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Long non-coding RNA H19: A key player in liver diseases

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Abstract

The rapid advance in deep sequencing technologies has identified numerous long non-coding RNAs (lncRNA) and their biological functions are increasingly being recognized as important regulators of gene expression and cell signaling pathways. H19 is the first lncRNA identified and characterized as the first imprinted gene in the pre-genomic era. During the last three decades, H19 has been extensively investigated as a multitasking lncRNA. H19 plays a crucial role in regulating many biological functions and is intimately involved in the pathogenesis of various human diseases. Here, we highlight the recent findings related to H19 in liver diseases. The unique features of H19 biogenesis and regulation make it an attractive diagnostic and prognostic biomarker and potential therapeutic target for certain liver diseases.

Conclusions: The roles of H19 in liver disease remains obscure. The rapid advance in new technologies offers promise for the understanding of the mechanisms of lncRNA H19 in physiological and pathological processes of liver diseases and for the development novel therapeutics.

Keywords

non-coding RNA; cholestasis; nonalcoholic fatty liver disease; hepatocellular carcinoma

Introduction

The completion of human genome sequencing in the early 2000s identified that most of the human genome (~93%) is transcribed, but less than 2% of transcripts encode proteins. Most of the transcripts represent non-coding RNAs (ncRNAs). Long non-coding RNAs (lncRNAs) are ncRNAs with more than 200 nucleotides. LncRNAs are further classified into sense, antisense, bidirectional, intronic, and intergenic lncRNA based on their topographic relation to the nearest protein-coding gene (1). Recent advance in next-generation sequencing has identified thousands of lncRNA loci and the number of lncRNAs which are linked to human diseases, including liver disease, is rapidly growing (2). However,

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compared to protein-coding genes and microRNAs (small ncRNAs with 20-24 nucleotides), lncRNAs are poorly characterized, and their biological functions remain largely unknown.

LncRNA H19 was the first lncRNA and first imprinted gene identified in eukaryotes as a hepatic fetal-specific non-translatable mRNA in the late 1980s (3). Its role in embryogenesis has been well characterized. The biological function of H19 as an RNA molecule remained a mystery until the identification of another lncRNA X-inactive-specific transcript in the early 1990s (4). During the last three decades, H19 has been well characterized as a multitasking lncRNA. The aberrant expression of H19 has been linked to various human cancers, including gastric, liver, and pancreatic cancers (5, 6). Recent studies also reported that H19 is involved in chronic liver diseases such as nonalcoholic fatty liver disease (NAFLD) and cholestatic liver disease, which are major global health issues. The high mortality and morbidity associated with hepatocellular carcinoma (HCC) and cholangiocarcinoma, the end stages of most chronic liver diseases, have imposed huge financial burdens on individuals and the health care systems (7). Therefore, there is an unmet need to identify novel diagnostic biomarkers and therapeutic targets for chronic liver diseases. In this concise review, we discussed the current understanding of H19 in the pathogenesis of chronic liver diseases.

Expression and regulation of H19 and functional mechanisms

The role of lncRNA H19 in the regulation of liver development has been well-documented (8). Genetic and molecular studies have shown that H19 is a parentally imprinted and maternally expressed gene, which is localized to chromosome 7 in mice and chromosome 11p15.5 in humans, respectively; downstream of another maternally imprinted and paternally expressed protein-coding gene, called insulin-like growth factor 2 (Igf2) (9). The H19 gene contains five exons and four small introns and encodes a ~2.3 kb fully capped, spliced, and polyadenylated transcript (8). The expression of H19 is controlled by a promoter and an imprinting control region (ICR), also called a differentially methylated domain (DMD) or a differentially methylated region (DMR). H19 and IGF2 are expressed in the same tissues, and their reciprocal expression is controlled by the zinc-finger protein, CCCTC binding factor (CTCF), which binds to unmethylated maternal ICR and prevents the activation of Igf2 by downstream enhancers. It also has been reported that H19 is the precursor of miRNA675, which is embedded in exon 1 of H19. The excision process of miRNA675 from H19 is regulated by RNA-binding protein (RBP) human antigen R (HuR) (Fig.1) (10). H19 is highly expressed during fetal development and down-regulated after birth, except in skeletal muscle. Aberrant expression of H19 has been linked to various human diseases, especially tumorigenesis (11). It also has been reported that H19 expression is upregulated by estrogen, c-Myc, hypoxia, and oxidative stress (12-15). The hepatic H19 expression level is very low under normal physiological conditions, but it can be upregulated under pathological conditions (16). The relative expression levels of H19 in different types of hepatic cells depend on the pathophysiological conditions. Despite the discrepancy found in the previous studies, there is consensus that H19 impacts various hepatic cells as a multitask regulator of gene expression. The mechanisms by which H19 regulates cellular functions include epigenetic regulation, sponge of miRs, production of miR-675, and regulation of target gene expression *via* binding to RBPs (17).

