Review

JHEP|Reports

Our emerging understanding of the roles of long non-coding RNAs in normal liver function, disease, and malignancy

Amin Mahpour,[1,2] Alan C. Mullen[1,2,3,*]

Summary

Long non-coding RNAs (lncRNAs) are important biological mediators that regulate numerous cellular processes. New experimental evidence suggests that lncRNAs play essential roles in liver development, normal liver physiology, fibrosis, and malignancy, including hepatocellular carcinoma and cholangiocarcinoma. In this review, we summarise our current understanding of the function of lncRNAs in the liver in both health and disease, as well as discuss approaches that could be used to target these non-coding transcripts for therapeutic purposes.

Keywords: lncRNA; Liver-specific lncRNAs; Liver fibrosis; Liver cancer; Hepatocellular carcinoma; Cholangiocarcinoma; Liver metabolism

Received 23 May 2020; received in revised form 6 August 2020; accepted 20 August 2020; available online 3 September 2020

Introduction

Seventy percent of the human genome is transcribed, yet only 2–3% of the genome encodes RNA transcripts that are translated into proteins.[1] Most biomedical research has focused on protein-coding genes, because proteins are considered the primary molecular building blocks that control the structure and function of cells. Recent advances in genome-wide RNA sequencing approaches have revealed the abundance and diversity of non-coding (nc) RNAs, and these RNA transcripts are increasingly recognised for their diverse biological functions in eukaryotic cells.

NcRNAs are primarily classified by their mechanism of transcription and mode of action. Most ncRNAs, including ribosomal and transfer RNA molecules, are derived through transcription by RNA polymerase (RNA Pol) I and RNA Pol III. RNA Pol II also transcribes various classes of small ncRNAs including microRNAs (miRNAs), small nucleolar RNAs, and piwi-interacting RNAs, which have been reviewed in detail previously.[2] The functions of longer RNA Pol II-transcribed ncRNAs, commonly referred to as long non-coding RNAs (lncRNAs) have more recently piqued the interest of researchers.[3]

What constitutes an lncRNA is still a matter of debate. However, lncRNAs are commonly defined as ncRNA transcripts that are greater than 200 nucleotides in length, which separates them from small ncRNAs such as miRNAs. Similar to messenger (m) RNAs, lncRNAs are 5′- capped and commonly contain a poly-adenylated tail at their 3′-end.[4] Interestingly, a few lncRNAs have been described that contain an RNase P-catalysed triple-helical structure at their 3′-ends instead of a poly-adenylated tail, presumably increasing transcript stability.[5]

In comparison to mRNAs, lncRNAs tend to be shorter transcripts and contain fewer exons.[6] The half-lives of lncRNAs are variable, but overall lncRNAs tend to be less stable than mRNAs.[7,8] Decreased stability of lncRNAs helps to explain why transcription at genes encoding lncRNAs is closer in level to transcription at protein-coding genes, while there is a greater difference in expression of mature mRNAs and lncRNAs, as measured by RNA sequencing.[9]

Many lncRNAs include one or multiple open reading frame (ORF) regions for protein synthesis.[10] Yet, possible ORFs in lncRNA transcripts are often not translated or, if translated, the resulting protein product is unstable and rapidly subjected to degradation; therefore these protein products are not thought to play a substantial biological role.[4]

The liver is an essential organ in the gastrointestinal system, which mediates numerous digestive and metabolic functions. Recent work from many research laboratories has led to an increased understanding of the role of lncRNAs in liver development, physiology, and pathology. We will review the identification and classification of lncRNAs, general mechanisms of action, our current understanding of lncRNA activities in the context of the liver, and the potential of lncRNAs as therapeutic targets in the treatment of liver disease.

Identification

Early efforts to identify and characterise lncRNAs employed sequencing of expressed cDNA libraries and expression sequence tags (ESTs).[11] However, because of the inherent laboriousness of this strategy, few lncRNAs were characterised.

