It is clear that miRNA plays a role in proliferation, persistence, invasion, metastasis, and prognostic indicators of hepatocellular carcinoma [36–39]. In liver cancer tissue, there are many abnormally expressed miRNAs, such as miR-224, miR-221, and miR-21 that influence hepatocellular carcinoma [40–42]. miR-224 has been shown to target HOXD10 RNA and enhance both PKA-mediated phosphorylation and MMP-9 to promote the invasion and metastasis of cancer cells [43]. MiR-224 can inhibit SMAD4 expression and promote cell proliferation [44]. Also, miR-224 can target ppp2r1b leading to excessive activation of the AKT signaling pathway, increasing the risk of liver cancer [45]. MiR-221 can target cell cycle kinase inhibitory proteins p27 and p57, thereby promoting the progression of HCC cell cycle [46, 47]. At the same time, miR-221 can interfere with the mTOR signaling pathway by inhibiting another target DDIT4, thus promoting tumor development [48]. MiR-221 expression levels can be raised by HCV infection, a process that relies on the activation of NF-kB signaling [49]. MiR-221 can also target SOCS1 and SOCS3, enhancing the activation of the downstream interferon signaling pathway, thus enhancing the effectiveness of interferon against HCV [50].

In HCC cells, miR-21 can target MAP2K3 and promote the proliferation of cancer cells [51]. In parallel, miR-21 can inhibit PDCD4, thus activating the expression of downstream c-Jun, MMP-2, and MMP-9. Furthermore, AP-1 can modulate the transcription of miR-21 in a positive feedback loop, further increasing the risk of liver cancer invasion and metastasis [52]. The role of abnormally expressed miRNAs in HCCs and in the serum of HCC patients is listed in Table 1. Clinical data has shown that miR-21 expression in tumors of HCC patients is relatively high [85]. The response to postoperative IFN-α/5-FU combined treatment is poor [86], suggesting that miR-21 may be a potential molecular marker for prognostic judgment and treatment of HCC. There are many abnormally expressed miRNAs in the serum of HCC patients. Among them, miR-16, let-7f, miR-21, miR-139, miR-122, and miR-1 show reduced expression [87–90]. High expression of mir-17-5p may be indicative of HBV (Hepatitis B virus) and the recurrence of HCC [90]. In HCV-infected sera from HCC patients, the specific low expression of mir-30c-5p, mir-223-3p, mir-302c-5p, and mir-17-5p, as well as the specific high expression of miR-221, also suggest potential biomarkers indicating the recurrence of HCC [91, 92].

3. Roles of IncRNAs in HCC

Ten of thousands of IncRNAs are transcribed in humans [93]. Importantly, detecting their targets is challenging in contrast to miRNA [94]. Indeed, IncRNAs often need to form complex secondary and tertiary structures to predict targets [95].

With the development of large scale parallel sequencing technology, IncRNA has been shown to play an important role in the development of human HCC [96]. So far, many HCC-related IncRNA disorders, such as HULC, HOTAIR, MALAT1, and H19, have been used as predictive biomarkers for human disease diagnosis or prognosis [97, 98].
Table 2: Signaling pathways in HCC regulated by lncRNAs.

<table>
<thead>
<tr>
<th>lncRNAs</th>
<th>Pathways</th>
<th>Functions</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>URHC</td>
<td>ERK/MAPK pathway</td>
<td>Regulates cell proliferation and apoptosis</td>
<td>[53]</td>
</tr>
<tr>
<td>HULC</td>
<td>RXRA pathway</td>
<td>Modulates abnormal lipid metabolism</td>
<td>[54]</td>
</tr>
<tr>
<td>LINC00152</td>
<td>mTOR pathway</td>
<td>Promotes proliferation in HCC</td>
<td>[55]</td>
</tr>
<tr>
<td>LINC01225</td>
<td>EGFR-dependent pathway</td>
<td>Promotes occurrence and metastasis of HCC</td>
<td>[56]</td>
</tr>
<tr>
<td>CCHE1</td>
<td>ERK/MAPK pathway</td>
<td>Promotes carcinogenesis of HCC</td>
<td>[57]</td>
</tr>
<tr>
<td>linc-cdh4-2</td>
<td>R-cadherin pathway</td>
<td>Inhibits the migration and invasion of HCC cells</td>
<td>[58]</td>
</tr>
<tr>
<td>lncARSR</td>
<td>PTEN-PI3K/Akt Pathway.</td>
<td>Promotes doxorubicin resistance in HCC</td>
<td>[59]</td>
</tr>
<tr>
<td>TSLNC8</td>
<td>Interleukin-6/STAT3 pathway</td>
<td>A tumor suppressor</td>
<td>[60]</td>
</tr>
<tr>
<td>PDIA3P1</td>
<td>p53 pathway</td>
<td>Promotes cell proliferation, migration and invasion, and suppresses apoptosis</td>
<td>[61]</td>
</tr>
<tr>
<td>lncRNA00673</td>
<td>Notch pathway</td>
<td>Promotes of proliferation and metastasis of HCC</td>
<td>[62]</td>
</tr>
<tr>
<td>Igf2as</td>
<td>ERK/MAPK pathway</td>
<td>Controls hepatocellular carcinoma progression</td>
<td>[63]</td>
</tr>
<tr>
<td>CCAL</td>
<td>Wnt/β-catenin pathway</td>
<td>Promotes HCC progression</td>
<td>[64]</td>
</tr>
<tr>
<td>LncDQ</td>
<td>EMT Pathway</td>
<td>Promotes HCC progression</td>
<td>[65]</td>
</tr>
<tr>
<td>Inc-Myd88</td>
<td>NF-κB and PI3K/AKT pathways</td>
<td>Promotes HCC progression</td>
<td>[66]</td>
</tr>
</tbody>
</table>

Furthermore, there is strong evidence that lncRNAs are associated with HCC via many signaling pathways. MyD88 levels were found to be elevated in multiple solid tumors, especially HCC [99]. Many ncRNAs, through a variety of mechanisms, regulate the location of binding sites of protein-coded genes. The abnormal increase in the expression of a new long noncoding Myd88 RNA (lnc-Myd88) is associated with HCC [66]. Chip analysis showed that lnc-Myd88 could increase Myd88 expression by enhancing the acetylation of H3K27 at the Myd88 gene promoter region, leading to the activation of NF-κB and PI3K/AKT signaling pathways [66]. Thus, lnc-Myd88 may be a new diagnostic and therapeutic target for HCC. Various examples of lncRNAs regulating signaling pathways in HCC are listed in Table 2. Hence, targeting these lncRNAs in combination with other therapeutic agents could have therapeutic potential for HCC.

In addition, a new concept suggests that lncRNAs could be used as a protein scaffold close together to form ribose nuclear proteins [100]. However, only a few scaffolding lncRNAs have been identified and the broad extent of this function is unknown. LncRNAs participate in a variety of biological processes and play an important role in various human diseases, including fibrosis diseases, liver diseases, and rare human diseases [101–104]. With the rise of RNA sequencing (RNA-Seq) technology, the number of lncRNAs identified has increased rapidly [105]. However, most lncRNAs have not been well annotated, and their regulatory mechanisms remain elusive. Furthermore, many lncRNAs are not evolutionarily conserved [106]. Therefore, it is vital to study the key function of the conserved lncRNAs.

### 4. circRNA and HCC

In addition to miRNA and lncRNA, other ncRNAs also influence the development of HCC [67, 107]. Circular RNAs (circRNAs) were discovered in mouse testes in 1993 [108] and represent another class of endogenous, noncoding RNA. In addition to being a biomarker for HCC, circRNA has recently been found to be an important gene expression and pathological network regulatory factor [109–111].

The interactions of hsa_circ_0005075-targeted miRNA genes, including miR-23b-5p, miR-93-3p, miR-581, miR-23a-5p, and their corresponding mRNA, have been studied [70]. One circRNA, Cdr1as, inhibits and absorbs microRNA-7 (miR-7), a suppressor of HCC [69]. CircMTO1 (hsa_circRNA_0007874/ hsa_circRNA_104135) suppresses HCC progression by absorbing oncogenic miR-9, thus promoting p21 expression [68]. Recently, circRNA_100338 was identified as a biomarker for HCC diagnosis and target for HCC therapeutics [67]. circRNAs function as microRNA sponges by targeting related genes shown in Figure 1 (modified from [108]) and Table 3. In addition, circ_0067934 directly inhibits miR-1324 that targets the 3′-UTR of FZD5 mRNA. Subsequently, the Wnt/β-catenin signaling pathway becomes...
Table 3: circRNAs function as microRNA sponges in HCC.

<table>
<thead>
<tr>
<th>circRNA</th>
<th>microRNA sponges</th>
<th>Cells</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>circRNA_10033</td>
<td>miR-141-3p, miR-9</td>
<td>HCC and MHCC97H</td>
<td>[67]</td>
</tr>
<tr>
<td>circMTO1</td>
<td>miR-7</td>
<td>HCC</td>
<td>[68]</td>
</tr>
<tr>
<td>CDR1as</td>
<td>miR-23b-3p, miR-93-3p, miR-581, miR-23a-3p</td>
<td>HCC cells, SMMC-7221, Hep3B, QGY-7703, and HepG2</td>
<td>[69]</td>
</tr>
<tr>
<td>hsa_circ_0005075</td>
<td>miR-1297</td>
<td>HCC cells, HepG2, and SNU449</td>
<td>[70]</td>
</tr>
<tr>
<td>hsa_circ_0001649</td>
<td></td>
<td>HCC cells, HepG2, and SMMC7721</td>
<td>[71]</td>
</tr>
</tbody>
</table>

Figure 1: circRNAs function as microRNA sponges by targeting related genes.

downregulated in HCC, which enhances the proliferation, migration, and invasion of HCC [112].

5. snoRNA and Their Host Genes in HCC

Small nucleolar RNAs (snoRNAs) are a novel molecular species that may have significant influence on the development and progression of HCC [78]. The expression of snord13-1 in HCC is significantly downregulated [72]. The reduction of snord13-1 in HCC was clearly associated with decreasing patient. In essence, snord13-1 functionally inhibits the growth of HCC cells [72]. Small nucleolar RNA host gene 20 (SNHG20) expression in sk-hep-1 cells significantly inhibited cell proliferation, migration, and invasion. This suggests that SNHG20 could be used as an independent prognostic predictor for HCC patients [74]. The potential roles of snoRNA or their host genes in liver cancer are listed in Table 4.

6. Conclusion

This review provides an overall view of ncRNA in hepatocellular carcinoma (HCC). In addition to the recent focus of research towards lncRNA and miRNA, other ncRNAs, including circRNA and snoRNA also influence liver cancer. Although the first miRNA was identified 20 years ago, other ncRNAs including lncRNAs, snoRNA, siRNA, and piRNAs were gradually discovered and proved to be important in the pathogenesis of cancer [113]. To fully grasp the complete picture of the role of ncRNAs in the pathogenesis of HCC,
Table 4: Potential role of snoRNA or their host genes in liver cancer.

<table>
<thead>
<tr>
<th>SnoRNA/their host genes</th>
<th>Potential role in liver cancer</th>
<th>Cells</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>SNORD1I3-1</td>
<td>A tumor suppressor role in HCC and a potential diagnostic and therapeutic target for HCC.</td>
<td>HepG2 and Huh7</td>
<td>[72]</td>
</tr>
<tr>
<td>SNHG3</td>
<td>Associated with malignant status and poor prognosis in HCC patients.</td>
<td>HCC</td>
<td>[73]</td>
</tr>
<tr>
<td>SNHG20</td>
<td>Up-regulated in patients with HCC, served as an independent prognostic predictor for HCC patients.</td>
<td>HCC and SK-Hep-1</td>
<td>[74, 75]</td>
</tr>
<tr>
<td>SNHG1</td>
<td>A prognostic biomarker and therapeutic target for HCC.</td>
<td>HCC and HepG2</td>
<td>[76, 77]</td>
</tr>
<tr>
<td>SNORD78</td>
<td>Associated with aggressive phenotype and poor prognosis of HCC</td>
<td>SK-Hep-1</td>
<td>[78]</td>
</tr>
<tr>
<td>SNHG6</td>
<td>Impacts HCC tumorigenesis by binding to up-frameshift protein 1 and regulating Smad7 expression.</td>
<td>HCC and L02</td>
<td>[79]</td>
</tr>
<tr>
<td>SNORD126</td>
<td>A therapeutic target</td>
<td>HepG2, LS174T and Huh7</td>
<td>[80]</td>
</tr>
<tr>
<td>SNHG12</td>
<td>A biomarker and a potential therapeutic target for HCC.</td>
<td>HCC</td>
<td>[81]</td>
</tr>
<tr>
<td>ACA11</td>
<td>A promising prognostic biomarker and therapeutic target for patients with HCC.</td>
<td>HCCLM9 and SK-Hepl</td>
<td>[82]</td>
</tr>
<tr>
<td>snoRA47</td>
<td>A valuable biomarker and a potential therapeutic target for HCC.</td>
<td>HCC</td>
<td>[83]</td>
</tr>
<tr>
<td>SNORD76</td>
<td>A promising prognostic biomarker in patients with HCC.</td>
<td>SK-Hepl and Huh7</td>
<td>[84]</td>
</tr>
</tbody>
</table>

it is paramount to explore the regulatory networks including circRNA-mRNA, miRNA-IncRNA, IncRNA/snoRNA-piRNA, and other networks that have not been found. To fully understand the biological functions of ncRNAs, we must ascertain the functions of all the proteins and ncRNAs of each cell type and the interactions among them. This full understanding is still a long way off, far more difficult than the genome project.

Conflicts of Interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

Authors’ Contributions

Huaixiang Zhou, Qiuran Xu, Chao Ni, and Xiaoge Hu contributed equally to this work.

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References


Review Article

A Systematic Review of Long Noncoding RNAs in Hepatocellular Carcinoma: Molecular Mechanism and Clinical Implications

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Hepatocellular carcinoma (HCC) has the second highest mortality rate worldwide among all cancers. Previous studies have revealed the significant involvement of long noncoding RNAs (lncRNAs) in numerous human cancers including HCC. Both oncogenic and tumor repressive lncRNAs have been identified and implicated in the complex process of hepatocarcinogenesis. They can be further explored as prospective diagnostic, prognostic, and therapeutic markers for HCC. An in-depth understanding of lncRNAs’ mechanism in HCC is therefore required to fully explore their potential role. In the current review, we will concentrate on the underlying function, molecular mechanisms, and potential clinical implications of lncRNA in HCC.

1. Introduction

Among all cancers, hepatocellular carcinoma (HCC) has the second highest mortality rate worldwide [1]. The risk factors, including HBV or HCV infection, alcoholism, liver cirrhosis, and metabolic diseases, contribute to HCC [2]. The molecular mechanism of hepatocarcinogenesis is highly complex and involves an interplay between dysregulated cell cycle, apoptosis, tumor cell invasion, and metastasis [2]. Despite advances in diagnosis and therapy, the incidence and mortality of liver cancer continue to increase [3]. It is vital therefore to illustrate the molecular mechanism of HCC in order to improve diagnosis, treatment, and overall prognosis.