LncRNA H19 in the nonalcoholic fatty liver disease (NAFLD)

The liver is the most important metabolic organ and plays many vital life functions. The incidence of NAFLD has rapidly increased during the last two decades due to the global pandemic of obesity. NAFLD can progress to steatohepatitis (NASH) and is the second most common indication for liver transplant and a major cause of HCC. Due to the complexity of disease pathology, no reliable diagnostic biomarkers and regulatory-approved drugs are available (18). There is compelling evidence supporting the “Multi-hit” over the “Two-hit” hypothesis of NAFLD pathogenesis (19). NAFLD is not a single organ disease. The progression from simple steatosis to NASH is correlated with systemic and adipose tissue inflammation, dysbiosis, and disruption of gut barrier function. It has been reported that overexpression of H19 disrupts the intestinal barrier function *via* miR675 (20). A recent study further showed that H19 inhibited the function of Paneth and goblet cells *via* suppressing autophagy (21). The role of H19 in NAFLD remains unexplored until the identification of its aberrant expression in the livers of NASH patients (22). By using $H19^{-/-}$ and AAV8-mediated overexpression of H19 mouse models, Liu, C *et al* reported that H19 promoted lipogenesis by facilitating polypyrimidine tract-binding protein 1 (PTBP1), a RBP, to stabilize sterol regulatory element-binding protein 1c mRNA and increase protein cleavage and nuclear translocation (23). Recently, two groups reported that H19 expression is induced by free fatty acids in hepatocytes and high-fat diet feeding *in vivo* (24, 25). Mechanistically, H19 promotes hepatic lipogenesis by downregulating miR130a, an inhibitor of peroxisome proliferator-activator receptor γ; or upregulating transcription factors, MLX-interacting protein-like (MLXIPL, also called carbohydrate-responsive element-binding protein), and PI3K/mTOR pathways (24, 25). These studies identified H19 as an essential player in diet-induced hepatic steatosis (Fig.2). However, it remains unclear whether and how H19 is involved in NAFLD/NASH disease progression.

LncRNA H19 in cholestatic liver disease

Cholestatic liver diseases, such as primary sclerosing cholangitis (PSC) and primary biliary cholangitis (PBC) in adults and biliary atresia (BA) and Alagille syndrome in children, are a significant cause of morbidity and mortality and liver transplant. Cholestasis is defined as the impairment of bile flow due to disruption of bile acid formation or excretion or obstruction of bile ducts (26). Bile acids are exclusively formed in hepatocytes and play critical roles in nutrient absorption *via* intrahepatic circulation. More importantly, bile acids function as signaling molecules in regulating lipid and glucose metabolism (27). Disruption of intrahepatic bile acid circulation or accumulation of bile acids in the liver can cause hepatocyte injury, cholangiocyte proliferation, ductal reaction, activation of hepatic stellate cells, and inflammation. Although cholestatic liver diseases are relatively rare compared to NAFLD, the incidence and prevalence are increasing. The available therapeutic agents are limited to Ursodeoxycholic acid and obeticholic acid for PBC, which are largely nonspecific and often ineffective. There is an urgent need to identify diagnostic biomarkers and new therapeutic targets for cholestatic liver diseases. LncRNAs are increasingly recognized as promising potential therapeutic targets for cholestatic liver diseases (28). Zhang Y, et al. first identified H19 as a key player in BDL-induced cholestatic liver injury (22). Several studies have reported upregulation of H19 in different hepatic cells, cholestatic mouse models, and

human PSC, PBC and BA patients (29-39). H19 not only functions as a sponge of miRNAs but also activates different signaling pathways involved in the activation of macrophages, cholangiocytes, and hepatic stellate cells (HSCs). Table 1 summarizes the key findings of the most recent studies, which assessed the roles of H19 in different hepatic cells and cholestatic animal models.