[1]*Gastrointestinal Unit, Massachusetts General Hospital, Boston, MA 02114, USA;* [2]*Harvard Medical School, Boston, MA 02115, USA;* [3]*Harvard Stem Cell Institute, Cambridge, MA 02138, USA*

* Corresponding author. Address: Massachusetts General Hospital, 55 Fruit Street, Boston, MA 02114, USA.
E-mail address: acmullen@mgh.harvard.edu (A.C. Mullen).

Subsequent approaches such as chromatin profiling by immunoprecipitation followed by high-throughput sequencing drastically improved the lncRNA discovery process. Regions containing specific histone marks, such as trimethylation of histone 3 lysine 4 (H3K4me3) and histone 3 lysine 36 (H3K36me3), define areas of active transcriptional initiation and elongation and were employed to identify lncRNA transcripts that do not overlap with protein-coding genes.[12]

With the advent of next-generation RNA-sequencing, lncRNA identification underwent a significant revolution.[13] With this approach, short RNA-sequencing reads were used to assemble a transcriptome model by mapping reads to a reference genome to identify exons and splice junctions between exons. The assembled transcriptome was then further subjected to a multitude of algorithms to filter out transcripts that have coding potential.[14] This computational strategy involved significantly less manual work than earlier approaches. However, this method relies heavily on the error-prone *de novo* construction of transcript sequences from short reads, which can lead to the assembly of incomplete transcripts. With this limitation in mind, defining the 5′ and 3′ ends of the lncRNA through approaches such as rapid amplification of cDNA ends (RACE)[15] and cloning the full-length lncRNA transcript in a specific cell type of interest are good starting points to define the exons of poorly characterised lncRNAs.

An alternative method that employs long-read sequencing is increasingly being utilised to sequence full-length transcripts.[16] The promise of this newer method is the increased accuracy of the exons and splice junctions contained in each transcript, but this comes at a cost of an increased rate of sequencing errors and decreased genome coverage when performed at the same price point as short-read sequencing.

With notable exceptions, most lncRNAs do not possess cross-species sequence homology.[17] However, this does not by any means suggest that lncRNAs are not or cannot be evolutionarily conserved. In contrast to the conservation of protein-coding genes, which rely heavily on the sequence homology to maintain amino acid sequence, lncRNA conservation may be enforced at the level of the RNA structure, function, and or syntenic expression pattern. The last criterion, which relies on conserved location in the genome relative to proximal genes, is often used to identify human orthologs of lncRNAs that are initially found in model organisms (*e.g.* mice).[18] Even taking these additional criteria into account, it is estimated that only a little over one-third of human lncRNAs have orthologous transcripts in mice.[19] Although these multiple levels need to be considered when addressing lncRNA conservation, it offers a far better insight into the mechanisms of lncRNA evolution across different organisms.

Through these efforts, a large collection of annotated lncRNAs have been described in an increasing number of organisms and are accessible through a multitude of sources, including NONCODE,[20] GENCODE,[21] RNAcentral,[22] and LNCipedia[23] databases. Fig. 1 shows the number of genes encoding lncRNAs and the number of different lncRNA transcripts annotated in different species. The increased number of lncRNAs in humans and mice compared to other vertebrates likely reflects the greater depth of sequencing analysis in these two species.

Diverse genomic origins of lncRNAs

LncRNAs are classified according to their sites of transcription relative to annotated protein-coding genes[24] (Fig. 2). Transcripts

Key points

- Long non-coding RNAs (lncRNAs) are emerging as critical biological mediators in the normal functioning of the liver.
- Aberrant expression of lncRNAs is associated with metabolic diseases, fibrosis, and malignancies involving the liver.
- LncRNAs exert their pathobiological effects through a multitude of mechanisms.
- Liver-specific targeting of lncRNAs is a promising novel treatment modality.
- LncRNAs have potential as biomarkers in liver disease.

that do not overlap with known protein-coding or small RNA-coding genes are termed long intervening non-coding (linc) RNAs.[12,17] LincRNAs are also often described as intergenic non-coding RNAs in the literature. This category of lncRNAs was easier to identify because of their distance from known genes, and many of the earliest described lncRNAs, including *H19*,[25] X-inactive specific transcript (*XIST*),[26,27] and HOX transcript antisense RNA (*HOTAIR*),[28] fall into this category.

