Review Article

The Diverse Mechanisms of miRNAs and lncRNAs in the Maintenance of Liver Cancer Stem Cells

Jing Zhao,1,2 Yan Fu,1 Jing Wu,3 Juan Li,1 Guangjian Huang,1 and Lunxiu Qin1,2

1Department of General Surgery, Huashan Hospital, Fudan University, Shanghai 200040, China
2Cancer Metastasis Institute, Fudan University, Shanghai 200040, China
3Department of Infectious Diseases, Huashan Hospital, Fudan University, Shanghai 200040, China

Correspondence should be addressed to Guangjian Huang; gjhuang@fudan.edu.cn and Lunxiu Qin; qinlx@fudan.edu.cn

Received 23 February 2018; Accepted 3 April 2018; Published 15 May 2018

Academic Editor: Kangsheng Tu

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 lncRNA, 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 lncRNAs 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 (lncRNA), 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, lncRNA has extremely complicated functions and mechanisms to manipulate gene expression in cis- and in trans-manners. LncRNA can interact with DNA, RNA,
and proteins to affect transcriptional machinery assembly, alternative splicing processes, and chromatin remodeling [14]. Dysregulated expression of miRNAs and IncRNAs 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 IncRNAs are essential for sustaining CSC properties in liver cancer, like miR-200b, miR-1246, Inc-TCF-7, Inc-DANC4, Inc-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 IncRNAs 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 simultaneously [17]. In addition, miR-24 can silence AXIN2 and GSK3β expression, leading to the nuclear accumulation and activation of β-catenin, which promotes stem cell-like phenotypes in 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+133+ in liver cancer cells via inhibiting FZD4 expression to inactivate the Wnt/β-catenin signaling cascade [25].

| miRNA   | Expression | Liver CSC subtype | Targets                        | Reference |
|---------|------------|------------------|--------------------------------|-----------|
| miR-181 | ↑          | EpCAM+           | CDX2, GATA6, NLK               | [17]      |
| miR-1246| ↑          | CD133+           | AXIN2, GSK3β                   | [18]      |
| Let-7b  | ↓          | CD24+CD133+      | FZD4                           | [25]      |
| miR-148a| ↓          | EpCAM+AFP        | ACVR1                          | [27]      |
| miR-200a| ↓          | Side population  | β-Catenin                      | [28]      |
| miR-214 | ↓          | EpCAM+           | β-Catenin, EZH2                | [29]      |
| Let-7a  | ↓          | Sphere formation | TCF4                           | [31]      |
| miR-25  | ↑          | CD133+           | PTEN/P13K/AKT/Bad              | [32]      |
| miRNA-21| ↑          | Side population  | PTEN, RECK, PDCD4              | [33]      |
| miR-612 | ↓          | EpCAM+CD133+     | SP1/Nanog                      | [34]      |
| miR-429 | ↑          | EpCAM+           | PBP4/E2F1/OCT4                 | [35]      |
| miR-145 | ↓          | CD133+           | Oct4                           | [36]      |
| miRNA-200b|↓          | CD133+CD24+     | BMI1, CD13, CD24              | [16]      |
| miR-142-3p|↓          | CD133+           | CD133                        | [37]      |
| miR-424, miR-222, miR-200b, let-7c|↓|α2δ1+      | PBX3                         | [38]      |
| miR-122 | ↓          | CD133+           | PDK4                           | [39]      |
| miR-130b| ↑          | CD133+           | TP53INP1                      | [40]      |
| miR-155 | ↑          | CD90+CD133+      | TP53INP1                      | [41]      |
| miR-152 | ↓          | CD133+           | KIT                           | [42]      |
| miR-589-5p|↓          | CD90+           | MAP3K8                        | [43]      |
| miR-150 | ↓          | CD133+           | c-Myb                         | [44]      |
| miR-148b| ↓          | Side population  | NRP1                          | [45]      |
| miR-137 | ↓          | CD133/44+EpCAM+ | ANT2                          | [46]      |
Li et al. defined the miR-148a-ACVR/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 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 PPBP4 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 spheroid 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 NR1I1 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 Smad-independent TGF-β pathway [67].