LncRNA H19 in HCC

HCC is the most common primary liver cancer and is often diagnosed at late stages due to the lack of diagnostic and prognostic biomarkers. Liver transplant remains the only therapeutic option. Aberrant up-regulation of H19 in tumorigenesis has been well established in different types of human cancers (11). However, the role of H19 in HCC is more complicated and remains controversial. Most studies with human HCC samples were limited by small sample size and variations in patient populations and tissue sampling. The major findings in the past three decades related to H19 in HCC and the potential reasons for the conflicting results are summarized and discussed in an excellent recent review (40). Studies with cultured HCC cell lines, *in vivo* animal models and human HCC patient samples indicate that H19 can be an oncogene or tumor suppressor by regulating different miRNAs, RBPs, and diverse signaling pathways (41-46). The most recent studies related to H19 in HCC are summarized in Table 2. The functions of lncRNAs are linked to their intracellular localization. More mechanistic and comprehensive studies are needed to define the role of H19 in the progression of HCC.

Conclusion and future perspectives

LncRNA H19 has gained increasing attention due to its broad spectrum of physiological and pathological functions. The ease of detection of lncRNAs in serum and other bodily fluids make them attractive biomarkers. Although H19 was discovered more than three decades ago, its potential roles in liver diseases remain largely obscure. Recent studies have solidified the idea that H19 represents a novel diagnostic and prognostic biomarker for various liver diseases. The fundamental role of H19 in promoting hepatic lipogenesis, inflammation, and epithelial-mesenchymal transition makes this lncRNA a promising therapeutic target in liver diseases. Recent advances in technologies for gene profiling and editing, as well as nanotechnology for RNA delivery, have rapidly moved the lncRNA research field forward. Considering the complexity of various liver diseases, it is essential to understand the comprehensive and systemic effects of H19 in different physiological and pathological settings. More mechanistic and translational studies with tissue- and cell type-specific $H19^{-/-}$ animal models are needed in order to validate H19 as a novel therapy target for liver diseases.

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Abbreviations:

ncRNAs	non-coding RNAs
lncRNAs	long non-coding RNAs
miRNAs	microRNAs
NAFLD	nonalcoholic fatty liver disease
HCC	hepatocellular carcinoma
Igf2	insulin-like growth factor 2
ICR	imprinting control region
DMD	differentially methylated domain
DMR	differentially methylated region
CTCF	CCCTC binding factor
RBP	RNA-binding protein
HuR	human antigen R
NASH	nonalcoholic steatohepatitis
PTBP1	polypyrimidine tract-binding protein 1
PSC	primary sclerosing cholangitis
PBC	primary biliary cholangitis
BDL	bile duct ligation
HSCs	hepatic stellate cells
EpCAM	epithelial cell adhesion molecule