Natural antisense transcripts (NATs) are widely expressed in the human genome. NATs are the result of transcription on the opposite strand to the sense protein-coding gene such that the non-coding transcript is the reverse complement of a region of the protein-coding transcript.[29] NATs are understood to function predominantly in cis (*i.e.* acting in proximity to where they are transcribed) by regulating the sense protein-coding transcript.[30] One mechanism proposed to explain how NATs regulate gene expression is through the formation of duplexes in which the sequence complementarity between NATs and the sense transcripts interferes with the recruitment of the splicing machinery, resulting in skipped exons or incomplete splicing through a process called RNA masking.[31] In an alternative mechanism, the RNA duplex may serve as an immediate substrate for either RNA editing[32] or promote degradation through the RNA interference pathway.[33]

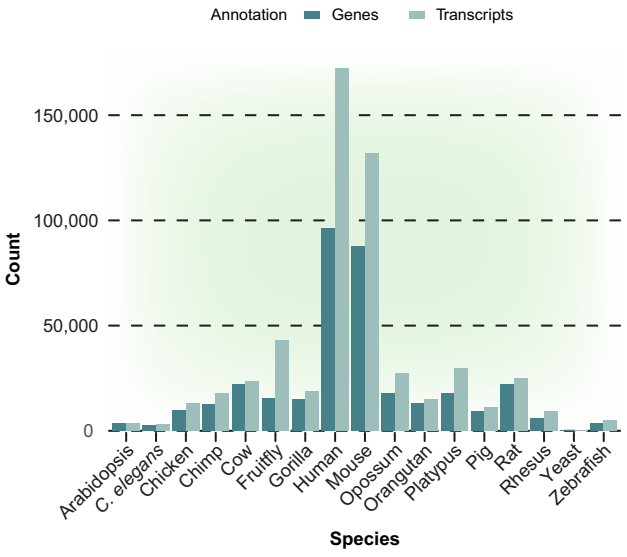

Fig. 1. Quantifications of lncRNA genes and their transcripts in multiple species as deposited in the release of NONCODE v.5 database. Transcripts consider the number of lncRNA isoforms identified for each lncRNA gene.

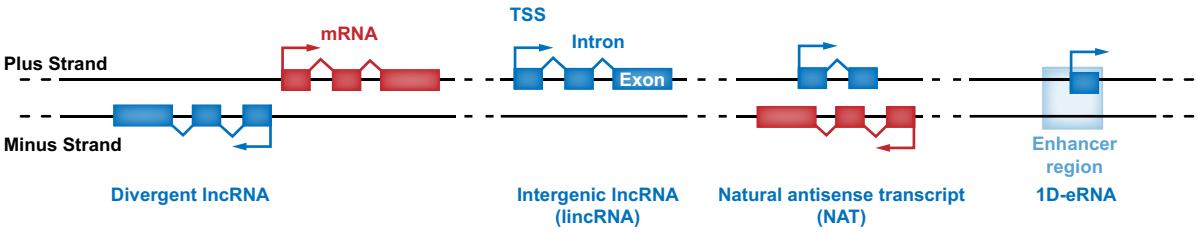

Fig. 2. LncRNAs are classified by genomic origins relative to protein-coding genes. Divergent lncRNAs are encoded on the opposite strand and direction from protein-coding genes. As the name suggests, lincRNAs are found in regions between genes. NATs are transcribed from the antisense strand of a protein-coding gene. 1D-eRNAs are lncRNAs transcribed from regions identified as enhancers and are distinct from 2D-eRNAs, which are divergently transcribed and non-polyadenylated transcripts produced from enhancers. eRNA, enhancer RNA; lincRNA, long intervening non-coding RNA; lncRNA, long non-coding RNA; NATs, natural antisense transcripts; TSS, transcription start site.