3. The Underlying Mechanisms of lncRNA 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 lncRNAs in the CD133+ CD13 cell population. Among these dysregulated lncRNAs, lncTCF7 and lnc-β-Catm are the most upregulated lncRNAs, which play critical roles in driving CSC self-renewal and tumor propagation via activating the Wnt/β-catenin pathway [19, 47]. IncTCF7 is located at chromosome 5, the neighboring TCF7 gene. IncTCF7 directly interacts with the SWI/SNF complex, and the evolutionally conserved SWI/SNF complex can hydrolyze ATP to provide energy for mobilizing nucleosomes and remodeling chromatin. Through the association, IncTCF7 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. IncTCF7-mediated Wnt activation leads to the self-renewal maintenance and tumorigenic capacity of liver CSCs [19]. Unlike the mechanism of IncTCF7, 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.

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. lncRNA DILC and lncSox4 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
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, IncSox4 upregulation has been identified in advanced liver cancer and poor prognostic samples. Further study has demonstrated that IncSox4 is primarily increased in the CD133+ liver cancer cell fraction and CSC spheroids, and IncSox4 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 IncRNA HULC and MALAT1 are dramatically upregulated in liver cancer cells. When MALAT1 is cooverexpressed with HULC in liver CSCs, the two IncRNAs 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 CREPT 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 IncRNA that can trigger the malignant transformation of hepatocyte-like cells through epigenetically remodeling TRF2, IncRNA HULC, and the β-catenin promoter structure to drive the expression of these oncopgenes [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 IncRNA 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]. IncRNA-mPvt1 is an oncofetal RNA that can promote stem cell-like properties in murine cells. Its human homologue IncRNA-hPVT1 is highly expressed in liver cancer cells and correlates with poor prognosis. IncRNA-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 IncRNA, termed IncCAMTA1, that is overexpressed in CD133+ CD133+ CSCs and induces liver CSC proliferation. The IncCAMTA1 transcription orientation is antisense to the tumor suppressive gene CAMTA1, and their expression is negatively correlated in liver cancer samples. IncCAMTA1 physically binds to the CAMTA1 promoter and mediates a repressive chromatin structure to decrease CAMTA1 expression. The IncCAMTA1-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 IncRNA 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 IncRNA HOTAIR can enhance liver cancer stem cell proliferation and malignant progression through downregulation of SETD2. The IncRNA 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
Figure 1: The diverse regulatory mechanisms of ncRNAs on Wnt signaling pathway. (A) Let-7b can target and silence FZD4 expression. (B) miR-148a can inhibit the expression of ACVR1 to block the formation of Wnt-FZD-LRP5/6 receptor complex. (C) miR-1246 can directly suppress AXIN2 and GSK3β expression. (D) miR-200a can silence the expression of β-catenin. (E) miR-214 can deplete β-catenin expression. (F) miR-612 can indirectly decrease the nuclear accumulation of β-catenin. (G) miR-181 can enhance TCF activity by decreasing its inhibitor NLK. (H) Let-7a can directly silence TCF4. (I) Inc-β-Catm can lead to the methylation of β-catenin, enhancing the stability of β-catenin protein. (J) IncRNA-DANCR can bind to 3'-UTR of β-catenin to increase its expression by preventing β-catenin from depletion by miR-214 and miR-320a. (K) Inc-TCF7 can recruit SWI/SNF complex to TCF7 promoter and further elevate the activation of TCF7.

cancer diagnosis [72]. Additionally, several miRNA-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 for remedying 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-Ctam, 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
This study was supported by the National Natural Science Foundation of China (81672378 and 81201521).

