Hepatocellular carcinoma (HCC) is one of the ten most commonly occurring solid cancers worldwide and is the second most prevalent cause of death from malignancy [1,2]. Hepatitis B Virus (HBV) or Hepatitis C Virus (HCV) infection, smoking, alcohol use and liver cirrhosis are the major causes of HCC [3,4]. Despite advances in the understanding of the molecular mechanisms underlying HCC and improved treatments for HCC, the median survival time is short. More than 60% of initially detected HCC in Japan are early stage with a 43% 5-year survival rate and a median survival time of 50 months [5].

In recent years, advances in genome-wide analyses of the mammalian transcriptome have revealed a novel class of transcripts, long noncoding RNAs (lncRNAs), which are pervasively transcribed in the genome [6]. LncRNAs are arbitrarily defined as transcripts of more than 200 nucleotides (nt) in length that lack significant open reading frames (ORF) and can be localized to both the nucleus and cytoplasm [7,8]. According to their location and orientation, lncRNAs are classified as intergenic lncRNAs (lincRNAs), genic and intragenic lncRNAs [9]. In the nucleus, lncRNAs mainly modulate gene transcription and mRNA splicing, whereas they are involved in RNA stability and activation of microRNAs (miRNAs) in the cytoplasm [10].

It is noteworthy that an increasing number of studies have demonstrated that lncRNAs act as a new class of regulatory molecules that are involved in the development and progression of hepatocellular carcinoma. Indeed, lncRNAs affect cell proliferation, migration, apoptosis, invasion, tumorigenicity, cell cycle, and metastasis [11-13]. Furthermore, evidence is accumulating to suggest that the aberrant expressions of lncRNAs have a clinical impact on the diagnosis of hepatocellular carcinoma. In this regard lncRNAs are associated with clinicopathological features including metastasis, invasion, TNM stage, prognosis, tumor size, and differentiation of patients.
with hepatocellular carcinoma. Among these features the greatest proportion are involved in metastasis and invasion. Hence, it has been proposed that these hepatocellular carcinoma-associated lncRNAs may be used as biomarkers for indicating metastasis of hepatocellular carcinoma [11,14-16].

For the purposes of this review, we present (Table 1) a comprehensive listing of reported dysregulated lncRNAs in HCC and the molecular mechanisms of known lncRNAs on hepatocellular carcinoma. Importantly, lncRNAs play crucial roles in hepatocellular carcinoma tumorigenesis and development by interacting with DNA, RNA, and proteins.

2 Interaction with DNA

Evidence suggest that lncRNAs combine with histone-modifying complexes and then target DNA. For example, HOX transcript antisense RNA (HOTAIR) occupies targeted double-stranded DNA (dsDNA) [17]. Additionally, long noncoding RNA MEG3 regulates the TGF-β pathway genes through formation of RNA-DNA triplex structures [18]. These triplex structures can serve as a specific recognition mechanism between lncRNA and genomic DNA. Indeed, it has been proposed that triplexes created between lncRNA and genomic DNA may decisively result in targeting specificity, as well as promoting favorable chromatin conformations that may contribute to the affinity.

3 Interaction with RNA

3.1 Interaction with mRNA

Accumulating evidence indicates that lncRNAs can influence mRNA processing and post-transcriptional regulation via forming lncRNA-mRNA duplexes. These effects depend on complementary base pairing, and involve the control of splicing, translation, and mRNA stability. Certain HCC-related lncRNAs have been demonstrated to bind directly and target mRNA to exert post-transcriptional regulation. For example, PCNA-AS1, antisense to PCNA, can increase PCNA mRNA stability via forming lncRNA–mRNA hybridization in HCC [19] (Figure 1a). Additionally, Yuan et al. found that lncRNA-ATB specifically increased the stability of IL-11 mRNA, which depends on the binding of IL-11 mRNA, in HCC [20] (Figure 1d).