Divergent lncRNAs are transcribed head-to-head from protein-coding genes so that the transcription start sites of the lncRNA and protein-coding genes are in close proximity and are oriented in opposite directions. Transcription of the divergent lncRNA can be facilitated by a shared bi-directional promoter that induces the expression of both lncRNA and protein-coding genes.[34] A criterion that is increasingly being used to define this class of lncRNAs is whether the lncRNA transcription starts within 300–500 base pairs of the transcription start site of a protein-coding gene.[24]

Enhancer RNAs (eRNA) are another novel class of RNA species and are exclusively expressed at enhancer regions.[35] Most eRNA transcripts are divergently transcribed (2D-eRNA) from enhancers, are composed of single exons, and are non-polyadenylated.[36] Because of the relatively open chromatin structure at enhancers, these eRNAs are considered to be the indirect effect of noisy transcriptional activity mediated by Pol II-containing transcriptional machinery as enhancers loop into proximity with promoters.[35] This transcriptional activity is primarily associated with divergently transcribed eRNAs that are not polyadenylated and has been exploited to identify novel enhancer regions by transcriptional profiling of eRNAs.[37] Whether or not these divergent, non-polyadenylated eRNA transcripts have functional activity has been the subject of multiple studies.[38–40] However, it is also imaginable that the process of eRNA transcription at the enhancer region could regulate enhancer activity by itself, through recruitment of activating transcription factors.[36] A less abundant class of eRNAs is unidirectional and polyadenylated (1D-eRNA).[41] These unidirectional eRNAs have similar features to lncRNAs but are encoded by genomic regions associated with an increased abundance of H3K4me1 relative to H3K4me3 when compared to regions encoding lncRNAs. The functional distinction between a non-coding transcript labelled as a unidirectional eRNA and one labelled as an lncRNA is still not well understood, and likely represents different RNA species across the same continuum.

Mechanisms of action: Transcript or transcription, that is the question

Perhaps the most studied question at the core of lncRNA biology is whether the non-coding transcript or the act of its transcription confers function to the lncRNA locus. In this line, evidence for each scenario is rapidly mounting for individual lncRNAs.

For some lncRNAs, genetic manipulation of the lncRNA locus in mouse models suggests that the mere act of transcription or related processes that include splicing allow cross-regulation of lncRNAs with that of nearby protein-coding genes.[42] However, the use of genetically manipulated models to study lncRNA function should be carefully interpreted, as complete or partial deletion of regulatory regions (*i.e.* promoters or enhancers) that control transcription of lncRNAs may adversely affect the expression of nearby genes. In contrast, other studies point to the direct role of transcripts, often exerted in trans, as opposed to the act of transcription, as described below. We will briefly review the range of functions attributed to lncRNA transcripts before discussing how specific lncRNAs function in the liver.

Interactions between lncRNAs and chromatin

Perhaps the earliest and the best-known lncRNA that directly interacts with chromosomes to exert its function is *XIST*.[43] One X chromosome is transcriptionally silenced in somatic cells of female mammals early in development in a process known as X-chromosome inactivation.[44] X-chromosome inactivation begins with the expression of the *XIST* transcript and distribution of the transcript along the entire X chromosome. *XIST* assists the formation of silent heterochromatin through the recruitment of two polycomb repressive complexes (PRCs) known as PRC1 and PRC2.[45]

A host of lncRNAs have also been described that function as more targeted activators or suppressors of gene expression through modification of chromatin or DNA. *HOTTIP* (HOXA transcript at the distal tip), for instance, functions through the adaptor protein WDR5 to recruit histone methyltransferases to trimethylate the fourth lysine residue of histone 3 protein (H3K4me3), promoting gene expression.[46] In contrast, immunoprecipitation of the PRC2 silencing complex provided the first indications that lncRNAs can direct regulatory complexes to specific loci in the genome to repress gene expression.[28] *HOTAIR* is expressed within the *HOXC* cluster[47,48] and functions in trans (*i.e.* function at distant sites from transcription) to silence the *HOXD* loci, which is located on a different chromosome.[28] Other lncRNAs such as *KCNQ1OT1* recruit DNA methyltransferases in cis to catalyse CpG dinucleotide methylation and suppress gene expression in the *KCNQ1* gene cluster of the paternal chromosome.[49]