References
[1] K. A. McGlynn, J. L. Petrick, and W. T. London, “Global epidemiology of hepatocellular carcinoma: an emphasis on demographic and regional variability,” Clinics in Liver Disease, vol. 19, no. 2, pp. 223–238, 2015.
[2] C. L. Chaffer and R. A. Weinberg, “A perspective on cancer cell metastasis,” Science, vol. 331, no. 6024, pp. 1559–1564, 2011.
[3] J. Bruix, G. J. Gores, and V. Mazzaferrro, “Hepatocellular carcinoma: clinical frontiers and perspectives,” Gut, vol. 63, no. 5, pp. 844–855, 2014.
[4] V. D’Andrea, A. Panarese, M. Tonda, M. Biffoni, and M. Monti, “Cancer stem cells as functional biomarkers,” Cancer Biomarkers, vol. 20, no. 3, pp. 231–234, 2017.
[5] J. Ji and X. W. Wang, “Clinical implications of cancer stem cell biology in hepatocellular carcinoma,” Seminars in Oncology, vol. 39, no. 4, pp. 461–472, 2012.
[6] J. S. Mattick, “RNA regulation: A new genetics?” Nature Reviews Genetics, vol. 5, no. 4, pp. 316–323, 2004.
[7] A. Bhan, M. Soleimani, and S. S. Mandal, “Long noncoding RNA and cancer: A new paradigm,” Cancer Research, vol. 77, no. 15, pp. 3965–3981, 2017.
[8] D. P. Bartel, “MicroRNAs: target recognition and regulatory functions,” Cell, vol. 136, no. 2, pp. 215–233, 2009.
[9] D. P. Bartel, “MicroRNAs: genomics, biogenesis, mechanism, and function,” Cell, vol. 116, no. 2, pp. 281–297, 2004.
[10] D. P. Bartel and C.-Z. Chen, “Micromanagers of gene expression: the potentially widespread influence of metazoan microRNAs,” Nature Reviews Genetics, vol. 5, no. 5, pp. 396–400, 2004.
[11] B. D. Harfe, “MicroRNAs in vertebrate development,” Current Opinion in Genetics & Development, vol. 15, pp. 410–415, 2005.
[12] J. Beermann, M.-T. Piccoli, J. Viereck, and T. Thum, “Noncoding rnas in development and disease: background, mechanisms, and therapeutic approaches,” Physiological Reviews, vol. 96, no. 4, pp. 1297–1325, 2016.
[13] A. N. Gargalionis and E. K. Basdra, “Insights in microRNAs Biology,” Current Topics in Medicinal Chemistry, vol. 13, no. 13, pp. 1493–1502, 2013.
[14] A. M. Schmitt and H. Y. Chang, “Long noncoding RNAs in cancer pathways,” Cancer Cell, vol. 29, no. 4, pp. 452–463, 2016.
[15] Q. Zhang, M. Su, G. Lu, and J. Wang, “The complexity of bladder cancer: long noncoding RNAs are on the stage,” Molecular Cancer, vol. 12, no. 1, article 101, 2013.
[16] S.-C. Tsai, C.-C. Lin, T.-C. Shih et al., “The miR-200b–ZEB1 circuit regulates diverse stemness of human hepatocellular carcinoma,” Molecular Carcinogenesis, vol. 56, no. 9, pp. 2035–2047, 2017.
[17] J. Ji, T. Yamashita, A. Budhu et al., “Identification of microRNA-181 by genome-wide screening as a critical player in EpCAM-positive hepatic cancer stem cells,” Hepatology, vol. 50, no. 2, pp. 472–480, 2009.
[18] S. Chai, K.-Y. Ng, M. Tong et al., “Octamer 4/microRNA-1246 signaling axis drives Wnt/β-catenin activation in liver cancer stem cells,” Hepatology, vol. 64, no. 6, pp. 2062–2076, 2016.
[19] Y. Wang, L. He, Y. Du et al., “The long noncoding RNA IncTCF7 promotes self-renewal of human liver cancer stem cells through activation of Wnt signaling,” Cell Stem Cell, vol. 16, no. 4, pp. 413–425, 2015.
[20] S. X. Yuan, J. Wang, F. Yang et al., “Long noncoding RNA DANCR increases stemness features of hepatocellular carcinoma by derepression of CTNNB1,” Hepatology, vol. 63, no. 2, pp. 499–511, 2016.
[21] F. Wang, J.-H. Yuan, S.-B. Wang et al., “Oncofetal long noncoding RNA PVT1 promotes proliferation and stem cell-like property of hepatocellular carcinoma cells by stabilizing NOP2,” Hepatology, vol. 60, no. 4, pp. 1278–1290, 2014.
[22] W. Yang, H.-X. Yan, L. Chen et al., “Wnt/β-catenin signaling contributes to activation of normal and tumorigenic liver progenitor cells,” Cancer Research, vol. 68, no. 11, pp. 4287–4295, 2008.
[23] T. Yamashita, J. Ji, A. Budhu et al., “EpCAM-positive hepatocellular carcinoma cells are tumor-initiating cells with stem/progenitor cell features,” Gastroenterology, vol. 136, no. 3, pp. 1012–1024, 2009.
[24] H. Clevers and R. Nusse, “Wnt/β-catenin signaling and disease,” Cell, vol. 149, no. 6, pp. 1192–1205, 2012.
[25] H. Cai, Y. Chen, X. Yang et al., “Let7b modulates the Wnt/B-catenin pathway in liver cancer cells via downregulated Frizzled4,” Tumour Biology the Journal of the International Society for Oncodevelopmental Biology & Medicine, vol. 39, 2017.
[26] H. Song, Q. Wang, J. Wen et al., “ACVR1, a therapeutic target of fibrodsysplasia ossificans progressiva, is negatively regulated by miR-148a,” International Journal of Molecular Sciences, vol. 13, no. 2, pp. 2063–2077, 2012.