With the development of human genome sequencing technology, about 20000 protein-coding genes have been identified, which account for less than 2% of the entire genome [4]. In fact, greater than 90% of the human DNA would be converted into noncoding RNAs (ncRNAs), which, despite not being translated into proteins, are involved in several cellular functions [5, 6]. The long ncRNAs (lncRNAs) with more than 200 nucleotides play significant roles in cell growth and differentiation, chromatin organization, and regulation of gene expression [7, 8]. lncRNAs are classified into intronic, intergenic, sense, and antisense types based on their genomic location [9] and into signaling, decoy, guide, and scaffold lncRNAs on a functional basis [10]. Signaling lncRNAs mainly act as transcription factors or as intermediates in various signaling pathways [10], and decoy lncRNAs act as “molecular sponges” by binding to and sequestering transcription factors away from their target genes [10]. Guide lncRNAs can regulate gene expression through chromatin remodeling by recruiting chromatin-modifying enzymes [10]. Finally, the scaffold lncRNAs act as recruiting platforms for multiple proteins and form lncRNA-ribonucleoprotein (lncRNA-RNP) complexes, which subsequently regulate downstream signaling [10].
Numerous lncRNAs have been identified recently with the help of high-throughput sequencing and microarrays. Most of them are aberrantly expressed in tumors like HCC, breast cancer, lung cancer, colorectal cancer, and others [11]. lncRNAs are known to regulate cell proliferation, epithelial-mesenchymal transition (EMT), angiogenesis, metastasis, autophagy, and so forth. Considering their cancer specific expression and detectable presence in clinical samples like blood and urine, lncRNAs are potential diagnostic markers for tumors. Therefore, a better understanding of HCC specific lncRNAs will greatly contribute to the diagnosis and treatment of HCC.

lncRNAs exhibit both tumor suppressive and oncogenic roles. In the present review, we will concentrate mainly on the functions, molecular mechanisms, and potential clinical implications of HCC-related lncRNAs that are abnormally expressed and therefore have critical roles in hepatocarcinogenesis.

2. Upregulated/Oncogenic lncRNAs in HCC

2.1. HULC. “Highly upregulated in liver cancer” or HULC, a 500 bp lncRNA, was the earliest lncRNA reported to be highly expressed in HCC [12]. In addition to the tumor tissues, significantly greater levels of HULC were also found in HCC cell lines and plasma of patients [12–16], indicating its potential role as a biomarker of HCC. HULC is involved in multiple cellular processes like proliferation, EMT, angiogenesis, autophagy, and chemoresistance (Table 1). Furthermore, HULC overexpression was linked with tumor size [17], clinical TNM stage [16], and recurrence and overall survival (OS) in HCC [18].

Wang et al. reported a decoy role of HULC wherein it downregulated miR-327 by its molecular sponge function [13]. HULC-induced miR-327 inhibition lifted the miR-327-mediated translational suppression of PRKACB, which consecutively activated the cAMP response element binding protein (CREB) [13]. CREB induced expression of HULC, thereby forming a CREB-HULC-PRKACB positive feedback loop [13]. HULC also acted as a molecular decoy to downregulate miR-186 which upregulated HMGA2 and lead to HCC progression. In this model, HULC expression was regulated by IGF2BP1 by accelerating HULC degradation [16]. Various studies have elucidated the pathways through which HULC promotes hepatocarcinogenesis: it activates angiogenesis via the HULC/miR-107/E2F1/SHPK1 axis [19], enhances EMT and metastasis via the HULC/miR-200a-3p/ZEB1 axis [18], induces autophagy via the HULC/USP22/Sirt3 axis [20], and augments cell proliferation by stabilizing COX-2 [21] (Table 2). HULC is also involved in hepatitis B virus (HBV) induced HCC, in which HBx plays an important role [22]. HBx markedly increased cell proliferation by upregulating HULC and inhibiting p18, while HULC inhibition abolished HBx-induced cell proliferation accompanied by p18 upregulation [14]. Taken together, HULC is a potential biomarker for diagnosing HCC.

2.2. HOTAIR. “HOX transcript antisense intergenic RNA” or HOTAIR is a lncRNA (2.2 kb length) which originates from the HOXC antisense strand [23]. HOTAIR is overexpressed in HCC cells and tissues [24–27] and is associated with worse prognosis, shorter recurrence-free survival, and increased risk of recurrence after hepatic transplantation [26, 28, 29]. Functionally, HOTAIR enhances proliferation, migration, glycolysis, autophagy, and chemoresistance in HCC cells (Table 1).

HOTAIR-mediated inhibition of miRNA-218 induced Bmi-1 expression and activated downstream PI4 and PI6 signaling, contributing to hepatocarcinogenesis [25]. FOXC1 upregulated HOTAIR in HCC cells via miR-1 inhibition, thereby increasing proliferation [30]. In addition, HOTAIR also increased cell proliferation by regulating OGFr [31]. HOTAIR silencing in Huh7 cells decreased proliferation and induced cisplatin resistance via inhibition of STAT3 and ABCB1, which was rescued by inhibiting STAT3 phosphorylation [32] (Table 1). Wei et al. showed that HOTAIR-induced upregulation of GLUT1 and activation of mTOR signaling pathway facilitate glycolysis in HCC cells [27], indicating a direct association between HOTAIR and glucose metabolism in cancer cells. RNAi-mediated HOTAIR knockdown in HCC cells upregulated the RNA binding motif protein 38 (RBM38) [33] (Table 1). Furthermore, knockdown of RBM38 could restore HOTAIR-knockdown-induced decrease in cell migration and invasion [33]. Thus, HOTAIR likely enables HCC metastasis and invasion by inhibiting RBM38. The PRC2 complex, consisting of SUZ12 and EZH2, plays a key role in hepatocarcinogenesis [34–36]. HOTAIR also acts as a scaffold by recruiting PRC2 to the LSD1/Co-REST/HDAC1 complex [37]. In addition, HOTAIR also promotes HBV-mediated HCC by accelerating the degradation of SUZ12 and ZNF198 [38] (Table 1). Finally, HOTAIR could also induce autophagy in HCC cells by upregulating ATG3 and ATG7 [39] (Table 1). Taken together, HOTAIR promotes hepatocarcinogenesis by multiple mechanisms.

2.3. MALAT1. Overexpressed “metastasis-associated lung adenocarcinoma transcript 1” or MALAT1 has been initially discovered in human non-small-cell lung cancer (NSCLC) [40]. MALAT1 is overexpressed in HCC tissues and cell lines [41, 42] and is linked with a higher tumor recurrence rate in patients after hepatic transplantation, indicating a predictive role of MALAT1 in HCC recurrence [42]. Functionally, MALAT1 promotes proliferation, invasion, metastasis, chemosensitivity, and autophagy in HCC cells (Table 1).

MALAT1 is upregulated by Sp1 and Sp3 and downregulated by MIT (Sp1 binding inhibitor), indicating a possibility of targeting MALAT1 in HCC patients by MIT [43]. High expression of MALAT1 is linked with 5-FU resistance in HCC cell line [44]. In addition, HIF-2α inhibits miR-216b through MALAT1, where the HIF-2α-MALAT1-miR-216b axis promotes autophagy with LC3-II upregulation and p62 downregulation, contributing to HCC chemosensitivity [44] (Table 1). MALAT1 also promotes arsenite-induced glycolysis via stabilizing HIF-1α in human hepatic L-02 cells [45]. Moreover, MALAT1, negatively regulated by p53, enhanced proliferation during liver regeneration through stimulation of the Wnt/β-catenin pathway [46] (Table 1). The mTOR signaling pathway is essential for the oncogenic role of...
### Table 1: Mechanisms and biological functions of upregulated LncRNAs in HCC.

<table>
<thead>
<tr>
<th>LncRNA</th>
<th>Full name</th>
<th>Mechanism</th>
<th>Function</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>HULC</td>
<td>Highly upregulated in liver cancer</td>
<td>Downregulating miR-372 and miR-186; downregulating p18, SPHK1, and ZEB1; activating USP22/C0X-2 axis and USP22/Sirtl axis; upregulating HMGA2</td>
<td>Proliferation(+), EMT(+), angiogenesis(+), metastasis(+), autophagy(+), chemoresistance(+)</td>
<td>[13, 14, 18–21, 85, 86]</td>
</tr>
<tr>
<td>HOTAIR</td>
<td>HOX transcript antisense RNA</td>
<td>Downregulating RBM38, miR-1, miRNA-218, SETD2, SUZ12, and ZNF198; activating P14, P16, GLUT1, MMP9, VEGF, ATG3, and ATG7</td>
<td>Proliferation(+), migration(+), invasion(+), glucose metabolism(+), autophagy(+)</td>
<td>[25–30, 33, 38, 39, 87]</td>
</tr>
<tr>
<td>MALAT1</td>
<td>Metastasis-associated lung adenocarcinoma transcript 1</td>
<td>HIF-2α-MALAT1-miR-216b axis; MALAT1/miR-143-3p-ZEB1 axis; MALAT1-miR-195-EGFR axis; HBx-MALAT1/ATBP3 axis; upregulating HIF-1α, Wnt/β-catenin pathway, SRSF1, and mTOR pathway</td>
<td>Proliferation(+), migration(+), invasion(+), chemoresistance(+), autophagy(+), metastasis(+)</td>
<td>[42, 44–51]</td>
</tr>
<tr>
<td>HOTTIP</td>
<td>HOXA transcript at the distal tip</td>
<td>miR-125b/HOTTIP axis; miR-192/-204-HOTTIP axis</td>
<td>Metastasis(+), proliferation(+)</td>
<td>[52–54]</td>
</tr>
<tr>
<td>MVIH</td>
<td>Microvascular invasion in HCC</td>
<td>Downregulating miR-199a</td>
<td>Angiogenesis(+)</td>
<td>[55, 56]</td>
</tr>
<tr>
<td>PVT1</td>
<td>Plasmacytoma variant translocation 1</td>
<td>PVT1/NOP2 axis; PVT1/EZH2/miR-214 axis</td>
<td>Proliferation(+), cancer cell stemness(+)</td>
<td>[58–61]</td>
</tr>
<tr>
<td>UCA1</td>
<td>Urothelial carcinoma associated-1</td>
<td>UCA1/miR-208/Snail2 axis; HBx-UCA1/EZH2-p7Kip1 axis; UCA1-miR-216b-FGFR1-ERK axis</td>
<td>Proliferation(+), invasion(+), EMT(+)</td>
<td>[88–91]</td>
</tr>
<tr>
<td>ATB</td>
<td>Activated by TGF-β</td>
<td>ATB/miR-200/ZEB1-ZEB2 axis</td>
<td>EMT(+), invasion(+), metastasis(+)</td>
<td>[92–94]</td>
</tr>
<tr>
<td>Linc-ROR</td>
<td>LincRNA regulator of reprogramming</td>
<td>Downregulating miR-145-HIF-1α</td>
<td>Chemoresistance(+), EMT(+), invasion(+), metastasis(+)</td>
<td>[95–97]</td>
</tr>
<tr>
<td>VLPLR</td>
<td>Very low density lipoprotein receptor</td>
<td>Downregulating ABCG2</td>
<td>Chemoresistance(+)</td>
<td>[98]</td>
</tr>
<tr>
<td>CCAT1</td>
<td>Colon cancer associated transcript 1</td>
<td>Downregulating let-7</td>
<td>Proliferation(+), migration(+), invasion(+)</td>
<td>[99]</td>
</tr>
<tr>
<td>Linc00974</td>
<td>Long intergenic non-protein-coding RNA 974</td>
<td>Upregulating KRT9, Notch, and TGF-β signaling</td>
<td>Proliferation(+), metastasis(+)</td>
<td>[100]</td>
</tr>
<tr>
<td>HNFIA-AS1</td>
<td>HNFIA antisense RNA 1</td>
<td>HNFIA-AS1-miR-30b axis; downregulating NKD1 and p21</td>
<td>Apoptosis(−), autophagy(+), proliferation(+)</td>
<td>[101, 102]</td>
</tr>
<tr>
<td>HEIH</td>
<td>Highly expressed in HCC</td>
<td>Upregulating EZH2</td>
<td>Proliferation(+), invasion(+)</td>
<td>[85, 103]</td>
</tr>
<tr>
<td>HBx-LINE1</td>
<td>Fusion of the human cellular long interspersed nuclear elements and HBx</td>
<td>Downregulating miR-122; upregulating Wnt signaling</td>
<td>EMT(+), invasion(+), metastasis(+)</td>
<td>[104, 105]</td>
</tr>
<tr>
<td>LincTCF7 (WSPAR)</td>
<td>WNT signaling pathway activating noncoding RNA</td>
<td>Upregulating Wnt signaling</td>
<td>EMT(+), invasion(+), metastasis(+), cancer stem cell self-renewal(+)</td>
<td>[106]</td>
</tr>
<tr>
<td>DANCR</td>
<td>Differentiation antagonizing non-protein-coding RNA</td>
<td>Downregulating CTNNB1</td>
<td>Cancer cell stemness(+)</td>
<td>[107, 108]</td>
</tr>
<tr>
<td>lncRNA</td>
<td>Full name</td>
<td>Mechanism</td>
<td>Function</td>
<td>References</td>
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<td>------------</td>
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</tr>
<tr>
<td>BANCR</td>
<td>BRAF-regulated IncRNA 1</td>
<td>Activating MEK</td>
<td>Invasion(+), metastasis(+)</td>
<td>[109]</td>
</tr>
<tr>
<td>ZEBI-AS1</td>
<td>ZEB1 antisense RNA 1</td>
<td>Upregulating ZEB1</td>
<td>EMT(+), invasion(+), metastasis(+), proliferation(+)</td>
<td>[110]</td>
</tr>
<tr>
<td>DBH-AS1</td>
<td>DBH antisense RNA 1</td>
<td>Activating MAPK signaling; upregulation of CDK6, CCND1, and CCNE1; downregulating p16, p21, and p27</td>
<td>Proliferation(+)</td>
<td>[111]</td>
</tr>
<tr>
<td>TUC338</td>
<td>Transcribed ncRNA encoding uc.338</td>
<td>TUC338/RASAL1 axis</td>
<td>Proliferation(+); chemoresistance(−)</td>
<td>[112]</td>
</tr>
<tr>
<td>TUG1</td>
<td>Taurine upregulated 1</td>
<td>TUG1/miR-332-Hedgehog axis; TUG1/miR-455-3p/AMPKβ2 axis</td>
<td>Proliferation(+), apoptosis(−), metastasis(+)</td>
<td>[113, 114]</td>
</tr>
<tr>
<td>ANRIL</td>
<td>CDKN2B antisense RNA 1</td>
<td>Downregulating miR-122-5p</td>
<td>Proliferation(+), apoptosis(−), metastasis(+)</td>
<td>[115, 116]</td>
</tr>
<tr>
<td>URHC</td>
<td>Upregulated in hepatocellular carcinoma</td>
<td>Downregulating ZAK; suppressing ERK/MAPK pathway</td>
<td>Proliferation(+), apoptosis(−)</td>
<td>[117]</td>
</tr>
<tr>
<td>AFAP1-AS1</td>
<td>AFAP antisense RNA 1</td>
<td>Upregulation of RhoA/Rac2 signaling</td>
<td>Proliferation(+), invasion(+)</td>
<td>[118]</td>
</tr>
<tr>
<td>PCNA-AS1</td>
<td>PCNA antisense RNA 1</td>
<td>Stabilizing PCNA</td>
<td>Proliferation(+)</td>
<td>[119]</td>
</tr>
<tr>
<td>CCAT2</td>
<td>Colon cancer associated transcript 2</td>
<td>Upregulating FOXM1 expression;</td>
<td>Proliferation(+), apoptosis(−), EMT(+)</td>
<td>[120–122]</td>
</tr>
<tr>
<td>SNHG1</td>
<td>Small nucleolar RNA host gene 1</td>
<td>Downregulating miR-195</td>
<td>Proliferation(+), invasion(+) , metastasis(+), migration(+), apoptosis(+)</td>
<td>[123, 124]</td>
</tr>
<tr>
<td>HCAL</td>
<td>HCC-associated IncRNA</td>
<td>HCA1-miR-15a/miR-196a/miR-196b-LAPT4M4B network</td>
<td>Proliferation(+), metastasis(+)</td>
<td>[125]</td>
</tr>
<tr>
<td>MUF</td>
<td>MSC-upregulated factor</td>
<td>Activating Wnt/β-catenin signaling</td>
<td>EMT(+)</td>
<td>[126]</td>
</tr>
<tr>
<td>HOXD-AS1</td>
<td>HOXD cluster antisense RNA 1</td>
<td>Upregulating SOX4, EZH2, and MMP2; HOXD-AS1/miR-199a/ARHGAP11a axis</td>
<td>Migration(+), invasion(+), apoptosis(−)</td>
<td>[127, 128]</td>
</tr>
<tr>
<td>AWPPH</td>
<td>None</td>
<td>Activating PIK3CA</td>
<td></td>
<td>[129]</td>
</tr>
<tr>
<td>SNHG12</td>
<td>Small nucleolar RNA host gene 12</td>
<td>SNHG12/miR-199a/b-5p/MLK3 axis</td>
<td>Proliferation(+), invasion(+), metastasis(+), apoptosis(−)</td>
<td>[130]</td>
</tr>
<tr>
<td>IncBRM</td>
<td>IncRNA for association with Brahma</td>
<td>Activating YAP1 signaling</td>
<td>Cancer stem cell self-renewal(+)</td>
<td>[131]</td>
</tr>
<tr>
<td>UniGene56159</td>
<td>None</td>
<td>UniGene56159/miR-140-5p/Slug axis</td>
<td>EMT(+), migration(+), invasion(+)</td>
<td>[132]</td>
</tr>
<tr>
<td>SNHG6-003</td>
<td>None</td>
<td>Sponge for miR-26a/b</td>
<td>Proliferation(+), chemoresistance(+)</td>
<td>[133]</td>
</tr>
<tr>
<td>Inc-β-Catm</td>
<td>None</td>
<td>Activating Wnt-β-catenin signaling</td>
<td>Proliferation(+), cancer stem cell self-renewal(+)</td>
<td>[134]</td>
</tr>
</tbody>
</table>