Irrespective of the reported direct interactions, an indirect mechanism between lncRNAs and their targeted mRNAs is illustrated by metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) [21,22]. By binding with associated proteins, MALAT1 alters splicing of mRNAs. The function of serine/arginine splicing factors (SF2/ASF) rely on MALAT1, and through the formation of a bipartite triple helix, MALAT1 promotes malignancy. A fragment near the 3’ end of MALAT1 may regulate the metastatic potential.

3.2 Interaction with miRNAs

It has been proposed that lncRNAs can function as competing endogenous RNAs (ceRNAs) and ‘sponges’ of miRNAs, thus regulating the expression of target mRNAs. MicroRNAs regulate the expression of over 60% of protein coding genes by targeting their mRNAs to AGO2-containing complexes in the cytoplasm and promoting their translational inhibition and/or degradation [23]. For example, clinical studies have demonstrated a link of miR-182 expression to poor prognosis in liver cancer patients. Mechanistically, multiple downstream genes including missing-in-metastasis, microphthal-
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associated transcription factor, FoxO1, cylindromatosis, and others, can be targeted by miR-182 and mediate its roles in a variety of cancers [24].

A typical example is HULC, a highly up-regulated IncRNA in liver cancer, transcribed from human chromosome 6p24.3 and containing a conserved target site of miR-372. Phospho-CREB, activated through the protein kinase A pathway, binds to the CREB binding site and induces upregulation of HULC. Subsequently, HULC directly binds to miR-372 and represses its expression and activity. The reduction of miR-372 induces increased levels of Prkacb (cAMP-dependent protein kinase catalytic subunit beta), which is a target mRNA of miR-372. Interestingly, Prkacb can facilitate phosphorylation of CERB in the protein kinase A pathway. Finally, an autoregulatory loop (CREB-HULC-miR372-Prkacb) is formed [25,26] (Figure 1g). Metastasis-associated lung adenocarcinoma transcript 1 (MALAT1) is also known as nuclear-enriched abundant transcript 2 (NEAT2) and is transcribed from chromosome 11q13. It is upregulated in many solid carcinomas and is associated with cell proliferation and migration through the modulation of caspase-3, caspase-8, Bax and Bcl-2. Leucci et al. demonstrated that miR-9 could directly bind MALAT1 RNA in vivo and regulate the MALAT1 in the nucleus in an AGO2-dependent manner [27] (Figure 1f). In addition, Takahashi et al. revealed that linc-RoR functioned as a miRNA sponge to limit endogenous miR-145 that can modulate the expression of key effectors of the hypoxia response, such as HIF-1a expression, in...
HCC [28]. In addition, IncRNA-ATB up-regulated ZEB1 and ZEB2 by competitively binding the miR-200 family and then induced EMT and the invasiveness of HCC [20]. H19 harbors both canonical and non-canonical binding sites for the let-7 family of microRNAs, which plays key roles in development and cancer. It has been reported that H19 modulates let-7 availability by acting as a molecular sponge using H19 knockdown and overexpression, as well as in vivo crosslinking and genome-wide transcriptome analysis [29] (Figure 1b). These suggest that blocking the associations between IncRNAs and their partners (RNAs or proteins) may play key roles in development and cancer.

Further, the expression of IncRNAs may be repressed by miRNAs. The IncRNA PTENP1 is a pseudogene of the tumor suppressor gene PTEN, which suppresses the oncogenic PI3K/AKT pathway, inhibits cell proliferation and migration/invasion, as well as inducing autophagy and apoptosis. The PTENP1 decoyed oncomirs miR-17 family, which would otherwise target PTEN, PHLP (a negative AKT regulator) and such autophagy genes as ULK1, ATG7 and p62 [30] (Figure 1c). HOTTIP is an antisense IncRNA mapped to the distal end of the HOXA gene cluster. Knockdown of HOTTIP significantly suppressed the expression of a number of HOXA genes Moreover, in human HCCs, HOTTIP expression negatively correlated with that of miR-125b, which is a post-transcriptional regulator of HOTTIP [31] (Figure 1e). miR-141 and miR-148a show an inhibitory effect on the expression of DNA methyltransferase 1 (DNMT1) and thus induces the overexpression of MEG3 [32] (Figure 2c). The examples about miRNAs’ regulating IncRNAs via epigenetic modification, and vice versa, can also be found in other parts of this article. In conclusion, IncRNAs can target multiple miRNAs, also can be targeted by miRNAs re-establishing the expression of a single IncRNA, then forming a feedback loop. A better understanding of the mechanism of regulation of ncRNAs network would accelerate the development of novel therapeutic strategies for the arrest of primary hepatic tumors.