[27] L. Li, Y. Liu, Y. Guo et al., “Regulatory MiR-148a-ACVR1/BMP circuit defines a cancer stem cell-like aggressive subtype of hepatocellular carcinoma,” Hepatology, vol. 61, no. 2, pp. 574–584, 2015.

[28] J. Liu, B. Ruan, N. You et al., “Downregulation of miR-200a induces EMT phenotypes and CSC-like signatures through targeting the β-catenin pathway in hepatic oval cells,” PLoS ONE, vol. 8, no. 11, Article ID e79409, 2013.

[29] H. Xia, L. P. J. Ooi, and K. M. Hui, “MiR-214 targets β-catenin pathway to suppress invasion, stem-like traits and recurrence of human hepatocellular carcinoma,” PLoS ONE, vol. 7, no. 9, Article ID e44206, 2012.

[30] J. Tang, Z. H. Tao, D. Wen et al., “MiR-621 suppresses the stemness of liver cancer via Wnt/B-catenin signaling,” Biochemical and Biophysical Research Communications, vol. 447, pp. 210–215, 2014.

[31] B. Jin, W. Wang, X.-X. Meng et al., “Let-7 inhibits self-renewal of hepatocellular cancer stem-like cells through regulating the epithelial-mesenchymal transition and the Wnt signaling pathway,” BMC Cancer, vol. 16, no. 1, article no. 863, 2016.

[32] X. Feng, J. Jiang, S. Shi, H. Xie, L. Zhou, and S. Zheng, “Knockdown of miR-25 increases the sensitivity of liver cancer stem cells to TRAIL-induced apoptosis via PTEN/PI3K/Akt/Bad signaling pathway,” International Journal of Oncology, vol. 49, no. 6, pp. 2600–2610, 2016.

[33] L. Zhou, Z.-X. Yang, W.-J. Song et al., “MicroRNA-21 regulates the migration and invasion of a stem-like population in hepatocellular carcinoma,” International Journal of Oncology, vol. 43, no. 2, pp. 661–669, 2013.

[34] Y. Liu, D.-L. Liu, L.-L. Dong et al., “MiR-621 suppresses stem cell-like property of hepatocellular carcinoma cells by modulating Spt/ Nanog signaling,” Cell Death & Disease, vol. 7, no. 9, Article ID e2377, 2016.

[35] L. Li, J. Tang, B. Zhang et al., “Epi genetic modification of MiR-429 promotes liver tumour-initiating cell properties by targeting Rb binding protein 4,” Gut, vol. 64, no. 1, pp. 156–167, 2015.

[36] Y. Jia, H. Liu, Q. Zhuang et al., “Tumorigenicity of cancer stem-like cells derived from hepatocarcinoma is regulated by microRNA-145,” Oncology Reports, vol. 27, no. 6, pp. 1865–1872, 2012.

[37] S. Chai, M. Tong, K. Y. Ng et al., “Regulatory role of miR-142-3p on the functional hepatic cancer stem cell marker CD133,” Oncotarget, vol. 5, no. 14, pp. 5725–5735, 2014.

[38] H. Han, Y. Du, W. Zhao et al., “PI3X is targeted by multiple miRNAs and is essential for liver tumour-initiating cells,” Nature Communications, vol. 6, article no. 8271, 2015.

[39] K. Song, H. Kwon, and C. Han, “Active glycolytic metabolism in CD133(+) hepatocellular cancer stem cells: regulation by MiR-122,” Oncotarget, vol. 6, no. 38, pp. 40822–40835, 2015.

[40] S. Ma, K. H. Tang, Y. P. Chan et al., “MiR-130b promotes CD133 liver tumor-initiating cell growth and self-renewal via tumor protein 53-induced nuclear protein 1,” Cell Stem Cell, vol. 7, no. 6, pp. 694–707, 2010.

[41] F. Liu, X. Kong, L. Lv, and J. Gao, “TGF-β1 acts through miR-155 to down-regulate TP53INP1 in promoting epithelial-mesenchymal transition and cancer stem cell phenotypes,” Cancer Letters, vol. 359, no. 2, pp. 288–298, 2015.

[42] H. Huang, M. Hu, P. Li, C. Lu, and M. Li, “MiR-152 inhibits cell proliferation and colony formation of CD133+ liver cancer stem cells by targeting KIT,” Tumor Biology, vol. 36, no. 2, pp. 921–928, 2015.