+: increase; −: decrease.
<table>
<thead>
<tr>
<th>lncRNA</th>
<th>Full name</th>
<th>Mechanism</th>
<th>Function</th>
<th>Reference</th>
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<tr>
<td>H19</td>
<td>None</td>
<td>Activating miR-200 family, downregulating AKT/GSK-3beta/Cdc25A pathway</td>
<td>Migration(–), invasion(–), metastasis(–), EMT(–)</td>
<td>[64, 68]</td>
</tr>
<tr>
<td>MEG3</td>
<td>Maternally expressed gene 3</td>
<td>Activating P53, MEG3/miR664/ADH4 axis</td>
<td>Proliferation(–), apoptosis(+)</td>
<td>[72, 73, 76]</td>
</tr>
<tr>
<td>Dreh</td>
<td>Downregulated by HBx</td>
<td>Downregulating vimentin</td>
<td>Proliferation(–), migration(–), metastasis(–)</td>
<td>[77]</td>
</tr>
<tr>
<td>LET</td>
<td>Low expression in tumor</td>
<td>Downregulating NF90</td>
<td>Invasion(–), metastasis(–)</td>
<td>[79]</td>
</tr>
<tr>
<td>ZNFX1-AS1</td>
<td>ZNFX1 antisense RNA 1</td>
<td>Upregulating miR-9</td>
<td>Proliferation(–), apoptosis(+)</td>
<td>[135]</td>
</tr>
<tr>
<td>PTENP1</td>
<td>Phosphatase and tensin homolog, pseudogene 1</td>
<td>Downregulating miR-17, miR-19b, and miR-20a</td>
<td>Proliferation(–), angiogenesis(–), apoptosis(+), autophagy(+)</td>
<td>[136]</td>
</tr>
<tr>
<td>AOC4P</td>
<td>Amine oxidase, copper containing 4, pseudogene</td>
<td>Downregulating vimentin</td>
<td>Proliferation(–), migration(–), invasion(–), EMT(–)</td>
<td>[137]</td>
</tr>
<tr>
<td>FTX</td>
<td>None</td>
<td>Downregulating miR-374a and Wnt/β-catenin signaling</td>
<td>Proliferation(–), invasion(–), metastasis(–), EMT(–)</td>
<td>[138]</td>
</tr>
<tr>
<td>XIST</td>
<td>X inactive specific transcript</td>
<td>XIST/miR-181/MPTEN axis; XIST/miR-92b/Smad7 axis</td>
<td>Proliferation(–), invasion(–), metastasis(–)</td>
<td>[139, 140]</td>
</tr>
<tr>
<td>LncRNA00364</td>
<td>None</td>
<td>LncRNA00364/STAT3/IFIT2 axis</td>
<td>Proliferation(–), apoptosis(+)</td>
<td>[141]</td>
</tr>
<tr>
<td>Linc-USP16</td>
<td>None</td>
<td>ceRNA for miR-21 and miR-590-5p and upregulating PTEN</td>
<td>Proliferation(–), migration(–)</td>
<td>[142]</td>
</tr>
<tr>
<td>CASC2</td>
<td>Cancer susceptibility candidate 2</td>
<td>Downregulating miR-24-3p, CASC2/miR-367/FBXW7 axis</td>
<td>Proliferation(–), apoptosis(+), invasion(–), EMT(–)</td>
<td>[143, 144]</td>
</tr>
<tr>
<td>LINC00657</td>
<td>None</td>
<td>LINC00657/miR-106a-5p/PTEN axis</td>
<td>Proliferation(–), migration(–), invasion(–)</td>
<td>[145]</td>
</tr>
<tr>
<td>FER1L4</td>
<td>Fer-1-like protein 4</td>
<td>Downregulating miR-106-5p</td>
<td>Proliferation(–), migration(–), apoptosis(+)</td>
<td>[146]</td>
</tr>
<tr>
<td>uc.134</td>
<td>None</td>
<td>uc.134/CUL4A/LATS1 axis</td>
<td>Proliferation(–), invasion(–), metastasis(–)</td>
<td>[75]</td>
</tr>
<tr>
<td>Lnc-DILC</td>
<td>lncRNA downregulated in liver cancer stem cells</td>
<td>IL-6/STAT3 axis</td>
<td>Proliferation(–)</td>
<td>[147]</td>
</tr>
</tbody>
</table>

+: increase; –: decrease.
MALAT1, which further mediates SRSF1 upregulation and mTOR activation [47]. MALAT1 promotes tumor growth, invasion, and metastasis of HCC as a decoy lncRNA through the MALAT1/miR-143-3p/ZEB1 axis and inhibiting miR-146b-5p [48, 49]. It can also act as a ceRNA for miR-195 and reverse miR-195-mediated EGFR inhibition and further promote cell proliferation by activating the PI3K/AKT and JAK/STAT pathways, indicating a role of MALAT1-miR-195-EGFR axis in HCC [50] (Table 1). Like HULC and HOTAIR, MALAT1 is also involved in HBx-mediated hepatocarcinogenesis [51]; it is upregulated by HBx and enhances proliferation and metastasis by activating LTBP3 [51], forming the HBx-MALAT1-LTBPI3 axis. Taken together, MALAT1 regulates multiple cellular processes through its decoy or ceRNA functions, indicating a potential target for HCC therapy.

2.4. HOTTIP. “HOXA transcript at the distal tip” or HOTTIP is greatly expressed in HCC tumor tissues and cells [52] and is linked with a greater threat of metastasis and poor OS [52]. Studies have shown the effect of HOTTIP on HCC proliferation, metastasis, and glutamine metabolism (Table 1) [52–54].

HOTTIP downregulates miR-125b, miR-192, and miR-204 and enhances the cell growth and migration through the miR-192/-204-HOTTIP axis [53, 54], while HOTTIP inhibition decreases growth of HCC cells [52, 54]. In addition, HOXA13 and GLS1 are further revealed to be the likely target genes of miR-192/-204-HOTTIP axis, and overexpression of miR-192 and miR-204 is associated with increased survival in the patients [54]. Taken together, these findings imply the oncogenic role of HOTTIP in hepatocarcinogenesis through miRNA interaction.

2.5. MVIH. “Microvascular invasion in HCC” or MVIH is situated at chromosome 10 and was firstly identified by Yuan et al. in HCC [55]. High levels of MVIH in HCC were correlated with enhanced invasion and poor prognosis with decreased RFS and OS [55]. As shown in Table 1, MVIH plays important roles in proliferation, migration, apoptosis, metastasis, and angiogenesis in HCC [55–57].

MVIH exerts its proangiogenic action by inhibiting PGK1 secretion [55]. It also acts like a sponge for miR-199, and MVIH-mediated inhibition of miR-199 leads to increased proliferation and apoptosis inhibition in HCC cells [56]. Recently, MVIH was reported to control proliferation and migration of HCC cells via modulation of ARID1A-mediated regulation of CDKN1A [57]. Taken together, these findings underscore the oncogenic role of MVIH in HCC.

2.6. PVT1. Murine PVT1 was first identified in the liver where it accelerated proliferation and cell cycling and enhanced stem-cell-associated properties [58]. Human PVT1 is overexpressed in HCC tumor tissues and cell lines and is linked with advanced TNM stage and poor prognosis as well as RFS [58–60], and upregulation of PVT1 can also predict HCC recurrence [59]. As shown in Table 1, PVT1 plays an oncogenic role in multiple cellular processes like proliferation and invasion and increases the stenness of HCC cells [58, 60, 61].

Functionally, through interaction between PVT1 and NOPO2, PVT1 enhances the expression of NOPO2 via stabilizing NOPO2, thus promoting proliferation, cell cycle, and stemness of HCC cells [58] (Table 1). In addition, PVT1 can also induce miR-214 inhibition via interaction with EZH2 to promote cell proliferation and invasion [60], forming a PVT1/EZH2/miR-214 axis (Table 1) [60]. The clearly oncogenic role of PVT1 indicates its potential use as a biomarker in diagnosing and predicting recurrence in HCC.

In addition to the lncRNAs mentioned above, several others are upregulated during hepatocarcinogenesis, including DANCR, HEIH, and Linc-ROR (Table 1).

3. Downregulated/Tumor Suppressive lncRNAs in HCC

3.1. H19. H19 is situated on chromosome 11p15.5 [62] and plays a key role in various cancers including HCC, where the abnormal expression of H19 is linked with later stages of cancer and poor DFS and outcome [63–65]. Functionally, H19 regulates proliferation, migration, invasion, EMT, metastasis, and chemoresistance in HCC cells [64, 66–68] (Table 2). H19 is upregulated in doxorubicin-resistant R-HepG2 cells [66] and induces drug resistance by modulating MDRI [66]. H19 overexpression enhanced the tumor growth in in vivo models of HCC, while H19 inhibition decreased [67]. HCC patients with elevated expression of H19 in the tumor tissues showed poor DFS, suggesting a predictive role of H19 in HCC prognosis [65]. However, some studies have shown H19 to be significantly downregulated in HCC [64, 65], which is correlated with poor prognosis [64]. In addition, H19 could also activate miR-200 and suppress tumor metastasis and EMT [64] (Table 2). H19 inhibition by miR-675 promoted metastasis of HCC via the AKT/GSK-3beta/Cdc25A pathway [68] (Table 2). Taken together, H19 seems to act as a tumor suppressor as well as an oncogene in HCC.

3.2. MEG3. “Maternally expressed 3” or MEG3 is a maternally inherited lncRNA presented on chromosome 14q32.3 [69] and was first identified by Miyoshi et al. [70]. MEG3 expression is reportedly low in human HCC cells [71–73] and is linked with reduced OS, suggesting a predictive role of MEG3 in HCC prognosis [73]. As shown in Table 2, MEG3 could regulate proliferation and apoptosis in HCC cells [72–75].

MEG3 can be negatively regulated by UHRF1 via modulating DNA methylation, since its promoter region is highly methylated [73]. One mechanism of MEG3 mediated tumor suppression is the activation of p53 by increasing its stability and modulating the downstream genes [72, 74] (Table 2). Using a novel delivery system, MEG3 was introduced into HCC cells and resulted in tumor growth inhibition via the p53 signaling, indicating a bona fide tumor suppressive role of MEG3 in HCC [74]. Furthermore, it acted as a molecular sponge for miR-664 and could inhibit cell proliferation by modulating miR-664-mediated regulation of ADH4 [76].
Table 3: LncRNAs as biomarkers in HCC.

<table>
<thead>
<tr>
<th>lncRNA</th>
<th>Expression in HCC</th>
<th>Potential implications</th>
<th>Sample</th>
<th>References</th>
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</thead>
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<tr>
<td>HULC</td>
<td>Up</td>
<td>Detection, metastasis, prognosis,</td>
<td>Plasma</td>
<td>[15, 17]</td>
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<td>Detection, metastasis</td>
<td>Plasma</td>
<td>[17, 148]</td>
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<td>Detection, HBV-related HCC</td>
<td>Serum</td>
<td>[149]</td>
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<td>Serum</td>
<td>[149]</td>
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<tr>
<td>PVT1</td>
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<td>Detection</td>
<td>Serum</td>
<td>[150]</td>
</tr>
<tr>
<td>uc002mbc.2</td>
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<td>Detection</td>
<td>Serum</td>
<td>[150]</td>
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<td>Plasma</td>
<td>[148, 151]</td>
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<td>Plasma</td>
<td>[148, 151]</td>
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<tr>
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<td>[88]</td>
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<td>Plasma</td>
<td>[108]</td>
</tr>
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<td>Detection</td>
<td>Serum</td>
<td>[152]</td>
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<tr>
<td>Linc00974</td>
<td>Up</td>
<td>Detection, metastasis</td>
<td>Plasma</td>
<td>[100]</td>
</tr>
</tbody>
</table>

(Table 2). Taken together, MEG3 is a tumor suppressor and might be considered a prospective diagnostic, predictive and therapeutic biomarker in HCC.

3.3. Dreh. “Downregulated expression by HBx” or Dreh was first identified by lncRNA microarray on WT and HBx-transgenic mice [77]. It is low expressed in the tumor tissues of HBV-related HCC patients and corresponding cell lines [77, 78]. Patients with decreased expression of Dreh showed poor survival [77]. As shown in Table 2, Dreh is linked with the proliferation and metastasis of HBV-related HCC.

A previous study revealed a negative correlation of Dreh expression with HBx and HBs [78]. Dreh is downregulated by HBx via downregulation of vimentin, which results in the suppression of HCC growth and migration [77, 78] (Table 2), thus underscoring the tumor suppressive role of Dreh in HBV-related HCC.

3.4. LET. “Low expression in tumor” or LET is present in significantly low levels in HCC tumor tissues [79] and is linked with metastasis [79]. As shown in Table 2, LET influences the invasiveness and metastasis of HCC cells.