Structural lncRNAs

NEAT1 (nuclear enriched abundant transcript 1) was first described as an abundant nuclear lncRNA.[50] Together, with other ncRNAs,[51] *NEAT1* plays a structural role to promote the formation of membrane-less nuclear bodies named paraspeckles.[52] Paraspeckles contain both proteins and RNAs and are thought to

regulate gene expression through the retention of mRNAs in the nucleus, as recently reviewed.[52,53] Similarly, another structural lncRNA *MALAT1* (metastasis-associated lung adenocarcinoma transcript 1) is associated with nuclear speckles,[54] which contain splicing factors.[55] Both *NEAT1* and *MALAT1* are present at hundreds of transcriptionally active regions and act as structural components of these two nuclear bodies.[56]

Unexpectedly, a crosstalk between *NEAT1* and mitochondria was recently described.[57] In response to mitochondrial stress, the transcription factor ATF2 is activated, inducing expression of *NEAT1*, leading to a change in nuclear paraspeckle morphology and an increase in the number of paraspeckles that retain mitochondrial mRNAs. This response results in decreased production of mitochondria and mitochondria-dependent apoptosis proteins. Thus, *NEAT1* affects mitochondrial function and dynamics through changes in the expression of mitochondrial proteins.

LncRNAs as miRNA sponges

One mechanism by which lncRNAs can regulate gene expression post-transcriptionally is to serve as miRNA sponges.[58] Because this class of lncRNAs competes with mRNAs for miRNA binding, these lncRNAs also belong to the category of competing endogenous RNAs (ceRNAs) and have been shown to regulate a vast number of genes and related pathways.[59] However, for an lncRNA to function as a miRNA sponge, it must be expressed at a high enough level to compete with mRNAs that contain the same miRNA seed sequence. LncRNAs are usually expressed at lower levels than mRNAs, and it is not clear how the stoichiometry could support this competing function for many examples of ceRNAs, even if an lncRNA contains multiple seed sequences for the same miRNA.[60]

LncRNAs in early liver development

Organ development and cell fate determination are driven by the coordinated regulation of hundreds to thousands of genes.[61] Owing to their ability to modulate gene regulatory pathways and the tight control of their expression in differentiation, lncRNAs have been shown to play important roles in development.[62]

Foetal liver development is a complex process that comprises multiple differentiation stages from cells derived from different lineages.[63] Lineage studies indicate that hepatocytes and cholangiocytes are derived from the mesendoderm lineage that in turn differentiates into the definitive endodermal (DE) layer of the early embryo. DE then organises in a tube along the anterior-posterior axis of the embryo, which ultimately forms the primitive digestive tract, including the foregut, midgut and hindgut. The foregut is the progenitor region for many internal organs including the liver, gastrointestinal system, lungs and thyroid.[64]

Differential expression analysis of lncRNAs during DE differentiation led to the identification of the DE-specific lncRNA *DEANR1* (definitive endoderm-associated lncRNA1).[65] Activin signalling drives embryonic stem cell differentiation towards DE through activation of the transforming growth factor-β (TGF-β) receptor signalling pathway and phosphorylation of the co-activators SMAD2 and SMAD3 (SMAD2/3). *DEANR1* recruits SMAD2/3 to the *FOXA2* promoter to induce FOXA2 expression and promote DE differentiation.