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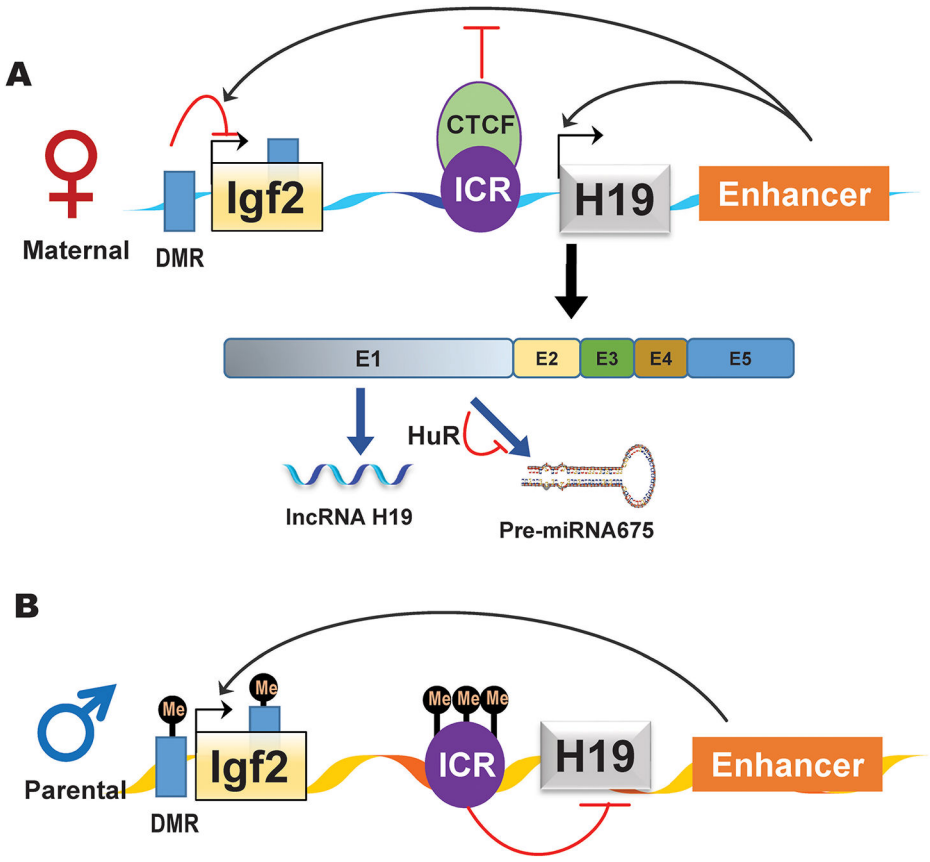

Fig.1. Regulation of H19 and Igf2 expression.

A) H19 is expressed from the maternal allele. The binding of CTCF to the unmethylated imprinting control region (ICR) prevents the down-stream enhancer from interacting with the promoter region of Igf2 but allows the enhancer to interact with the H19 promoter. The H19 transcript contains five exons. In exon 1, there is the coding region for miR675. The production of pre-miR675 is inhibited by RBP HuR. B) In the parental allele, the ICR is methylated, which prevents the binding of CTCF and allows the enhancer to interact with the promoter region of Igf2. DMR; DNA methylation region; ICR: imprinting control region; CTCF: CCCTC binding factor.