LET is downregulated by HDAC3 [79], and LET inhibition increases the stability of NF90, thus promoting hypoxia-induced invasion [79] (Table 2). This was successfully validated in an HCC clinical sample with abnormal histone acetylation, downregulation of LET, and upregulation of NF90. These findings suggest a tumor suppressive role of LET centered around regulating metastasis under hypoxia.

As shown in Table 2, along with the lncRNAs discussed above, several others have been indicated to influence hepatocarcinogenesis, such as ZNFXI-ASI, PTENP1, and XIST.

4. lncRNAs as Diagnostic Biomarkers and Drug Targets in HCC

Increasing evidence shows critical roles of various lncRNAs in hepatocarcinogenesis, either as tumor suppressors or as oncogenes. Abnormal expression of lncRNAs is significantly linked with cancer proliferation, metastasis, OS, DFS, RFS, and the tumor TNM stage. Multivariate analyses have further revealed that lncRNAs can independently predict recurrence and outcomes of HCC. With the rapid development of molecular diagnostics such as sequencing technology, qRT-PCR, microarrays, and RNA immunoprecipitation, lncRNAs can be easily detected in various body fluids, thus paving the way for lncRNA as novel diagnostic and prognostic markers of HCC. For example, the oncogenic HULC is significantly upregulated in plasma of patients as well the HCC tumor tissues; thus, it could serve as a novel diagnostic biomarker for HCC (Table 3) [15, 17]. In addition to plasma, serum and exosomes can also be used for lncRNA detection. For example, HEIH, an oncogenic lncRNA expressed highly in HCC tissues, was also found to be overexpressed in the serum and exosomes of patients with HCV-related HCC (Table 3). In addition to HULC and HEIH, many other lncRNAs could also serve as biomarkers of HCC which are shown in Table 3.

Since various lncRNAs are abnormally expressed in HCC and affect many downstream genes and related signaling pathways through oncogenic or tumor suppressive action, restoring these lncRNAs to their normal expression level is a therapeutic option worth considering, especially as an alternative to the chemotherapeutic drugs which usually result in chemoresistance [80]. Pharmaceutical companies have recently shown a great interest in lncRNA-targeted therapy and have already taken actions [81, 82]. lncRNAs could be upregulated by exogenous overexpression and directly targeted by their specific siRNAs or antisense oligonucleotides [83, 84]. For example, the tumor suppressor MEG3 introduced into HCC tumor through a novel delivery system effectively induced apoptosis in HCC cells [74], presenting a potential lncRNA-targeted therapy with fewer side effects. Therefore, clarifying the specific mechanism of lncRNA action will greatly promote the advancement of lncRNA-based diagnosis and therapy for HCC.

Conflicts of Interest

The authors declare that they have no conflicts of interest.
Authors’ Contributions
Xiaoge Hu and Jiahong Liang contributed equally to this work.

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References

BioMed Research International


S. Cheng, L. Wang, C.-H. Deng, S.-C. Du, and Z.-G. Han, “ARID1A represses hepatocellular carcinoma cell proliferation”


[143] F. Zeng, Y. Le, J. Fan, and L. Xin, "LncRNA CASC2 inhibited the viability and induced the apoptosis of hepatocellular carcinoma cells through regulating miR-24-3p," *Journal of Cellular Biochemistry*.


Research Article

Analysis of the Cancer Genome Atlas Data Reveals Novel Putative ncRNAs Targets in Hepatocellular Carcinoma

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Hepatocellular carcinoma (HCC) is the prevalent type of primary liver malignancy. Different noncoding RNAs (ncRNAs) that negatively regulate gene expression, such as the microRNAs and the long ncRNAs (lncRNAs), have been associated with cell invasiveness and cell dissemination, tumor recurrence, and metastasis in HCC. To evaluate which regulatory ncRNAs might be good candidates to disrupt HCC proliferation pathways, we performed both unsupervised and supervised analyses of HCC expression data, comparing samples of solid tumor tissue (TP) and adjacent tissue (NT) of a set of patients, focusing on ncRNAs and searching for common mechanisms that may shed light in future therapeutic options. All analyses were performed using the R software. Differential expression (total RNA and miRNA) and enrichment analyses (Gene Ontology + Pathways) were performed using the package TCGABiolinks. As a result, we improved the set of lncRNAs that could be the target of future studies in HCC, highlighting the potential of FAM170B-AS1 and TTN-AS1.

1. Introduction

Epidemiologic data from the International Agency for Research on Cancer of the World Health Organization reveals that liver cancer comprises 5.6% of worldwide cancer incidence and 9.1% of all cancer-associated mortality [1]. Hepatocellular carcinoma (HCC) is the most prevalent type of primary liver malignancy [2]. The high lethality of HCC can be attributed to the lack of diagnostic markers for an early detection and late stages high heterogeneity [3]. HCC has been epidemiologically associated with chronic Hepatitis B Virus (HBV) or Hepatitis C Virus (HCV) [4], as well as alcoholic and nonalcoholic fatty liver disease, which are its major risk factors [2]. Currently, the most effective treatment is either surgical tumor resection or liver transplantation [5].

Multiple studies have shown the potential of different microRNAs (miRNAs) as prognostic and diagnostic biomarkers in many types of cancer, including HCC [6–8]. miRNAs are noncoding RNAs that negatively regulate gene expression by leading mRNAs to target degradation or translational repression after binding to its 3’UTR (for review see [9]). In cancer, their role has been either as tumor suppressors or as enhancers (oncomiRs) [10].

In HCC, different miRNAs have been associated with cell invasiveness by repressing TET gene expression, leading to silencing of several invasion-suppressors via hypermethylation [11], and cell dissemination by regulating differentiation, hence increasing metastatic potential [12]. They are even implicated in improvement of HBV and HCV viral replication and tumor-supporting mechanisms [13, 14]. This
multifaceted miRNA capacity of influencing in the HCC environment proves the importance of studies describing expression profiles of miRNAs during tumor occurrence.

Another class of noncoding (nc) RNAs, the long ncRNAs (lncRNAs), are > 200 nucleotides’ RNA molecules with multiple regulatory roles that cannot be inferred by their sequence. These roles comprise, among others, chromatin organization affecting the gene expression [15]. HOTAIR, an antisense lncRNA, has been associated with HCC recurrence and metastasis [16], HULC and FTX (HCC) are also upregulated in tumoral samples [17].

Here, differently from previous works that focused on viral infection (HBV or HCV) comparing primary solid tumor tissue (TP) and adjacent tissue (NT) [6, 8, 18, 19], or focused on the mutation found [20], we *in silico* compared TP and NT of a set of patients in sense to discover the pathways that differentiate both groups of samples and the regulatory ncRNAs and their putative targets. As a result, we improve the set of lncRNAs that could be the target of future studies.

2. Material and Methods

All analyses were performed using the R software (v. 3.4.0) [21]. The differential expression (mRNA and miRNA) analysis was performed using the package TCGA-Biowlks (v. 2.7.1) [22]. First, we downloaded HCC harmonized data (hg38) from The Cancer Genome Atlas (TCGA) using the function GDCdownload with the option legacy = FALSE. We analyzed a total of 41 participants that have expression data of both primary solid tumor and adjacent tissue samples. It is worth noticing that in the database adjacent tissue is referred to as normal; however this is hardly the case as all patients were cirrhotic. Thus we use the term adjacent, as this is not a sample from a normal liver. To select these individuals, we used only the participant ID of the TCGA barcode as query barcode (e.g., participant ID in bold: TCGA-BC-A10Q-01A-I1R-A13I-07). The sampling comprises a group of men and women, white, black, or Asian, showing or not the presence of risk factors such as fat liver disease. Not all samples had a positive diagnostic for HBV or HCV. All data is available at TCGA web portal.

For total RNA differential expression, we followed the standard pipeline. The samples were highly correlated after an outlier check (TCGAAnalyze_Preprocessing function). Except one sample (0.85 < r < 0.9) all other samples showed an r > 0.9. Then, we followed a normalization step using both GC content and gene length (TCGAAnalyze_Normalization) and gene filtering by quantile (TCGAAnalyze_Filtering) as recommended in [23]. Differentially expressed genes (DEGs) were accessed by the function TCGAAnalyze_DEA considering a log2 fold change (logFC) of > 1 or < -1 and false discovery rate (FDR) of 0.01. Enrichment analyses of DEGs and top 10 categories’ plot were performed by the functions TCGAAnalyze_EA_Complete and TCGAAnalyze_EABarpot, respectively.

Heat maps were plotted using the function heatmap.2 from package gplots (v. 3.0.1) [24] considering the gene expression information of the top genes based on significant FDR or all differentially expressed transcripts of the categories miRNA, precursor microRNA (pre-miRNA), and lncRNA. Hierarchical cluster analyses were performed using the package pvclust (v. 2.0-0) [25] with 1000 bootstrap replications. Clusters with approximately unbiased grouping support p values (%) (au – red values) of 95 were considered as statistically significant groups.

For the differentially expressed transcripts, we performed a Spearman correlation to detect which regulatory RNAs are negatively correlated with other RNAs. We accepted those with r < -0.8 and p value < 0.05 as statistically significantly correlated. These correlated transcripts were used as interactions to input the network on Cytoscape (v. 3.5.1) [26], where the edges represent the statistically significant r values. The miRNAs and their putative targets were used to predict their interaction using the online software TargetScan (release 7.1) [27]. Interactions not found in TargetScan were also tested in miRDB [28] and TarBase (v. 8) [29]. The interactions found by either TargetScan or TarBase were confirmed by two other tools: miRWalk v. 3.0 (http://mirwalk.umn.uni-heidelberg.de/) [30] considering a binding probability cut-off of 0.8, and mirDIP v. 4.1 (http://ophid.utoronto.ca/mirDIP/index.jsp) [31, 32] considering a “medium” cut-off of scores. Gene Ontology Biological Processes of the proteins associated with the network were evaluated using the Cytoscape plugin BINGO [33]. For the interest in lncRNAs, we performed a supervised prediction model using the Area Under the Curve of the Receiver Operating Characteristic (AUC-ROC) using the package pROC v. 1.11.0 [34].

3. Results and Discussion

In this study we performed a supervised analysis of HCC expression data focusing on ncRNAs searching for common mechanisms that may shed light in future therapeutic options. The majority of statistically significant differentially expressed ncRNAs are higher expressed on tumor samples, suggesting that these RNAs are necessary to tumor progression/maintenance. Additionally, tumor samples showed a more diverse expression profile in comparison to those from adjacent tissues. Such pattern has been reported also for gastric [35] and colorectal cancers [36].

We found a total of 1739 DEGs in total RNA-seq among tumor and normal samples. From these, 1276 were upregulated in tumor (Figure 1(a), Figure S1A, and Table S1). miRNA differential expression (DE) revealed 234 DE miRNAs, of which 169 were upregulated in tumor (Figure 1(b), Figure S1B, and Table S1). Other noncoding regulatory RNAs resulted in 92 pre-miRNAs (73 upregulated in tumor) and 122 lncRNAs (90 upregulated in tumor) (Figure 1(c), Table S2). Considering the fold change of DEGs and DE miRNAs, the top ten up and downregulated genes in tumoral samples are shown in Table 1.

The enrichment analysis (Gene Ontology + Pathways) revealed that the most represented pathways in differentially expressed transcripts from total RNA-seq are involved in bile metabolism, fear behavioral response, and immune-related categories (Figure 2). To infer putative expression relationship, we plotted a network based on Spearman’s correlation,
Figure 1: Heat maps of differentially expressed noncoding regulatory RNAs. (a) miRNA. (b) Pre-miRNA. (c) LncRNA. Tumor samples in brown and adjacent samples in blue. Hierarchical clusterization based on transcript log10 scale expression.

considering only the negative interactions. These interactions involved a total of 18 highly correlated regulatory ncRNAs of all types (miRNA, pre-miRNA, and lncRNA) with their putative targets (Figure 3(a)). In the case of miRNAs and pre-miRNAs, the miRNA-target interactions were predicted as explained in the Material and Methods. These highly negative targets are most involved in programmed cell death, immune response, and Molybdenum cofactor biosynthesis processes (Figure 3(b)). For the lncRNAs in the network, we calculated the AUC-ROC values and found four lncRNAs with potential to correctly discriminate TP and NT samples: CCND2-ASI (AUC = 0.792, 95% confidence interval: 0.6834-0.8903), FAM170B-ASI (AUC = 0.917, 95% confidence interval: 0.8387-0.9758), TTN-ASI (AUC = 0.901, 95% confidence interval: 0.84-0.9539), and SYNPR-ASI (AUC = 0.939, 95% confidence interval: 0.8798-0.9823).

The DEGs’ enrichment analysis suggested that bile metabolism and fear behavioral response immune-related categories are the most represented pathways. Immune-related categories are usually disrupted in cancer. For example, CD274, upregulated in our TP samples, confers immune resistance to tumor cells by the inactivating cytotoxic T-cell
Table 1: Top ten differentially expressed genes (DEGs) from total RNA-seq and microRNA RNA-seq.

<table>
<thead>
<tr>
<th>Total RNA-seq DEGs</th>
<th>Upregulated in tumor</th>
<th>Fold change</th>
<th>Downregulated in tumor</th>
<th>Fold change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transcript</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<table>
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</table>

^†putative regulatory lncRNA.

Figure 2: Enrichment analysis of differentially expressed transcripts from total RNA data. GO: Gene Ontology. The red lines represent the ratio of genes found for the pathway over the total number of genes for that specific pathway. Inside each bar, n: number of genes. Bar sizes are in agreement to the -log10 of the FDR of the enriched ontology/pathway.
[37], DACT1, which encodes for an antagonist of beta catenin 1, and DVL2, a dishevelled protein family member, are respectively, down- and upregulated in tumor tissues, suggesting that the Wnt signaling is active [38]. Additionally, CDK14 and GSK3B are upregulated, reinforcing the Wnt signaling activation, which is related to cell polarity category [39]. This signaling pathway has been associated with malignant transformation [40].

GO group classified as fear behavioral response includes a series of genes neurotransmitters (such as glutamate, dopamine, and serotonin receptors), which comes as no surprise since many studies have shown the impact of serotonin, GABA, and sympathetic neurotransmitters in hepatocyte proliferation [41–43]. It also includes MECP2 and transcription factors associated with chromatin remodeling. Finally, bile acids are also known to act as potential carcinogens and deregulation of bile acids homeostasis has been linked to HCC formation [44].

Another transcription factor, FXR2, is supposed to act as heterodimer (or larger complexes) with TP53 or FXR1, suppressing tumor development. However, TP53 and FXR1 expressions were not detected after normalization process. Still, FXR2 can interact as homodimer or as a larger complex [45]. The absence of FXR1 expression could be a consequence of GSK3B upregulation, once FXR1 phosphorylation by GSK3B leads to FXR1 downregulation [46].