Combined analysis of SMAD3 gene occupancy and expression profiling of DE differentiation led to the identification of *DIGIT* (divergent to goosecoid, induced by TGF-β family signalling).[66] The transcription of *DIGIT,* which is divergently transcribed from the gene encoding goosecoid (*GSC*), is induced during DE differentiation in both human and mouse models of embryonic stem cell differentiation. *DIGIT* interacts with BRD3 at sites of histone 3 lysine 18 acetylation (H3K18ac), regulating gene expression and promoting DE differentiation.[67]

LncRNAs in normal liver function and lipid processing

The liver is responsible for homeostasis and regulation of lipid metabolism in mammals, and disruption of normal lipid metabolism can lead to hepatic steatosis.[68] Many lncRNAs have been described that affect lipid metabolism and are reviewed elsewhere.[69,70] We will focus our discussion on lncRNAs that have been shown to regulate key steps in lipid metabolism (Fig. 3).

LncRNAs in lipid metabolism

Cholesterol is imported into the liver from LDL lipoproteins via LDL receptors on hepatocytes. This influx is counterbalanced by the efflux of cholesterol through ATP-binding cassette transporter A1 (ABCA1), which delivers cholesterol to HDL lipoprotein.[71] Intracellular cholesterol is processed by cholesterol 7α-hydroxylase (also called cytochrome P450 family 7 subfamily A member 1 [CYP7A1]) to form 7α-hydroxycholesterol,[72] which is then modified by cytochrome p450 family 8 subfamily b member 1 (CYP8B1) to eventually form cholic acid.[73] Cholic acid, in addition to chenodeoxycholic acid, is then conjugated to taurine or glycine for excretion in the bile.[74] CYP8B1 activity shifts the ratio of cholic acid:chenodeoxycholic acid in favour of cholic acid, which has a weaker stimulatory effect on the farnesoid X receptor (FXR),[75] a sensor of bile acid composition.[76–79]

Lnc-HC was discovered as an upregulated transcript in the livers of rats with high-fat diet-induced metabolic syndrome.[80] Although *Lnc-HC* is not highly evolutionarily conserved, syntenic transcripts have been found in other organisms. The study of protein interactors of this lncRNA revealed that *Lnc-HC* interacts with hnRNPA2B1 and forms a ribonucleoprotein complex. Analysis of data from RNA-immunoprecipitation showed that transcripts encoding CYP7A1 and ABCA1 are present in the *Lnc-HC*-hnRNPA2B1 complex. These two genes have previously been shown to function as key regulators of lipid and specifically cholesterol catabolism.[81] *In vitro* experiments using hepatocytes indicated that interaction with the *Lnc-HC*-hnRNPA2B1 complex induces nuclear retention and eventual degradation of *CYP7A1* and *ABCA1* transcripts, culminating in cholesterol accumulation in hepatocytes.

LncLSTR (lncRNA liver-specific triglyceride regulator) is a murine lncRNA whose depletion results in reduced plasma triglyceride levels.[82] *LncLSTR* interacts with TDP-43 and interferes with the ability of TDP-43 to suppress the expression of CYP8B1, which is an essential enzyme in cholesterol metabolism. Thus, depletion of *lncLSTR* leads to decreased expression of CYP8B1, which affects bile acid composition.[83,84] Activation of apolipoprotein C2 (APOC2) and the reduction in plasma triglyceride levels observed with depletion of *lncLSTR* were attenuated with depletion of FXR.[76–79] Together these results suggest that *lncLSTR* modulates bile acid composition to regulate APOC2 expression, via FXR,[85] and to control serum triglyceride levels.