[43] X. Zhang, P. Jiang, L. Shuai et al., “miR-589-5p inhibits MAPK8 and suppresses CD90+ cancer stem cells in hepatocellular carcinoma,” Journal of Experimental & Clinical Cancer Research, vol. 35, no. 1, p. 176, 2016.

[44] J. Zhang, N. Luo, Y. Luo, Z. Peng, T. Zhang, and S. Li, “MicroRNA-150 inhibits human CD133-positive liver cancer stem cells through negative regulation of the transcription factor c-Myb,” International Journal of Oncology, vol. 40, no. 3, pp. 747–756, 2012.

[45] Q. Liu, Y. Xu, S. Wei et al., “miRNA-148b suppresses hepatic cancer stem cell by targeting neuropilin-1,” Bioscience Reports, vol. 35, no. 4, Article ID e00229, 2015.

[46] A.-Q. Lu, B. Lv, F. Qiu, X.-Y. Wang, and X.-H. Cao, “Upregulation of miR-137 reverses sorafenib resistance and cancer-initiating cell phenotypes by degrading ANT2 in hepatocellular carcinoma,” Oncology Reports, vol. 37, no. 4, pp. 2071–2078, 2017.

[47] P. Zhu, Y. Yang, G. Huang et al., “Lnc-β-Catm elicits EZH2-dependent β-catenin stabilization and sustains liver CSC self-renewal,” Nature Structural & Molecular Biology, vol. 23, no. 7, pp. 631–639, 2016.

[48] X. Wang, W. Sun, W. Shen et al., “Long non-coding RNA DILC regulates liver cancer stem cells via IL-6/STAT3 axis,” Journal of Hepatology, vol. 64, no. 6, pp. 1283–1294, 2016.

[49] Z.-Z. Chen, L. Huang, Y.-H. Wu, W.-J. Zhai, P.-P. Zhu, and Y.-F. Gao, “LncSox4 promotes the self-renewal of liver tumour-initiating cells through Stat3-mediated Sox4 expression,” Nature Communications, vol. 7, Article ID 12598, 2016.

[50] M. Wu, Z. Lin, X. Li et al., “HULC cooperates with MALAT1 to aggravate liver cancer stem cells growth through telomere repeat-binding factor 2.” Scientific Reports, vol. 6, Article ID 36045, 2016.

[51] T. Li, Q. Zheng, J. An et al., “SET1A cooperates with CUDR to promote liver cancer growth and hepatocyte-like stem cell malignant transformation epigenetically,” Molecular Therapy the Journal of the American Society of Gene Therapy, vol. 24, no. 2, pp. 261–275, 2016.

[52] H. Pu, Q. Zheng, H. Li et al., “CUDR promotes liver cancer stem cell growth through upregulating TERT and C-Myc,” Oncotarget, vol. 6, no. 38, pp. 40775–40798, 2015.

[53] P. Zhu, Y. Wang, J. Wu et al., “LncBRM initiates YAP1 signalling activation to drive self-renewal of liver cancer stem cells,” Nature Communications, vol. 7, Article ID 13608, 2016.

[54] L.-J. Ding, Y. Li, S.-D. Wang et al., “Long noncoding RNA IncCAMT1 promotes proliferation and cancer stem cell-like properties of liver cancer by inhibiting CAMTA1,” International Journal of Molecular Sciences, vol. 17, no. 10, article no. 1617, 2016.

[55] W. Guo, S. Liu, Y. Cheng et al., “ICAM-1-related noncoding RNA in cancer stem cells maintains ICAM-1 expression in hepatocellular carcinoma,” Clinical Cancer Research, vol. 22, no. 8, pp. 2041–2050, 2016.

[56] H. Li, J. An, M. Wu et al., “LncRNA HOTAIR promotes human liver cancer stem cell malignant growth through downregulation of SETD2,” Oncotarget, vol. 6, no. 29, pp. 27847–27864, 2015.

[57] J. Nichols, B. Zevnik, K. Anastassiadis et al., “Formation of pluripotent stem cells in the mammalian embryo depends on the Pou transcription factor Oct4,” Cell, vol. 95, no. 3, pp. 379–391, 1998.
[58] X. Q. Wang, W. M. Ongkeko, L. Chen et al., “Octamer 4 (Oct4) mediates chemotherapeutic drug resistance in liver cancer cells through a potential Oct4-AKT-ATP-binding cassette G2 pathway,” *Hepatology*, vol. 52, no. 2, pp. 528–539, 2010.