From the negative correlation network (Figure 3), we can highlight the immune-related categories, as occurred in the DEGs enrichment analysis. The expression of NAT1 has been recently reported to show high expression in breast cancer and be associated with steroid biosynthetic pathway genes [47]. Here, NAT1 is also upregulated in TP samples. This gene's
expression is also negatively correlated with two lncRNAs, both antisense RNAs: SYNPR-AS1 and CCND2-AS1. CCND2-ASI is known to promote glioma cell proliferation by activating Wnt/β-catenin signaling [48], but it is downregulated in HCC. CASPI, usually downregulated in cancer cells once it promotes apoptosis [49], showed a high expression pattern in TP samples and is negatively correlated with CCND2-ASI. NLRPI expression is associated with tumor inflammassomes and suppression of apoptosis in metastatic melanoma [50]. However, NLRPI is downregulated in our tumor data and negatively correlated with the pre-miRNA MIR3667. This miRNA is known to disrupt the oncogenic activity of PCAT-I/IMYC in prostate cancer [51] and thus its low expression in TP samples is expected. GRIK2, correlated with tumor progress [52], ERCl, which is upregulated in TP samples and its expression is associated with tumor progression once it is necessary to focal adhesion turnover [53], and PRDX3, whose overexpression is highly connected to prostate cancer [54] by protecting tumoral cells from oxidative stress [55], are all upregulated in TP samples of HCC and negatively correlated with the expression of the antisense RNA FAM170B-ASI. ERCI is also negatively correlated with SYNPR-ASI, hsa-mir-139, which is known to play antitumoral roles in HCC [56], and the pre-miRNA MIR320A plays antitumoral roles in breast cancer [57]. SLC17A8 is upregulated in prostate cancer [58] and in HCC and negatively correlated with SYNPR-ASI. HSD3B7 is associated with bie acid and did not change its expression in CTNNBI mutated HCC samples [59]. However, here HSD3B7 is upregulated in TP samples and negatively correlated with LINC01493.

NLRPI/CASPI form a complex that induces pyroptosis [60], a cell death dependent on CASPI and associated with many pathological conditions, including cancer [61]. Bearing in mind that we can not interpret gene expression as active protein production or enzymatic activity, still it seems like pyroptosis pathway is disrupted in HCC in comparison to other cancer types and that CCND2-ASI might play a role by regulating CASPI expression in this process.

AQP9 overexpression decreased the PIK3CB levels in normal tissues, reducing the cell proliferative potential by increasing FOXO1 levels and reducing PCNA expression [62]. In HCC, AQP9 levels are low [63] inducing PIK3CB activity and cell proliferation [62]. In agreement with these authors, AQP9 is downregulated in our TP samples profile, while PIK3CB is upregulated. PCNA is also upregulated but did not pass the logFC cut-off. AQP9 is negatively correlated with TTN-ASI, which was recently described as an oncogene highly expressed in esophageal squamous cell carcinoma progression and metastasis [64].

Hierarchical cluster analysis of the differentially expressed total transcripts, miRNAs, pre-miRNAs, and lncRNAs, shows that statistically significant groups are created in all cases, discriminating most adjacent from tumoral samples. This kind of distinction was not found when trying to differentiate samples also by viral types (HBV or HCV) (data not shown). It is worth noticing that DEG, but especially ncRNA analysis, was able to perfectly discriminate between TP and NT, although it was not able to separate HBV and HCV-infected samples. This suggests that the mechanisms depicted here are common to HCC regardless of its causative injury. Even though risk factors for HCC are well-known, it remains as an important cause of death worldwide. Although tumor surveillance in cirrhotics is highly recommended by international guidelines [65], late diagnosis is quite common. Moreover, advanced liver disease and parenchymal dysfunction further prevent curative therapies [66].

4. Conclusions

Our data suggests that neither HBV nor HCV infection changes overall gene expression (including those genes encoding for ncRNAs) in TP samples. Pyroptosis pathway is misregulated in HCC if compared to other cancer types and the lncRNA CCND2-ASI might be involved in this misregulation, revealing a singular characteristic of HCC. Additionally, FAM170B-ASI and TTN-ASI emerge as new candidates to tests to disrupt HCC homeostasis by turning cancer cells susceptible to oxidative stress or affecting cancer cell proliferation, respectively. Also, these lncRNAs show remarkable expression signatures, differentiating TP from NT samples with high AUC-ROC values.

Data Availability

All data used in this work is publicly available at The Cancer Genome Atlas (TCGA) database <https://cancer-genome.nih.gov/>.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors’ Contributions

Tiago Falcon and Martiela Freitas contributed equally to this work.

Acknowledgments

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Supplementary Materials

Figure S1: volcano plot representing differentially expressed transcripts (primary solid tumor x normal tissues). (A.) Total RNA. (B.) MicroRNA. Blue dots: upregulated transcripts in tumor samples. Red dots: downregulated transcripts in tumor samples. Black dots: statistically nonsignificant expressed transcripts. logFC: log2 fold change. Vertical gray lines: cut-off of logFC < -1 and logFC > 1. Horizontal purple line: FDR cut-off of 0.01. Table S1: differentially expressed data from total RNA. logFC: log2 fold change. Table S2:
differentially expressed miRNAs. logFC: log2 fold change. (Supplementary Materials)

References


Liver cancer is the second leading cause of cancer-related death worldwide. The high frequency of recurrence and metastasis is the main reason for poor prognosis. Liver cancer stem cells (CSCs) have unlimited self-renewal, differentiation, and tumor-regenerating capacities. The maintenance of CSCs may account for the refractory features of liver cancer. Despite extensive investigations, the underlying regulatory mechanisms of liver CSCs remain elusive. miRNA and IncRNA, two major classes of the ncRNA family, can exert important roles in various biological processes, and their diverse regulatory mechanisms in CSC maintenance have acquired increasing attention. However, to the best of our knowledge, there is a lack of reviews summarizing these findings. Therefore, we systematically recapitulated the latest studies on miRNAs and IncRNAs in sustaining liver CSCs. Moreover, we highlighted the potential clinical application of these dysregulated ncRNAs as novel diagnostic and prognostic biomarkers and therapeutic targets. This review not only sheds new light to fully understand liver CSCs but also provides valuable clues on targeting ncRNAs to block or eradicate CSCs in cancer treatment.

1. Introduction

Liver cancer is one of the most common malignancies and is ranked as the second leading cause of cancer-related death around the world [1]. Despite great progress in prevention, diagnosis, and treatment, the prognosis of liver cancer remains dismal due to frequent recurrence and metastasis [2]. Similar to most malignancies, liver cancer is composed of a heterogeneous cell hierarchy, in which a distinct small subset referred to as cancer stem cells (CSCs) resides [3]. CSCs have unlimited proliferation, self-renewal, differentiation, and tumor-regenerating capacities, which lead to tumor initiation, relapse, metastasis, and drug resistance [4]. Liver CSCs are the main obstacle to the cure for refractory liver cancer. Recently, several surface markers have been widely used to isolate liver CSCs, including CD13, CD133, CD24, EpCAM, CD44, CD90, and OV6. In addition, the normal stemness-related transcriptional factors and developmental signaling pathways also exert critical roles in the maintenance of CSCs, such as the AKT, Wnt/β-catenin, and IL-6/STAT3 cascades [5]. Though numerous coding genes are reported to be involved in liver CSCs, these regulatory mechanisms are largely inadequate for the complete understanding and eradication of CSCs.

With the development of whole genome and transcriptome sequencing technologies, numerous noncoding RNAs (ncRNAs) without protein-coding potential have been identified [6]. ncRNAs are no longer treated as “evolutionary junk.” Increasing evidence demonstrates that ncRNAs play important regulatory roles in various biological processes, including cancer development [7–11]. According to the relative length of nucleotides, ncRNAs are briefly classified into two categories: small or short RNA with lengths less than 200 nucleotides and long noncoding RNA (IncRNA), which is longer than 200 nucleotides in length. miRNA, as the representative member of small RNAs, has been well studied [12]. Through imperfectly binding to the 3′-untranslated region (3′-UTR) of target mRNAs, miRNA can induce mRNA degradation or inhibit translation to silence target gene expression [13]. Unlike miRNA, IncRNA has extremely complicated functions and mechanisms to manipulate gene expression in cis- and in trans-manners. IncRNA can interact with DNA, RNA,
and proteins to affect transcriptional machinery assembly, alternative splicing processes, and chromatin remodeling [14]. Dysregulated expression of miRNAs and lncRNAs has been found in multiple cancers, and these aberrant ncRNAs have oncogenic or tumor suppressive functions as well as acting as coding genes [15]. Accumulating investigations have demonstrated that miRNAs and lncRNAs are essential for sustaining CSC properties in liver cancer, like miR-200b, miR-1246, lncTCF-7, lnc-DANCR, lnc-PVT1, and so on [16–21]. These findings provide new insights regarding the complexity of liver CSCs and help foster an understanding of the underlying mechanisms. In this review, we systematically summarize the latest findings of miRNAs and lncRNAs on liver CSCs and illustrate their diverse mechanisms. Accordingly, we may develop ncRNA-based novel approaches to conquer CSCs in the future.

2. The Underlying Mechanisms of miRNAs in the Regulation of Liver CSCs

miRNAs, with a length of 19–25 nucleotides, usually function as negative regulators to repress gene expression via base-pair complementation with the 3′-UTR of target genes. Currently, many miRNAs alterations have been documented to participate in liver CSC regulation via various targets, as listed in Table 1. We summarize the detailed mechanisms as follows.

2.1. miRNAs Regulate Liver CSCs by Affecting the Wnt/β-Catenin Cascade. The Wnt/β-catenin signaling pathway has been shown to play an important role in regulating stem cell and tumorigenic properties in liver cancer. Inhibition of the Wnt pathway has also been shown to be effective in eliminating CSC-like features [22, 23]. When a Wnt ligand binds to its receptor FZD or LRPs, cytoplasmic Dvl is phosphorylated, which dissociates β-catenin from the Axin/APC/GSK3β destructive complex. Then, β-catenin accumulates and is translocated into the nucleus to form the β-catenin/LEF/TCF transcriptional complex, which initiates the transcription of downstream genes to regulate liver CSC maintenance [24]. Several miRNAs are reported to affect the Wnt/β-catenin cascade to regulate liver CSCs.

The miR-181 family is critical for maintaining the “stemness” of EpCAM+ liver CSCs. These miRNAs can directly bind to the 3′-UTR of CDX2, GATA6, and NLK mRNAs and inhibit their expression. CDX2 and GATA6 are the transcriptional regulators for hepatic differentiation, and NLK functions as the inhibitor of Wnt/β-catenin signaling. Therefore, miR-181 can enhance the self-renewal ability of EpCAM+ liver CSCs and maintain the undifferentiated state of CD133+ liver CSCs. Moreover, the important CSC transcriptional factor OCT4 can directly bind to the miR-1246 promoter to increase its expression [18]. Enhancing let-7b has also been reported to decrease the ratio of CD24+ in liver cancer cells via inhibiting FZD4 expression to inactivate the Wnt/β-catenin signaling cascade [25].

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Expression</th>
<th>Liver CSC subtype</th>
<th>Targets</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-181</td>
<td>↑</td>
<td>EpCAM+</td>
<td>CDX2, GATA6, NLK</td>
<td>[17]</td>
</tr>
<tr>
<td>miR-1246</td>
<td>↑</td>
<td>CD133+</td>
<td>AXIN2, GSK3β</td>
<td>[18]</td>
</tr>
<tr>
<td>Let7b</td>
<td>↓</td>
<td>CD24+/CD133+</td>
<td>FZD4</td>
<td>[25]</td>
</tr>
<tr>
<td>miR-148a</td>
<td>↓</td>
<td>EpCAM+ AFp</td>
<td>ACVR1</td>
<td>[27]</td>
</tr>
<tr>
<td>miR-200a</td>
<td>↓</td>
<td>Side population</td>
<td>β-Catenin</td>
<td>[28]</td>
</tr>
<tr>
<td>miR-214</td>
<td>↓</td>
<td>EpCAM+</td>
<td>β-Catenin, EZH2</td>
<td>[29]</td>
</tr>
<tr>
<td>Let-7a</td>
<td>↓</td>
<td>Sphere formation</td>
<td>TCF4</td>
<td>[31]</td>
</tr>
<tr>
<td>miR-25</td>
<td>↑</td>
<td>CD133+</td>
<td>PTEN/PI3K/AKT1/Bad</td>
<td>[32]</td>
</tr>
<tr>
<td>miRNA-21</td>
<td>↑</td>
<td>Side population</td>
<td>PTEN, RECK, PDCD4</td>
<td>[33]</td>
</tr>
<tr>
<td>miR-612</td>
<td>↓</td>
<td>EpCAM+CD133+</td>
<td>SPC/NANOG</td>
<td>[34]</td>
</tr>
<tr>
<td>miR-429</td>
<td>↑</td>
<td>EpCAM+</td>
<td>PBBP4/E2F1/OCT4</td>
<td>[35]</td>
</tr>
<tr>
<td>miR-145</td>
<td>↓</td>
<td>CD133+</td>
<td>Oct4</td>
<td>[36]</td>
</tr>
<tr>
<td>miRNA-200b</td>
<td>↓</td>
<td>CD13+CD24+</td>
<td>BMI1, CD13, CD24</td>
<td>[16]</td>
</tr>
<tr>
<td>miR-142-3p</td>
<td>↓</td>
<td>CD133+</td>
<td>CD133</td>
<td>[37]</td>
</tr>
<tr>
<td>miR-424, miR-222, miR-200b, let-7c</td>
<td>↓</td>
<td>α2δ1+</td>
<td>PBX3</td>
<td>[38]</td>
</tr>
<tr>
<td>miR-122</td>
<td>↓</td>
<td>CD133+</td>
<td>PDK4</td>
<td>[39]</td>
</tr>
<tr>
<td>miR-130b</td>
<td>↑</td>
<td>CD133+</td>
<td>TP53INP1</td>
<td>[40]</td>
</tr>
<tr>
<td>miR-155</td>
<td>↑</td>
<td>CD90+/CD133+</td>
<td>TP53INP1</td>
<td>[41]</td>
</tr>
<tr>
<td>miR-152</td>
<td>↓</td>
<td>CD133+</td>
<td>KIT</td>
<td>[42]</td>
</tr>
<tr>
<td>miR-589-5p</td>
<td>↓</td>
<td>CD90+</td>
<td>MAP3K8</td>
<td>[43]</td>
</tr>
<tr>
<td>miR-150</td>
<td>↓</td>
<td>CD133+</td>
<td>c-Myb</td>
<td>[44]</td>
</tr>
<tr>
<td>miR-148b</td>
<td>↓</td>
<td>Side population</td>
<td>NRP1</td>
<td>[45]</td>
</tr>
<tr>
<td>miR-137</td>
<td>↓</td>
<td>CD133/44+EpCAM+</td>
<td>ANT2</td>
<td>[46]</td>
</tr>
</tbody>
</table>
Li et al. defined the miR-148a-ACVR1/BMP circuit as having a regulatory role in liver CSCs. ACVR1 is an important receptor of the bone morphogenetic protein (BMP) that is closely implicated in the regulation of BMP/Wnt signaling. These authors found that miR-148a can inhibit the expression of ACVR1 by binding to the 3'-UTR of ACVR1, further leading to the downregulation of the direct downstream targets of the Wnt signaling pathway [26, 27]. There are also several other miRNAs that are reported to inhibit the stemness of liver CSCs through the Wnt/β-catenin pathway. For example, miRNA-200a can directly repress β-catenin; miRNA-214 can directly target β-catenin and indirectly inhibit β-catenin through EZH2 together; miR-612 can indirectly decrease the nuclear accumulation of β-catenin, and let-7a can deplete TCF4 [28–31].