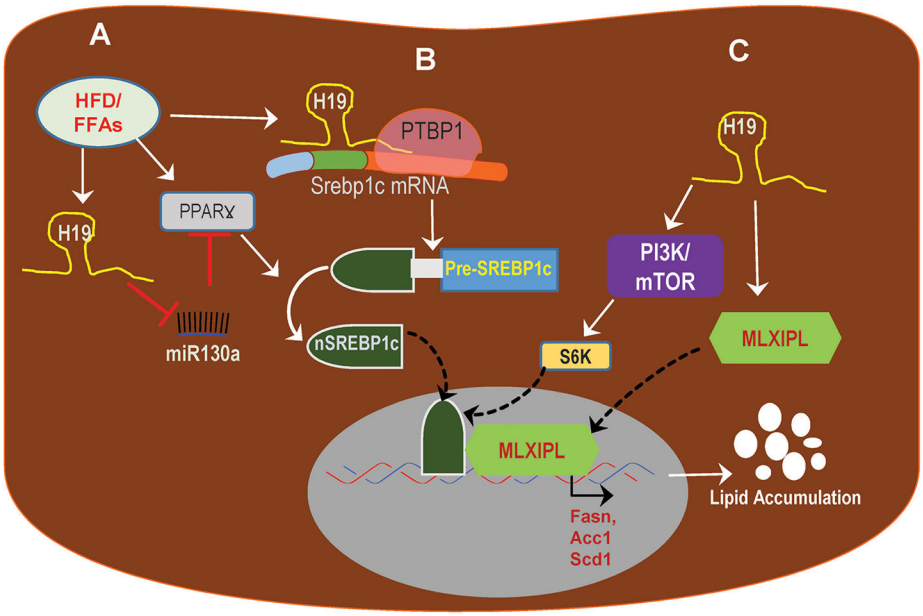

Fig.2. Potential mechanisms of H19-induced hepatic lipid accumulation

HFD/FFAs induce upregulation of H19 in hepatocytes. A) H19 inhibits miR130a expression, an inhibitor of PPARɣ, and results in activation of PPARɣ and hepatic lipogenesis. B) H19 facilitates RBP, PTBP1, to stabilize the Srebp1c mRNA and promotes SREBP1c protein cleavage and nuclear translocation of the activated nuclear form, nSREBP1c and results in the increase of transcription of lipogenic genes. C) H19 induces activation of PI3K/mTOR pathway and upregulates lipogenic transcription factor, MLXIPL, resulting in increased lipid accumulation. HFD: High-fat diet; FFAs: free fatty acids; PPARɣ: peroxisome proliferator-activated receptor ɣ; RBP: RNA binding protein; PTBP1: Polypyrimidine Tract Binding Protein 1; Srebp1c: Sterol regulatory element-binding protein 1c; Mlxipl: MLX interacting protein-like; nSREBP1, the nuclear form of SREBP1c; PI3K/mTOR: phosphoinositide 3-kinase/mammalian target of rapamycin.

Table 1.

Potential targets of lncRNA H19 in cholestatic liver fibrosis

Animal models or Human Samples	*In vitro* models	Targets	Effects	Ref # (Year)
C57BL/6 & $H19^{-/-}$ mice. 2-week BDL	HepG2, Huh7, Hep3B, H69, Mz-Cha-1, CCLP-1, HuCCT1, SG231. Mouse Hepa1, MLC, and MSC.	ZEB1 EpCAM SOX9	BDL-induced H19 suppressed ZEB1 expression, which resulted in the de-repression of EpCAM by ZEB1 and cholestatic liver fibrosis.	(32)(2017)
C57 male mice 3 and 4-week CCl4	Primary mouse hepatocytes, AML12 cell line	Sox9	Sox9-mediated upregulation of H19 is responsible for CCl4-induced liver fibrosis.	(5)(2017)
Sprague-Dawley male rats, 12-week CCl4 model	HSC-T6 cell line	DNMT1 ERK	DNMT1-mediated epigenetic regulation of H19 and H19-mediated activation. ERK1/2 promoted HSC activation and liver fibrosis	(38)(2018)
$Mdr2^{-/-}$, $H19^{-/-}$ (Δ Exon1/+) PSC liver samples (n=16) 8-week CCL4 mouse model	Primary mouse hepatocytes, cholangiocytes, and Kupffer cells; MLC cell line	FXR/SHP S1PR2/ ERK1/2	Bile acid/estrogen-induced H19 expression in cholangiocytes is responsible for gender disparity of cholestatic liver injury in $Mdr2^{-/-}$ mice by downregulation of SHP and activation of S1PR2 /ERK1/2 signaling pathways.	(16, 29) (2017, 2018)
C57/BL6 male mice 8-week CCl4 mouse model	Primary mouse HSCs, human LX2 and L02 cell lines	miR148a, USP4 TGF-β/SMAD	H19 promoted hepatic fibrosis by activating HSCs *via* sponging miR148a and upregulating USP4, which enhanced the TGF-β-mediated activation of SMAD in HSCs.	(37)(2018)
C57/BL6 $Mdr2^{-/-}$, $H19^{-/-}$ (Δ Exon1/+) and DKO mice; 2-week BDL model	Primary mouse hepatocytes, HSCs, cholangiocytes, and Kupffer cells; MLC, H69 and LX2 cell lines	CyclinD1/p21 CCL2/CCR2	Cholangiocyte-derived exosomal H19 is preferentially taken up by HSCs and Kupffer cells significantly promoted the activation of HSCs and macrophages in liver fibrotic progression.	(30,31)(2019, 2020)
C57/BL6 male mice BDL for 2, 4, and 6 weeks.	JS-1 murine HSC cell line	PI3K/AKT/mTOR	H19 promoted autophagy by interacting with the PI3K/AKT/mTOR pathway, which was responsible for IGFBPrP1-induced activation of HSC and liver fibrosis.	(39)(2019)
Human BA patient liver samples (n=57) $Mdr2^{-/-}$, $H19^{-/-}$ and DKO 2-week BDL model	MLC cell line HUCCT1	S1PR2 Let7/ HMGA2	H19 expression level is correlated to disease severity in BA patients. H19 promotes cholangiocyte proliferation and fibrotic liver injury by regulating S1PR2 and let-7/ HMGA2–mediated pathways.	(35)(2019)
ICR male mice 8-week CCl4 mouse model	Primary mouse HSCs and human LX2 cell lines	ADH3/ALDH1 RARα/RXRβ AMPKα/LKB1	H19 induced HSC activation by upregulation of ADH3/ALDH1 and retinoic acid signaling pathways and by activation of AMPKα *via* facilitating the formation of AMPKα/LKB1 complex.	(33,34)(2020)
C57/BL6j $H19^{-/-}$male mice 1-week BDL model	Human Huh7, Mouse Hepa1, MSC, and MLC cell lines	PTBP1 and Let7	H19 suppressed the expression of PTBP1, which inhibited the biogenesis of Let7, but enhanced the bioavailability to their targets.	(36)(2020)