4 Interaction with proteins

4.1 Interaction with histone-modifying complexes

A large number of studies have revealed that many HCC-related IncRNAs exert their function through interaction with proteins or protein complexes, especially with epigenetic complexes, such as polycomb repressive complex 1 (PRC1) and polycomb repressive complex 2 (PRC2).

HOTAIR was the first demonstrated lncRNA shown to coordinate gene silencing via assembly of PRC2. The structural domains of HOTAIR formed in its 5’ region and 3’ region are bound to enhancer of zeste 2 (EZH2, PRC2 subunit) and lysine specific demethylase 1 (LSD1), respectively [33]. HOTAIR preferentially occupies a GA-rich DNA motif to enable the formation of a RNA-dsDNA triplex [17]. This occurs independently of EZH2. Simultaneously, HOTAIR is required for the occupancy of suppressor of zeste 12 homolog (SUZ12, PRC2 subunit) on MIR-34A loci [34], then epigenetically modify their targeted genes, including Snail and vimentin (Figure 2b). HOTAIR also could promote migration and invasion of HCC cells by inhibiting RBM38 [35] (Figure 2d). H19 can specifically associate with enhancer of zeste homolog 2 (EZH2), a key subunit of the PRC2 complex, and inhibit E-cad expression by directly suppressing E-cad transcription and by indirectly activating Wnt signaling [36] (Figure 2e); H19 associated with the protein complex hnRNP U/PCAF/RNAPol II, activating miR-200 family by increasing histone acetylation. These results demonstrate that H19 can alter the miR-200 pathway [37], thus contributing to mesenchymal-to-epithelial transition and to the suppression of tumor metastasis (Figure 2g). KCNQ1OT1 could interact with chromatin and with the H3K9- and H3K27-specific histone methyltransferases G9a and the PRC2 complex in a lineage-specific manner [38]. LncRNA-HEIH also can associate with EZH2, and this association is required for the repression of EZH2 target genes in HCC, involving p15, p16, p21 and p57 [39] (Figure 2f). Kaneko et al. demonstrated that MEG3 interacted with PRC2 mainly through the RBR of JARID2 and MEG3 acts in trans on PRC2 and JARID2 by facilitating their recruitment to a subset of target genes [40]. PVT1 silenced P15/P16 in in trans by recruiting EZH2 [41]. The upregulated EZH2 enforces hepatocellular carcinoma cell proliferation (Figure 2a). LncRNAs also recruit activating chromatin modifiers such as lysine (K)-specific methyltransferase 2A (MLL). LncRNA HOTTIP could bind the adaptor protein WDR5 directly and targets WDR5/MLL complexes across HOXA, driving H3K4 trimethylation and gene transcription [42]. LncRNA-LET could bind to NF90, a double-stranded RNA-binding protein that has been implicated in the stabilization, transport, and translational control of many target miRNAs, and decreases HIF1-a and CDF42 mRNA stability through its association with NF90 under hypoxic and normoxic conditions, respectively [43]. In these examples, IncRNAs are required for the assembly of different histone-modifying complexes or and DNA methyltransferase, which in turn modify the neighboring chromatin. As many as 38% lincRNAs cooperate with at least one of multiple
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this suggests that one
lncRNA may harbor several types of binding elements for chromatin modifiers. The specific locus is subject to the ensuing histone modification caused by the occupancy of histone-modifying complexes.