### 2.2. miRNAs Regulate Liver CSCs by Affecting the PTEN/PI3K/akt/Bad Signaling Pathway

PTEN is a well-known tumor suppressor that serves as the natural inhibitor of PI3K to negatively regulate AKT. MiR-25 can directly target PTEN to stimulate the PI3K/akt pathway, which enables liver CSCs to resist apoptosis [32]. MiR-21 is upregulated in liver CSCs and can promote the migration and invasion of liver CSCs. A mechanistic study revealed that miR-21 can target the tumor suppressors PTEN, RECK, and PDCD4 to reduce their protein expression without affecting the mRNA levels [33].

### 2.3. miRNAs Affect Liver CSC Maintenance via Stemness-Related Transcriptional Factors and Markers

miR-612 can target and decrease the expression of SPI, which is an important transcriptional activator of the stemness-factor, Nanog. Through silencing SPI to reduce Nanog expression, miR-612 can shrink the number and size of liver CSCs [34]. OCT4 is another critical transcriptional factor for the maintenance of stem cells and liver CSCs [37, 58]. Li et al. reported that the overexpression of miR-429 endows EpCAM+ liver CSCs to increase stem-cell-associated gene expression, self-renewal, chemotherapeutic resistance, and tumorigenicity capacities. They found that miR-429 can inhibit the expression of PBP4 by binding to its 3'-UTR, which promotes the transcription activity of EZF1 on OCT4. Successively, the increased OCT4 is recruited to the EpCAM promoter and enhances its expression to strengthen liver CSCs’ properties [35]. It was also reported that miR-145 participates in the regulation of the stemness of liver CSCs through direct modulation of OCT4 [36]. Surface markers, such as CD13 and CD24, are functionally implicated in tumor development and progression. CD13 can help HCC CSCs achieve resistance to chemotherapy by inducing cells into dormancy and decreasing the accumulation of reactive oxygen species, DNA damage, and cell death [59, 60]. CD24 can help to activate the STAT3 signal, subsequently inducing Nanog expression to sustain CSC traits [61]. miR-200b is found to inhibit liver CSC formation via two independent mechanisms. On the one hand, miR-200b can directly suppress BMI1 expression, a stemness-related transcriptional factor. On the other hand, miR-200b can directly target ZEB1, which acts as a transcriptional activator to promote CD13 and CD24 expression [16]. CD133 is another type of surface marker that plays an important role in maintaining liver tumorigenesis [62]. miR-142-3p can bind to the 3'-UTR of CD133 and inhibit its expression, thereby attenuating the stemness of CD133+ liver CSCs [37]. Han et al. found that α2δ1 may serve as a novel liver CSC marker. miR-424, miR-222, miR-200b, and let-7c are downregulated in α2δ1+ liver CSCs, which synergistically play important roles in the acquisition and maintenance of liver CSC properties. These researchers found that low expression levels of the four miRNAs can increase the expression of PBX3, which can activate critical genes for liver CSCs, including CACNA2D1, EpCAM, SOX2, SALL2, NOTCH3, and WNT10A [38].

### 2.4. miRNAs Regulate Liver CSCs through Metabolic Reprogramming

Metabolic reprogramming is one of the most common cancer hallmarks. For example, the famous “Warburg effect” indicates that cancer cells prefer to elevate glycolysis and lactate production even in the presence of oxygen [63]. Song et al. reported that enhanced glycolysis is associated with CD133+ liver CSC characteristics, and the downregulation of miR-122 plays an important role in the abnormal metabolic process. Downregulation of miR-122 leads to the upregulated expression of its direct target, pyruvate dehydrogenase kinase 4 (PDK4). PDK4 can stimulate glycolysis and further increase the stemness gene expression and sphere formation capacity in CD133+ liver CSCs [39].

### 2.5. miRNAs Regulate Liver CSCs by Affecting Tumor-Associated Genes

TP53INP1 is a tumor suppressor and has antiproliferative and proapoptotic activities [64, 65]. miR-130b, upregulated in CD133+ liver CSCs, can repress TP53INP1 expression by directly targeting its 3'-UTR of TP53INP1, which helps CSCs to enhance proliferation, resist the chemotherapeutic drug, doxorubicin, and increase the expression of a series of stem cell-associated genes, including β-catenin, Notch-1, and Nestin [40]. MiR-155 is also reported to regulate liver CSCs via targeting TP53INP1 [41]. KIT, a well-established oncogene, can be suppressed by miR-152 directly by binding to the 3'-UTR of KIT, thus inhibiting cell proliferation and colony formation of CD133+ liver CSCs [42]. MAP3K8 is a well-known oncogene in various human tumors. miR-589-5p can target MAP3K8 and decrease its expression to suppress stemness-associated genes, including Oct4, Sox2, and Nanog, thereby reducing the capacity of forming self-renewal spheres and tumorigenicity [43]. miR-150 is able to target the oncogene C-Myb, by which miR-150 can inhibit C-Myb downstream genes, such as cyclin D1 and Bcl-2, to induce cell cycle arrest and apoptosis in CD133+ cells [44]. miR-148b is downregulated in liver CSCs, which can repress the oncogene NRPI to inhibit proliferation, metastasis, tumorigenesis, and drug resistance in liver CSCs [45]. miR-137 has a tumor suppressive role and can target the adenosine nucleotide translocator ANT2. The downregulation of miR-137 enhances the sphere-forming ability and resistance to sorafenib therapy as well as increasing CD133, CD44, and EpCAM expression, which accounts for the phenotypes of the liver CSCs [46]. TGF-β is an antimitogenic cytokine that becomes oncogenic in advanced tumors [66]. The restoration of miR-122 has been reported to be able to
induce a dormant state of stem-like HCC through the TGF-β pathway [67].

3. The Underlying Mechanisms of IncRNA in the Regulation of Liver CSCs

In contrast to miRNAs, IncRNAs have versatile mechanisms to control gene expression at both the transcriptional and posttranscriptional levels. IncRNAs can serve as a scaffold to recruit transcriptional factors within the promoter region to affect gene expression. IncRNAs can directly bind to DNA and RNA via a complementary sequence to impact transcriptional initiation or RNA stability. Additionally, IncRNA can modulate the posttranslational modification of proteins. Numerous IncRNAs have been demonstrated to be deregulated in cancers and exert critical roles in cancer development, such as malignant proliferation, metastasis, invasion, antiapoptosis, therapeutic resistance, and CSC formation. Recently, accumulating studies have focused on the regulation of IncRNAs in liver CSCs, as listed in Table 2. The underlying mechanisms are addressed as follows.

### 3.1. IncRNAs Sustain Liver CSCs by Activating the Wnt/β-Catenin Pathway

CD133 and CD13 are two widely used liver CSC surface markers, and the double-positive cell fraction exhibits obvious CSC properties, including a strong self-renewal capacity and chemical drug resistance. The transcriptome microarray analysis identified many differentially expressed IncRNAs in the CD133+ CD13+ cell population. Among these dysregulated IncRNAs, Inc-TCF7 and Inc-β-Catm are the most upregulated IncRNAs, which play critical roles in driving CSC self-renewal and tumor propagation via activating the Wnt/β-catenin pathway [19, 47]. Inc-TCF7 is located at chromosome 5, the neighboring TCF7 gene. Inc-TCF7 directly interacts with the SWI/SNF complex, and the evolutionarily conserved SWI/SNF complex can hydrolyze ATP to provide energy for mobilizing nucleosomes and remodeling chromatin. Through the association, Inc-TCF7 recruits the SWI/SNF to the promoter region of TCF7 and enhances TCF7 expression to increase the Wnt7a/Wnt4/Wnt2b levels, which triggers the Wnt pathway. Inc-TCF7-mediated Wnt activation leads to the self-renewal maintenance and tumorigenic capacity of liver CSCs [19]. Unlike the mechanism of Inc-TCF7, Inc-β-Catm can strengthen β-catenin protein stability via posttranscriptional modification to activate the Wnt signaling pathway. Inc-β-Catm is located on chromosome 1q, which frequently occurs as copy-number amplification in liver cancer cells. Inc-β-Catm is highly expressed in liver CSCs and positively correlated with tumor aggressiveness and poor prognosis in liver cancer. Inc-β-Catm functions as a scaffold to associate β-catenin and the methyltransferase EZH2 in liver CSCs. Successively, EZH2 methylates β-catenin at Lys49, which abolishes its polyubiquitination and protects β-catenin from proteasomal degradation. β-Catenin subsequently forms a complex with TCF and acts as a cotranscriptional factor to initiate Wnt signaling. The Inc-β-Catm and EZH2-dependent β-catenin stabilization is required for oncosphere formation in vitro and increases tumorigenic cell frequency in vivo [47]. Additionally, IncRNA-DANCR has a novel mechanism to enhance liver CSC features by regulating β-catenin and stimulating the Wnt pathway. IncRNA-DANCR is dramatically overexpressed in liver CSCs and acts as an independent predictor for poor prognostic outcome in liver cancer. Instead of forming a complex with proteins, DANCR interacts with the β-catenin mRNA transcript within its 3' UTR that harbors miR-214 and miR-320a binding sites. The competitive occupation enables DANCR to block miRNA-mediated β-catenin depletion and raises the β-catenin reservoir to elevate its downstream AXIN2, NOTUM, and OAT levels. Through derepressing β-catenin and eliciting Wnt signaling, DANCR enhances the expansion and maintenance of liver CSCs [20].

### 3.2. IncRNAs Regulate Liver CSCs through the IL-6/STAT3 Cascade

Apart from the Wnt pathway, the IL-6/STAT3 signaling cascade plays important roles in regulating liver CSC maintenance as well. IncRNA DILC and IncSox4 have been established to control CSC features via the STAT3 pathway in liver cancer. Inc-DILC, which is downregulated in EpCAM+, CD24+, or OV6+ liver CSCs, has a

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**Table 2: IncRNAs participate in the regulation of liver CSCs.**

<table>
<thead>
<tr>
<th>IncRNAs</th>
<th>Expression</th>
<th>Liver CSC subtype</th>
<th>Regulatory partners</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>IncRNATCF7</td>
<td>↑</td>
<td>CD133(^+)CD133(^+)</td>
<td>SWI/SNF complex</td>
<td>[19]</td>
</tr>
<tr>
<td>Inc-β-catm</td>
<td>↑</td>
<td>CD133(^+)CD133(^+)</td>
<td>β-catenin, EZH2</td>
<td>[47]</td>
</tr>
<tr>
<td>IncRNA-DANCR</td>
<td>↑</td>
<td>EpCAM(^+), CD90(^-)</td>
<td>CTNNB1 mRNA</td>
<td>[20]</td>
</tr>
<tr>
<td>Inc-DILC</td>
<td>↓</td>
<td>EpCAM(^+)CD24(^+)OV6(^+)</td>
<td>IL-6</td>
<td>[48]</td>
</tr>
<tr>
<td>IncSox4</td>
<td>↑</td>
<td>EpCAM(^+)CD133(^+)</td>
<td>STAT3</td>
<td>[49]</td>
</tr>
<tr>
<td>HULC, MALAT1</td>
<td>↑</td>
<td>CD133(^+)CD44(^+)CD24(^+)EpCAM(^+)</td>
<td>TRF2</td>
<td>[50]</td>
</tr>
<tr>
<td>Inc-CUDR</td>
<td>↑</td>
<td>CD133(^+)CD44(^+)CD24(^+)EpCAM(^+)</td>
<td>CTCF</td>
<td>[51]</td>
</tr>
<tr>
<td>Inc-CUDR</td>
<td>↑</td>
<td>CD133(^+)CD44(^+)CD24(^+)EpCAM(^+)</td>
<td>Cyclin D1</td>
<td>[52]</td>
</tr>
<tr>
<td>IncBRM</td>
<td>↑</td>
<td>CD133(^+)CD13(^+)</td>
<td>BRM</td>
<td>[53]</td>
</tr>
<tr>
<td>LncPVT1</td>
<td>↑</td>
<td>Sphere formation</td>
<td>NOP2</td>
<td>[21]</td>
</tr>
<tr>
<td>IncCAMTAI</td>
<td>↑</td>
<td>CD133(^+)CD13(^+)</td>
<td>CAMTA1</td>
<td>[54]</td>
</tr>
<tr>
<td>ICR</td>
<td>↑</td>
<td>ICAM-1(^-)</td>
<td>ICAM-1 mRNA</td>
<td>[55]</td>
</tr>
<tr>
<td>HOTAIR</td>
<td>↑</td>
<td>CD133(^+)CD44(^+)CD24(^+)EpCAM(^+)</td>
<td>CREB-P300-RNA polII complex</td>
<td>[56]</td>
</tr>
</tbody>
</table>
tumor suppressive function. A stem cell signaling PCR array revealed that Inc-DILC can modulate the JAK2/STAT3 cascade. A gain in Inc-DILC expression decreases the activated phospho-STAT3 protein levels, attenuates STAT3 nuclear translocation, and represses the transcriptional activity of STAT3-responsive elements. Consistently, increased phospho-STAT3 is observed in Inc-DILC-silenced spheroid-formed xenografts. Mechanistic studies have revealed that Inc-DILC binds to the IL-6 promoter and inhibits its transcription, while IL-6 is a critical cytokine in the activation of the JAK2/STAT3 axis. Of note, IL-6 can be induced by the inflammatory factors, TNF-α and IL-1β, and the inflammatory microenvironment is a major aspect for liver cancer progression. In CSC spheroids, the downregulation of Inc-DILC also enhances TNF-α and IL-1β-induced IL-6 expression. These findings suggest that Inc-DILC can coordinate the crosstalk between inflammatory signaling and the autocrine IL-6/STAT3 pathway to promote liver CSC expansion [48]. Through comprehensive analysis of GSE datasets, lncSox4 upregulation has been identified in advanced liver cancer and poor prognostic samples. Further study has demonstrated that lncSox4 is primarily increased in the CD133+ liver cancer cell fraction and CSC spheroids, and lncSox4 is essential for liver CSC self-renewal and tumorigenic capacity. Mechanistically, IncSox4 interacts with STAT3 and recruits it to the Sox4 promoter region, inducing H3K4me3 and H3K27ac modification to drive the Sox4 promoter activation and augments Sox4 expression. The IncSox4/STAT3-dependent Sox4 expression exerts an indispensable function in sustaining liver CSC propagation, which may serve as an important target for CSC eradication [49].

3.3. IncRNAs Induce Liver CSCs via the Telomere-Related Pathway. Wu et al. found that lncRNA HULC and MALAT1 are dramatically upregulated in liver cancer cells. When MALAT1 is cooverexpressed with HULC in liver CSCs, the two lncRNAs can cooperate to promote liver CSC growth via the telomere repeat-binding factor 2 (TRF2). MALAT1 and HULC coexpression facilitates the TRF2 promoter and enhancer to form a loop, recruiting P300, RNA pol II, and CREP into the loop, which enhances TRF2 expression and its phosphorylation and SUMOylation. Then, the excessive TRF2 forms a complex with HULC and MALAT1 on the telomeric region to protect telomeres from degradation. As a result, telomerase activity and microsatellite instability (MSI) are obviously induced in the liver CSCs [50]. CUDR is a novel lncRNA that can trigger the malignant transformation of hepatocyte-like cells through epigenetically remodeling TRF2, lncRNA HULC, and the β-catenin promoter structure to drive the expression of these oncogenes [51]. Furthermore, CUDR has been reported to be highly expressed in CD133+/CD44+/CD24−/EpCAM+ liver CSCs. Mechanistically, CUDR interacts with cyclin D1 to increase IncRNA H19 expression and enhance the association between TERT and TERC, thereby promoting telomerase activity and prolonging telomere length. Additionally, with the help of CTCF, the CUDR-cyclin D1 complex is recruited to the C-Myc gene promoter region to increase C-Myc expression. Synergistically, the excessive TERT and C-Myc expression account for liver cancer stem cell proliferation [52].