[59] N. Haraguchi, H. Ishii, K. Mimori et al., “CD13 is a therapeutic target in human liver cancer stem cells,” *The Journal of Clinical Investigation*, vol. 120, no. 9, pp. 3326–3339, 2010.

[60] M. Yamashita, H. Wada, H. Eguchi et al., “A CD13 inhibitor, ubenimex, synergistically enhances the effects of anticancer drugs in hepatocellular carcinoma,” *International Journal of Oncology*, vol. 49, no. 1, pp. 89–98, 2016.

[61] T. K. Lee, A. Castilho, V. C. Cheung, K. H. Tang, S. Ma, and I. O. Ng, “CD24+ liver tumor-initiating cells drive self-renewal and tumor initiation through STAT3-mediated NANOG regulation,” *Cell Stem Cell*, vol. 9, no. 1, pp. 50–63, 2011.

[62] K. H. Tang, S. Ma, T. K. Lee et al., “CD133+ liver tumor-initiating cells promote tumor angiogenesis, growth, and self-renewal through neurotensin/interleukin-8/CXCL1 signaling,” *Hepatology*, vol. 55, no. 3, pp. 807–820, 2012.

[63] O. Warburg, “On the origin of cancer cells,” *Science*, vol. 123, no. 3191, pp. 309–314, 1956.

[64] S. Okamura, H. Arakawa, T. Tanaka et al., “p53DINP1, a p53-inducible gene, regulates p53-dependent apoptosis,” *Molecular Cell*, vol. 8, no. 1, pp. 85–94, 2001.

[65] R. Tomasini, A. Azizi Samir, M.-J. Pebusque et al., “p53-dependent expression of the stress-induced protein (SIP),” *European Journal of Cell Biology*, vol. 81, no. 5, pp. 294–301, 2002.

[66] P. M. Siegel and J. Massagué, “Cytostatic and apoptotic actions of TGF-β in homeostasis and cancer,” *Nature Reviews Cancer*, vol. 3, no. 11, pp. 807–820, 2003.

[67] L. Boix, J. M. López-Oliva, A. C. Rhodes, and J. Bruix, “Restoring Mir122 in Human Stem-Like Hepatocarcinoma Cells, Prompts Tumor Dormancy through Smad-Independent Tgf-B Pathway,” *Journal of Hepatology*, vol. 64, no. 2, pp. S568–S569, 2016.

[68] S. Liu, N. Li, X. Yu et al., “Expression of intercellular adhesion molecule 1 by hepatocellular carcinoma stem cells and circulating tumor cells,” *Gastroenterology*, vol. 144, no. 5, pp. 1031-1041.e10, 2013.

[69] J. Hayes, P. P. Peruzzi, and S. Lawler, “MicroRNAs in cancer: biomarkers, functions and therapy,” *Trends in Molecular Medicine*, vol. 20, no. 8, pp. 460–469, 2014.

[70] J. R. Evans, F. Y. Feng, and A. M. Chinnairyan, “The bright side of dark matter: IncRNAs in cancer,” *The Journal of Clinical Investigation*, vol. 126, no. 8, pp. 2775–2782, 2016.

[71] J. Ji, J. Shi, A. Budhu et al., “MicroRNA expression, survival, and response to interferon in liver cancer,” *The New England Journal of Medicine*, vol. 361, no. 15, pp. 1437–1447, 2009.

[72] V. Vlaeminck-Guillem, A. Ruffion, J. André, M. Devonec, and P. Paparel, “Urinary prostate cancer 3 test: toward the age of reason?” *Urology*, vol. 75, no. 2, pp. 447–453, 2010.

[73] A. L. Kasinski and F. J. Slack, “MiRNA-34 prevents cancer initiation and progression in a therapeutically resistant K-ras and p53-induced mouse model of lung adenocarcinoma,” *Cancer Research*, vol. 72, no. 21, pp. 5576–5587, 2012.

[74] J. Elmén, M. Lindow, A. Silahtaroglu et al., “Antagonism of microRNA-122 in mice by systemically administered LNA-antimiR leads to up-regulation of a large set of predicted target mRNAs in the liver,” *Nucleic Acids Research*, vol. 36, no. 4, pp. 1153–1162, 2008.

[75] T. Zhan, N. Rindtorff, and M. Boutros, “Wnt signaling in cancer,” *Oncogene*, vol. 36, no. 11, pp. 1461–1473, 2017.