4.2 Interaction with other proteins

With the exception of the direct interaction with proteins, how do lncRNAs interact with other proteins is interesting. Evidence suggests that lncRNA-MVIH can activate angiogenesis by interacting with PGK1, a protein secreted by tumor cells which inhibits angiogenesis, and preventing its secretion [44]. LncRNA-DREH could specifically associate with the protein vimentin, a type III intermediate filament (IF) and the major cytoskeletal component of mesenchymal cells [13]. GAS5 positively influences YBX1 protein stability without increasing its transcription [45]. The putative stem-loop structure formed by exon 12 of GAS5 is responsible for its interplay with YBX1. YBX1 possesses the capacity of complexing with IGF2BP1, which combines with GHET1 and prevents mRNA degradation. The exon 12 is a GAS5’s predominant structural domain for mimicking binding domain of glucocorticoid receptor (GR). GAS5, GR, YBX1, and P53 may collaborate as a complex to influence cell cycle regulation.

Figure 2: LncRNAs regulate cell proliferation, migration, apoptosis, invasion, tumorigenicity, cell cycle, and metastasis by histone modification. a. The P15/P16 is silenced by the occupancy of EZH2, which is recruited by PVT1. b. Through the formation of dsDNA/RNA triplex, HOTAIR recruits SUZ12 and EZH2 to the MiR-34A loci and then silences the transcription of miR-34a. HOTAIR also forms complexes with LSD1. HOTAIR attenuates Snail, N-cadherin and vimentin protein levels. All of these are targets of miR-34a. c. DNMT1-mediated MEG3 hypermethylation caused the loss of MEG3 expression. d. HOTAIR could promote migration and invasion of HCC cells by inhibiting RBM38. e. H19 guides EZH2 to the E-cadherin promoter loci. f. IncRNA-H19 is found to interact with enhancer of zeste homolog 2 (EZH2), a key component of PRC2, then recruit PRC2 to p16 promoter and repress the expression of p16 gene, thereby contributing to cell cycle arrest. g. H19 associated with the protein complex hnrNP U/PCAF/POLII, activating miR-200 family by increasing histone acetylation, thus contributing to the suppression of tumor metastasis. Abbreviations: RBM38, RNA binding motif protein 38; ZEB1/2, zinc finger E-box-binding protein 1/2; LSD1, lysine-specific demethylase 1; PRC2, polycomb repressive complex 2; EZH2, enhancer of zeste homologue 2.
5 Conclusion

LncRNAs are characterized by the complexity of their mechanisms. This review summarizes the potential impact of dysregulated LncRNAs in HCC and the known interactions between LncRNAs and DNA, RNA, and proteins in hepatocellular carcinoma. Sun et al. [46] proposed a global network-based computational framework to infer potential human LncRNA–disease associations by implementing the random walk with restart method on a LncRNA functional similarity network. In total, they observed 371 LncRNA–LncRNA functional associations between 117 LncRNAs in the LncRNA functional similarity network (LFSN).

Molecular-based tumor predictors are essential for individualized HCC diagnosis. Cancer-specific miRNAs have been widely regarded as potential biomarkers for the diagnosis and prognosis of cancers as they are easily detectable in the blood, urine, sputum and other biological fluids of patients. Similarly, LncRNAs that are found in body fluids have demonstrated a utility to be used as fluid-based non-invasive markers for clinical use. Therapeutic benefit can be obtained through RNA-based therapeutic strategies, such as siRNA and microRNA, or using small molecule compounds designed specifically to interact with target LncRNAs or ribonucleoprotein complexes [47]. It is tempting to speculate that a multitude of LncRNAs may interrupt specific steps in numerous tumor suppressive and oncogenic pathways. The revelation of the underlying mechanisms of LncRNAs may benefit our understanding of hepatocellular carcinoma's pathogenesis.

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References

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