3.4. IncRNAs Affect Liver CSC Properties through Multiple Other Mechanisms. IncBRM is another upregulated lncRNA identified in the CD133+ CD133+ transcriptome microarray and is required for liver CSCs to maintain the self-renewal potential and initiate tumorigenicity. IncBRM can facilitate BRG1-embedded BAF complex formation and recruit the complex to the YAP1 promoter to trigger YAP1 transcription in a KLF4-dependent manner, thereby driving liver CSC properties [53]. lncRNA-mPvt1 is an oncofetal RNA that can promote stem cell-like properties in murine cells. Its human homologue lncRNA-hPVT1 is highly expressed in liver cancer cells and correlates with poor prognosis. lncRNA-PVT1 can interact with NOP2 and stabilize NOP2 from proteasomal degradation, which promotes malignant cell proliferation and self-renewal of spheroids [21]. Ding et al. identified a lncRNA, termed lncCAMTA1, that is overexpressed in CD133+ CD133+ CSCs and induces liver CSC proliferation. The lncCAMTA1 transcription orientation is antisense to the tumor suppressive gene CAMTA1, and their expression is negatively correlated in liver cancer samples. lncCAMTA1 physically binds to the CAMTA1 promoter and mediates a repressive chromatin structure to decrease CAMTA1 expression. The lncCAMTA1-dependent CAMTA1 downregulation accounts for liver CSC properties [54]. ICAM-1 is an established CSC marker in liver cancer [68]. lncICR is the ICAM-1-related lncRNA and is overexpressed in the ICAM-1+ liver CSC population. lncICR can form an RNA duplex with the ICAM-1 transcript via their complementary sequence, which increases the stability of ICAM-1 mRNA and augments its expression to maintain the liver CSC feature [55]. Lu et al. reported that the lncRNA HOTAIR can enhance liver cancer stem cell proliferation and malignant progression through downregulation of SETD2. The lncRNA HOTAIR can block the recruitment of the CREB-P300-RNA pol II complex to the SETD2 promoter to inhibit the expression and phosphorylation of SETD2, leading to the decreased formation of the hMSH6-H3k36me3-Skp2 complex to inhibit the DNA damage repair. In addition, the microsatellite instability (MSI) and abnormal expression of cell cycle related genes triggered by HOTAIR overexpression also contribute to the malignant growth of liver CSCs [56].

4. Perspective and Conclusion

miRNA and IncRNA are two major classes of ncRNAs. Previous studies have demonstrated that miRNAs and IncRNAs are stable in body fluids, and they can be easily and noninvasively accessible. Some of the miRNAs and IncRNAs have tissue- or disease-specific expression patterns. Therefore, miRNAs and IncRNAs have been recognized to be ideal biomarkers for early diagnosis, prognostic prediction, and therapeutic evaluation in cancer [69, 70]. For example, we have reported that low expression of miRNA-26a may predict poor prognosis and response to adjuvant INF-α treatment in liver cancer [71]. The prostate-specific IncRNA PCA3 has become the first FDA-approved IncRNA-based biomarker for prostate
mRNA-targeted treatments have reached clinical trial, such as miRNA-34 mimics for treating cancer (phase I clinical trials) [73] and anti-miRs targeted miR-122 forremedying hepatitis (phase II clinical trials) [74]. Given the diverse regulatory mechanisms of ncRNAs in liver CSCs mentioned in this review, these dysregulated ncRNAs have great potential to be applied in diagnosis and prognosis. Furthermore, it may be feasible to target these aberrant ncRNAs to block or eradicate liver CSCs in cancer treatment.
In this review, we emphasized the effects of ncRNAs on signaling pathways, finding that many miRNAs or lncRNAs control liver CSC properties by targeting different components of the Wnt/β-catenin pathway, like miR-148, miR-1246, miR-200a, lncRNA TCF-7, lncRNA β-Cam, and so on (Figure 1). Recently, the Wnt/β-catenin axis has become an attractive therapeutic target because of its important functions in cancer. Several antibodies and small molecular inhibitors are undergoing preclinical or clinical trials, such as OMP-18R5 (antibody against FZD7), OMP54F28 (soluble Fzd decoy receptor), and PRI-724 (inhibitor of TCF-CBP interaction) [75]. However, there are still no applicable candidates in clinical practice because of the serious side effects, as proper Wnt/β-catenin activity is essential to sustain normal cell survival. How to precisely regulate Wnt/β-catenin is a great conundrum. Targeting these regulatory miRNAs and lncRNAs may be an alternative approach to accomplish precise modulation of Wnt/β-catenin activation.

In conclusion, we overview the multiple functions and diverse mechanisms of miRNAs and lncRNAs in liver CSCs and highlight their potential clinical applications as novel diagnostic and prognostic biomarkers and therapeutic targets. Our review provides new insights to understand liver CSCs and delineates new clues to develop a ncRNA-based therapeutic strategy for liver cancer.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors’ Contributions

Jing Zhao and Yan Fu contributed equally to this work.

Acknowledgments

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References


H. Han, Y. Du, W. Zhao et al., “PPIX3 is targeted by multiple miRNAs and is essential for liver tumour-initiating cells,” Nature Communications, vol. 6, article no. 8271, 2015.


MicroRNA-219-5p Promotes Tumor Growth and Metastasis of Hepatocellular Carcinoma by Regulating Cadherin 1

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MicroRNAs play significant roles in the development of cancer and may serve as promising therapeutic targets. In our previous work, miR-219-5p was identified as one of the important metastasis-related microRNAs in HCC. Here we demonstrated that miR-219-5p expression was elevated in HCC tissues and was associated with vascular invasion and dismal prognosis. In multivariate analysis, miR-219-5p was identified as an independent prognostic indicator for HCC patients. Functional mechanism analyses showed that miR-219-5p promoted HCC cell proliferation and invasion in vitro, as well as in vivo, tumor growth and metastasis in nude mice models bearing human HCC tumors. In addition, cadherin 1 (CDH1) was revealed to be a downstream target of miR-219-5p in HCC cells. In conclusion, miR-219-5p promotes tumor growth and metastasis of HCC by regulating CDH1 and can serve as a prognostic marker for HCC patients.

1. Introduction

Hepatocellular carcinoma (HCC) is one of the most common causes of cancer-related deaths worldwide, with high incidence of tumor recurrence and metastasis [1]. Identification of molecular markers plays a critical role in predicting the clinical outcome and promoting individual therapies for patients with HCC [2, 3].

MicroRNAs (miRNAs) have been implicated in regulation of pathogenesis of human tumors and could be potential biomarkers for diagnosis and prognosis [4, 5]. Recent studies have demonstrated that miRNAs participate in diverse human cancers processes including cell differentiation, proliferation, and apoptosis, as well as invasion and metastasis. For instance, miR-125 is a tumor suppressor that can decrease cell proliferation and metastasis through suppressing LIN28B expression in HCC [6], while miR-122a exerts tumor promoting effects on HCC by p53-dependent way [7]. Thus, cancer-specific miRNAs might be promising targets for cancer therapy [8].

Recently, miRNAs are demonstrated to function as critical regulators of cancer invasion and metastasis [9]. In our previous work, miR-219-5p is identified as one of the significant metastasis-related miRNAs in HCC [10]. However, little is known of the possible mechanism of miR-219-5p involved in HCC metastasis. In the present study, we found that miR-219-5p was upregulated in HCC tissues, was related to overall survival (OS) time of HCC patients, and promoted the proliferation and metastasis of HCC cells via downregulating CDH1. These results provide a clear understanding of the underlying mechanism by which miR-219-5p promotes HCC metastasis.

2. Materials and Methods

2.1. Clinical Tissue and Cell Culture. HCC tissues were obtained from patients who are treated with surgical resection in Huashan Hospital, Fudan University, and each patient had specific clinical-pathological information.
Before surgical operations and collections of clinical tissues, all individuals wrote informed consent.

Human HCC cell lines Hep3B, Huh7, HepG2, MHCC-97H, and HCCLM3 were cultured in DMEM (Gibco) with 10% FBS. And they were propagated at 37°C in 5% CO₂.

2.2. Cell Transfection. HepG2 and MHCC-97H cells were transfected with miR-219-5p mimic (50 nM) and miR-219-5p antagonist (400 pmol/ml) according to the manufacturer instructions. miR-219-5p mimic, antagonist, and their corresponding negative controls were purchased from Ribobio (Shanghai, China).

2.3. RNA Extraction and Quantitative Reverse Transcription-Polymerase Chain Reaction (qRT-PCR). RNA of samples was obtained by TRIzol reagent (Invitrogen, USA). Then, we reversely transcribed RNA into cDNA according to the instruction of PrimeScript RT Master Mix and Mir-X miRNA First-Strand Synthesis Kit (TaKaRa, Shanghai, China). Next, cDNA was quantified by application of SYBR Premix Ex Taq II (TaKaRa) with gene-targeted or miR-specific primers. We applied the delta-delta Ct method to conduct quantification as well as calculation of the relative expression of each mRNA or miRNA. Primer sequences are listed in Table S1. Each sample was carried out three times.

2.4. Cell Proliferation Assay. The cell proliferation assay was conducted with Counting Kit-8 (CCK-8) (Tongren, Shanghai, China). 5000 cells (transfected with miR-219-5p mimic, miR-ctrl, miR-219-5p antagonist, antagonist NC) were planted in 96-well plates. Then 10% CCK-8 solution was added. The absorbance of each sample was assessed by a microplate reader set at 450 nM. Each sample was performed three times.

2.5. Cell Cycle Analysis and Apoptosis Assay. Cell cycle analysis was conducted with each sample fixed into 70% ethanol at 4°C. We added Propidium iodide (PI) and RNase to samples according to manufacturers’ instructions (Beyotime, Shanghai, China). After staining, cells were measured by flow cytometry (BD Bioscience, MA, USA). We analyzed results by Cell Quest software (BD Biosciences). Apoptosis assay was conducted by samples bound with Annexin V-FITC and 7-AAD according to the manufacturers’ instructions (BD Bioscience). Then we analyzed samples by means of flow cytometry as described above. Each sample was replicated in triplicate.

2.6. Dual-Luciferase Reporter Assay. The wild-type sequence containing the predicted target sites of miR-219-5p in the 3’ UTR of CDH1 mRNA was synthesized by JIELI corporation (Shanghai, China). We mutated the target sites from CUC-CAC to GACCAG. After plasmid transfection, luciferase activities were assessed according to the manufacturer’s instruction (Promega, USA). All samples were independently repeated three times.

2.7. Western Blotting. Samples were obtained with RIPA lysis buffer added with protease inhibitors. After quantification with bicinchoninic acid (BCA) assay (Weiao, Shanghai, China), we separated each protein through 10% SDS-PAGE and then moved them onto PVDF membranes (Millipore, USA). Then, samples were blocked with 5% nonfat milk. After incubation with primary antibodies against GAPDH and CDH1 (Cell Signaling Technology, Danvers, MA, USA) and secondary antibodies, protein levels were detected with ImageQuant™ LAS 4000 (GE Healthcare Life Sciences). Each sample was analyzed three times.

2.8. Cell Migration and Invasion Assay. The methods of cell migration and invasion assays were constructed as previously described [11].

2.9. Animal Model. Subcutaneous HCC model was established by injecting 5 × 10⁶ MHCC-97H cells (transfected with antagonist NC or antagonist miR-219-5p) into BALB/c nude mice (Shanghai SLAC Laboratory Animal Co.). And after 6 weeks, the tumor of each group was isolated. To establish in vivo tumor metastasis model, we transplanted tumors tissues (~2 mm³) from the above subcutaneous HCC model to the livers of BALB/c mice. After 6 weeks, the tumors and lung tissues were obtained.

Tumor size was measured twice weekly with a calliper and the volume was calculated in mm³.

2.10. Statistical Analysis. Data was reported as mean ± SD. Data analysis was conducted by IBM SPSS Statistics Version 22. X² and t-test were applied to measure differences between groups. Results were determined to be statistically significant when P < 0.05.

3. Results

3.1. miR-219-5p Upregulation Is Associated with Metastasis and Dismal Prognosis of HCC. We analyzed the expression levels of miR-219-5p in 191 paired HCC tissues and corresponding noncancerous liver tissues by using qRT-PCR and found that miR-219-5p was significantly increased in HCC tissues compared with the nontumor liver tissues (P < 0.001) (Figure 1(a)). The expression levels of miR-219-5p were remarkably higher in HCC patients with metastasis in comparison to those without metastasis (P < 0.001) (Figure 1(b)). To further validate the role of miR-219-5p in HCC metastasis, we analyzed miR-219-5p in various HCC cell lines with different metastatic potentials and found that miR-219-5p levels in the HCC cells with high metastatic potentials were higher than those nonmetastatic cell lines (Supplementary Figure 1(A)). These results indicated that miR-219-5p upregulation is correlated with HCC metastasis. Moreover, elevated miR-219-5p expression was found to be correlated with vascular invasion (P = 0.003) and worse differentiation (P = 0.011) of liver tumor, as well as severe liver cirrhosis (P < 0.001) (Table 1). Kaplan–Meier analysis showed that miR-219-5p overexpression was associated with poorer overall survival and higher recurrence...
Figure 1: The association of miR-219-5p upregulation with metastasis and prognosis of HCC. (a) Relative expressions of miR-219-5p in 191 paired liver cancer tissues and paracancerous tissue samples. (b) The comparison of miR-219-5p levels between metastatic and nonmetastatic HCC tissues. Patients with high miR-219-5p level had a trend of worse overall survival (c) and significantly high recurrence rates compared with those with low miR-219-5p (d). Data are shown as mean ± SD. ***P < 0.001 versus the control.

Univariate analysis showed that miR-219-5p, tumor size, tumor encapsulation, and vascular invasion were related to overall survival (OS) (Table 2); miR-219-5p, HBsAg, tumor size, vascular invasion, and tumor number were associated with HCC recurrence (Table 3). Multivariate analysis showed that miR-219-5p, vascular invasion, and tumor size were independent prognostic indicators for overall survival and tumor recurrence. Therefore, these results suggested that miR-219-5p upregulation can be a predictor of metastasis and dismal prognosis of HCC patients.