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Table 2.

Potential targets of lncRNA H19 in HCC

Animal models or Human Samples	*In vitro models*	Targets	Potential mechanisms	Ref# (Year)
C57/BL6J mice transplanted with TICs from DEN-treated $Tgfbr2^{fl/fl}$ mice by splenic injection followed by ip injection of CCl4 for 3 weeks and tail vein injection of Ad-Cre.	TICs isolated from B6.129S6-$Tgfbr2^{fl/fl}$ mice (male, 14-day-old) injected with DEN (25 mg/kg).	TGFβ/ Tgfbr2-Sox2	TGFβ regulated H19 expression *via* suppression of SOX2. Inactivation of Tgfbr2 in TICs simultaneously increased SOX2 and H19 levels, which are responsible for HCC development and progression.	(43)(2019)
Human HBV patient liver tissues and matched normal tissue (n=20)	Human L02 cell line	miR675/PPARα Akt/ mTOR	HBV x protein-induced H19/miRNA675 is responsible for HBV-associated hepatitis and liver injury by activating PPARα and Akt/mTOR signaling pathways.	(42)(2019)
16-month Female and 17.7-month male C57/BL6 $Mdr2^{-/-}$and $Mdr2^{-/-}$/$H19^{-/-}$DKO mice. Human HCC patient liver tissues (n=242) with matched non-tumor tissues (n=298)	Primary cells from mouse non-tumor liver tissues	H19 is pro-oncogenic in inflammation-mediated HCC.	The single-cell transcriptome analysis of the non-tumor tissue of 18-month old female $Mdr2^{-/-}$ mouse indicated that H19 was mainly expressed in hepatocytes, endothelial cells and macrophages. H19 expression level in HCC tumor inversely correlated to the patient's survival.	(41)(2020)
Human HCC liver tissues	Huh7, Hep3B, SNU-449, and SNU-387 cell lines	miR675	High H19 expression is negatively related to sorafenib sensitivity by upregulation of miR675 in HCC cells.	(45)(2020)
Human HCC liver tissues and matched non-cancerous liver tissues (n=55)	HepG2 cell line	G3BP1 Myc	NSUN2-mediated m^5C-modified H19 promotes HCC by recruiting G3BP1 oncoprotein, which leads to MYC accumulation.	(44)(2020)
Human HCC liver tissues (n=64) and TCGA cohort (N=393)	Hep-G2, Hep2B2, THP-1, SK-OV-3 and NCI-H520	miRNA-193b MAPK1 axis	TAM-derived H19 promotes tumor cell migration and invasion and immune cell infiltration by hijacking miR-193b as a sponge and activating MAPK1.	(46)(2020)