3.2. The Effects of miR-219-5p on In Vitro Proliferation and Invasion of HCC Cells. To investigate the biological significance of miR-219-5p, we treated human HCC cell lines with miR-219-5p mimic or antagomir that would lead to different expression levels of miR-219-5p. Upregulation of miR-219-5p in HepG2, which had a low endogenous expression level, by miR-219-5p mimic induced significant increases in the abilities of proliferation (Figure 2(a); Supplementary Figure 2(A)). On the other hand, knockdown of miR-219-5p in MHCC-97H (with a high endogenous miR-219-5p level) by miR-219-5p antagomir (Supplementary Figure 2(A)) significantly inhibited the proliferation of cells (Figure 2(a)). What is more, the cell cycle distribution analysis showed that the cell number in G1 phase of HepG2 cells treated with miR-219-5p mimic was obviously decreased, and the cell number in S phase was increased compared with the ctrl. Cell cycle arrest at the G1 to S transition was found in MHCC-97H cells after treated with miR-219-5p antagomir (Figure 2(b); Supplementary Figure 2(B)). Furthermore, miR-219-5p mimic transfection significantly suppressed the apoptosis of HepG2 cells compared with ctrl, while miR-219-5p downregulation induced by miR-219-5p antagomir markedly promoted the apoptosis of MHCC-97H cells (Figure 2(c)). Next, we performed transwell assays to evaluate the invasion and migration abilities of HepG2 and MHCC-97H cells. Results showed that miR-219-5p upregulation significantly enhanced the migration and invasion abilities of HepG2, and miR-219-5p knockdown induced by miR-219-5p antagomir led to reduced number of migrated and invaded cells (Figure 2(d); Supplementary Figure 2(C)). Taken together, these data suggested that miR-219-5p can promote the proliferation, cell cycle transition of G1 into S phase, antiapoptotic potentials, and metastatic phenotype of HCC cells.
Table 1: Relationship between miR-219-5p level and clinicopathologic features.

<table>
<thead>
<tr>
<th>Variables</th>
<th>miR-219-5p expression</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low (n = 96)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>High (n = 95)</td>
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</tr>
<tr>
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</tr>
<tr>
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<td>13</td>
</tr>
<tr>
<td>Male</td>
<td>78</td>
<td>82</td>
</tr>
<tr>
<td>Age (years)</td>
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<td>32</td>
</tr>
<tr>
<td></td>
<td>&lt;50</td>
<td>64</td>
</tr>
<tr>
<td>HBV status</td>
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<td>94</td>
</tr>
<tr>
<td></td>
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</tr>
<tr>
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<td>16</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>80</td>
</tr>
<tr>
<td>AFP (ng/mL)</td>
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</tr>
<tr>
<td></td>
<td>≤20</td>
<td>32</td>
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<tr>
<td>Tumor size (cm)</td>
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</tr>
<tr>
<td></td>
<td>≤5</td>
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</tr>
<tr>
<td>Tumor number</td>
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</tr>
<tr>
<td></td>
<td>Single</td>
<td>92</td>
</tr>
<tr>
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<td>52</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>44</td>
</tr>
<tr>
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<td>Yes</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>65</td>
</tr>
<tr>
<td>Tumor differentiation</td>
<td>I–II</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>III–IV</td>
<td>31</td>
</tr>
</tbody>
</table>

3.3. Effects of miR-219-5p on In Vivo Tumor Growth and Lung Metastasis of HCC Xenografts. To further validate promoting roles of miR-219-5p in HCC progression, we established HCC xenografts models by subcutaneous implantation of MHCC-97H cells (transfected with miR-219-5p antagomir or antagomir NC). The average tumor volume of the miR-219-5p antagomir-treated group was obviously smaller than that of antagomir NC group (P < 0.05) (Figures 3(a) and 3(b)). To further validate its effect on the lung metastasis of HCC, the tumor tissues (1-2 mm³) were obtained from subcutaneous xenografts to establish orthotopic implantation models of nude mice. The average volume of orthotopic tumors in the miR-219-5p antagomir group was significantly smaller than that in the antagomir NC group (P < 0.05) (Figure 3(c)). Moreover, the total number of lung metastases in the miR-219-5p antagomir group was decreased compared with the antagomir NC (P < 0.01) (Figure 3(d)). These results suggested that miR-219-5p plays a crucial role in promoting in vivo tumor growth and lung metastasis of HCC.

3.4. CDH1 Is a Direct Target of microRNA-219-5p. Next, we searched for putative target genes of miR-219-5p in microRNA.org. We identified CDH1 as a direct target of miR-219-5p and the potential binding sequence in CDH1 3’UTR (Figure 4(a)). We carried out a dual-luciferase reporter assay to prove that CDH1 is a direct target of miR-219-5p. The reporter vector containing wild-type (CDH1-WT) or mutated-type binding sequence (CDH1-MT) was transfected into HEK293T cells along with miR-219-5p mimic or ctrl. Results showed that cotransfection of miR-219-5p with CDH1-WT, rather than with CDH1-MT, resulted in a significant decrease in luciferase activity compared with ctrl group (P < 0.05) (Figure 4(b)). To further validate the influence of miR-219-5p on CDH1, we overexpressed miR-219-5p in HepG2 cells and knocked down miR-219-5p in MHCC-97H cells, finding that miR-219-5p upregulation led to a significant decrease of CDH1 expression at both mRNA and protein levels (P < 0.01). And, miR-219-5p knockdown resulted in enhanced CDH1 expression (P < 0.01) (Figures 4(c) and 4(d)). Moreover, the linear regression analysis showed a negative relevance between miR-219-5p and CDH1 in HCC tissues (R² = 0.4225; P < 0.001) (Figure 4(e)). These suggested that miR-219-5p is closely associated with negative regulation of CDH1 and CDH1 is a direct target of miR-219-5p.

4. Discussion

Metastatic relapse remains one of the major reasons for the dismal prognosis of HCC, which is a complicated process including cell adhesion, migration, and getting to target organs. Many molecules have been determined to be related to HCC metastasis [12]. However, the mechanism of HCC metastasis is not fully understood yet. Thus, characterizing the metastasis-related molecules and signaling pathways may provide more clues to the understanding of HCC metastasis. The clinical relevance and biological functions of miRNAs expression have been confirmed in various human solid tumors [13]. Thus, miRNAs were identified as superior molecular markers. Recently, an increasing number of studies have reported the indispensable roles of miRNAs in HCC [14–16]. In our previous study, miR-219-5p was found to be a promoter for HCC metastasis [10]. However, some studies demonstrated inconsistent results in other kinds of cancers. For example, miR-219-5p was reported to function as a tumor suppressor in colorectal and gastric cancers [17, 18]. The real reason is not clear. These results stimulate us to investigate the role of miR-219-5p in regulating aggressive phenotype of HCC cells.

In the present study, we found that miR-219-5p expression levels were remarkably upregulated in HCC tissues compared with the nontumor liver tissues, and high miR-219-5p levels were significantly associated with metastasis and dismal prognosis of HCC. Using gain- and loss-functional analyses,
Table 2: Univariate and multivariate analyses of factors associated with overall survival (OS) in patients with hepatocellular carcinoma (HCC).

<table>
<thead>
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<th>Features</th>
<th>Univariate $P$</th>
<th>Overall survival</th>
<th>Multivariate 95% CI</th>
<th>$P$</th>
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<td>Sex</td>
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</tr>
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</tr>
<tr>
<td>&gt;50 versus ≤50</td>
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<tr>
<td>HBsAg</td>
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<tr>
<td>Positive versus negative</td>
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<tr>
<td>AFP</td>
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<tr>
<td>20 ng/ml versus ≤20 ng/ml</td>
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<tr>
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<td></td>
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<td>Yes versus no</td>
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<td>Tumor number</td>
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<td>1.433–3.903</td>
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</tr>
</tbody>
</table>

Table 3: Univariate and multivariate analyses of factors associated with recurrence in patients with hepatocellular carcinoma (HCC).

<table>
<thead>
<tr>
<th>Features</th>
<th>Univariate $P$</th>
<th>Recurrence</th>
<th>Multivariate 95% CI</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td>NA</td>
</tr>
<tr>
<td>Male versus female</td>
<td>0.331</td>
<td></td>
<td>NA</td>
<td></td>
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<tr>
<td>Age</td>
<td>0.832</td>
<td></td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>&gt;50 versus ≤50</td>
<td>0.043</td>
<td></td>
<td>0.761</td>
<td>0.419</td>
</tr>
<tr>
<td>HBsAg</td>
<td></td>
<td></td>
<td>0.392–1.476</td>
<td></td>
</tr>
<tr>
<td>Positive versus negative</td>
<td>0.615</td>
<td></td>
<td>NA</td>
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<tr>
<td>AFP</td>
<td></td>
<td></td>
<td>0.542–1.193</td>
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<td>20 ng/ml versus ≤20 ng/ml</td>
<td>0.065</td>
<td></td>
<td>NA</td>
<td></td>
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<tr>
<td>Liver cirrhosis</td>
<td></td>
<td></td>
<td>0.010</td>
<td>0.005</td>
</tr>
<tr>
<td>Yes versus no</td>
<td></td>
<td></td>
<td>1.579</td>
<td></td>
</tr>
<tr>
<td>Tumor size</td>
<td></td>
<td></td>
<td>0.751–3.318</td>
<td></td>
</tr>
<tr>
<td>&gt;5 cm versus ≤5 cm</td>
<td></td>
<td></td>
<td>0.014</td>
<td></td>
</tr>
<tr>
<td>Tumor encapsulation</td>
<td></td>
<td></td>
<td>1.870</td>
<td></td>
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<tr>
<td>Yes versus no</td>
<td></td>
<td></td>
<td>1.205–2.902</td>
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</tr>
<tr>
<td>Tumor number</td>
<td></td>
<td></td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td>Multiple versus single</td>
<td></td>
<td></td>
<td>1.663</td>
<td></td>
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<tr>
<td>Vascular invasion</td>
<td></td>
<td></td>
<td>1.072–2.577</td>
<td></td>
</tr>
<tr>
<td>Yes versus no</td>
<td></td>
<td></td>
<td>0.023</td>
<td></td>
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<tr>
<td>Tumor differentiation</td>
<td></td>
<td></td>
<td>1.072–2.577</td>
<td></td>
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<tr>
<td>I–II versus III–IV</td>
<td></td>
<td></td>
<td>0.023</td>
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<td>miR-219-5p</td>
<td></td>
<td></td>
<td>0.023</td>
<td></td>
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<tr>
<td>High versus low</td>
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<td></td>
<td>0.023</td>
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</table>

Abbreviations. HBsAg: hepatitis B surface antigen; HR: hazard ratio; CI: confidence interval.
Figure 2: The effects of miR-219-5p on in vitro proliferation and invasion of HCC cells. (a) The alterations in cell proliferation of HepG2 cells after upregulation of miR-219-5p by mimic transfection (left) and MHCC-97H cells after knockdown of miR-219-5p by antagomir (right) were detected by CCK8 assay. (b) The cell cycle distribution of HepG2 and MHCC-97H cells after transfection with miR-219-5p mimic or antagomir. (c) Representative pictures of apoptosis of HepG2 and MHCC-97H cells after transfection with miR-219-5p mimic or antagomir detected by flow cytometry. (d) Migration and invasion of cells were determined by transwell assay in HepG2 and MHCC-97H cells treated with miR-219-5p mimic/antagomir and the corresponding negative control (magnification ×100). Data are shown as mean ± SD. *P < 0.05, **P < 0.001 versus the control.
FIGURE 3: Effects of miR-219-5p on in vivo tumor growth and lung metastasis of HCC. (a, b) The differences in tumor size and volume of the subcutaneous implantation models of MHCC-97H cells after transfection with miR-219-5p antagomir or antagomir NC. (c) Comparison of the tumor volumes in the orthotopic implantation models of MHCC-97H cells after transfection with antagomir to knockdown miR-219-5p. (d) Representative images of lung metastasis (left) and comparison of the numbers of lung metastatic nodes in orthotopic implantation nude mice models of MHCC-97H cells after transfected with miR-219-5p antagomir or antagomir NC (magnification ×200). Data are shown as mean ± SD. *P < 0.05, **P < 0.01.
miR-219-5p 3’ UCUUAACG —CAAACCUGUUAGU 5’
WT CDH1 3’UTR 5’... UGAUUUCAACUUUGACAAUCA... 3’
MT CDH1 3’UTR 5’... UGAUUUCAACUUUGACAGCCA... 3’

(a)

Figure 4: CDH1 is identified as a downstream target of miR-219-5p. (a) Sequences of CDH1 3’ UTR and miR-219-5p according to the prediction of microRNA.org. Wild-type and mutated-type binding sequences of CDH1 3’ UTR are shown. (b) Relative luciferase activity in HEK293T cells transfected with reporter vector containing wild-type or mutated-type binding sequence along with miR-219-5p mimic or negative control. CDH1 protein (c) and mRNA levels (d) in HCC cells treated with miR-219-5p mimic or antagonir. (e) Linear regression analysis between miR-219-5p and CDH1 in HCCs. (f) Working model for the role of miR-219-5p in regulation of CDH1 in HCC. Data are shown as mean ± SD. * P < 0.05, ** P < 0.01.
we found that miR-219-5p could promote in vitro proliferation, migration, and invasion of HCC cells. Furthermore, using loss-functional assays, we demonstrated that miR-219-5p promoted in vivo tumor growth and distal pulmonary metastasis of HCC. These provided more evidence to support that miR-219-5p is an important promoter for HCC growth and metastasis.

Another significant finding of the present study is that CDH1 is identified as a downstream target of miR-219-5p. CDH1, a suppressive oncogene, encodes the epithelial cell adhesion molecule, E-cadherin, which contributes to cell polarity and cell-cell adhesion [19–21]. Low expression levels of CDH1 were found to be correlated with aggressive clinicopathological factors and poor survival [22, 23]. Also, CDH1 inactivation resulted in the loss of cell-cell adhesion, which contributes to metastasis in a variety of tumors [24–26]. Furthermore, there is increasing evidence that multiple mechanisms are involved in the expression of CDH1, including epigenetic DNA methylation, somatic mutations, chromosomal deletions, and protein modification [11, 27, 28]. Recently, miRNAs have been determined to play gene-regulatory roles [28]. In this study, we found the following: (1) Bioinformatic analysis indicated that CDH1 can be a potential downstream target of miR-219-5p. (2) In a dual-luciferase reporter assay, cotransfection of miR-219-5p with CDH1 containing wild-type rather than mutated-type binding sequence resulted in a significant decrease in luciferase activity. (3) miR-219-5p upregulation led to a significant decrease of CDH1 expression; miR-219-5p knockdown resulted in enhanced CDH1 expression. (4) The linear regression analysis showed a negative relevance between miR-219-5p and CDH1 in HCCs. These indicated that miR-219-5p is closely associated with negative regulation of CDH1 and CDH1 is a direct target of miR-219-5p. Collectively, miR-219-5p promotes HCC growth and metastasis by downregulating CDH1 (Figure 4(f)).

In conclusion, these data suggest that miR-219-5p upregulation is an independent prognostic indicator for HCC patients. It plays an important role in promoting HCC growth and metastasis by downregulating CDH1. These provide more clues to develop novel strategies to combat HCC metastasis.

Supplementary Materials

Table S1: The primers listed were used for qPCR. Supplementary Figure 1: (A) miR-219-5p expressions in six liver cancer cell lines. GAPDH served as internal control. Data are shown as mean + SD. *P < 0.05, **P < 0.01. Supplementary Figure 2: the relative expression levels of miR-219-5p in HepG2 and MHCC-97H cells were examined after the cells were treated with miR-219-5p mimic, antagonir, or negative control (A) for 48 h using RT-qPCR. The analysis of distribution of cells with miR-219-5p mimic/antagomir was shown by histogram (B). The histogram of migration and invasion shows the mean ± SD of three independent experiments (C). Data are shown as mean ± SD. *P < 0.05, **P < 0.01. (Supplementary Materials)

References


Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors’ Contributions

Jing Yang and Yuan-Yuan Sheng contributed equally as first authors.

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