Steroid response binding proteins (SREBPs) are a family of transcription factors that bind sterol response elements (SREs)

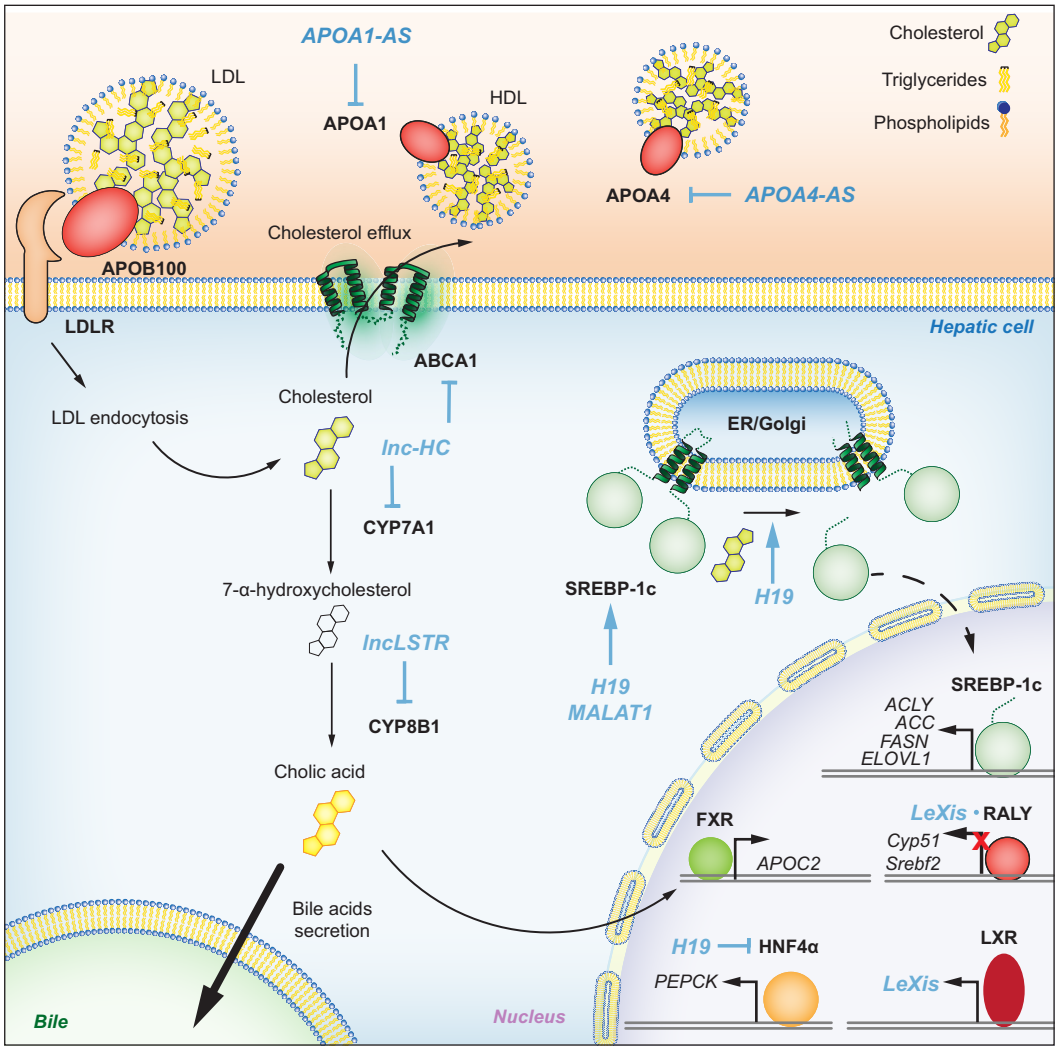

Fig. 3. LncRNAs regulate various aspects of lipid metabolism. Extrahepatic cholesterol enters hepatocytes through the LDL receptor (LDLR). lncRNAs *lnc-HC* and *lncLSTR* control key enzymes in cholesterol catabolism. *H19* and *MALAT1* regulate SREBP-1c stability. SREBP-1c controls the expression of genes regulating fatty acid synthesis including ACLY (ATP citrate synthase), ACC (acetyl-CoA carboxylase), FASN (fatty acid synthase) and ELOVL1 (ELOVL fatty acid elongase 1).[186] HNF4α, which regulates critical genes such as PEPCK in gluconeogenesis, is inhibited by *H19*. Changes in the ratio of bile acids triggers FXR activation as a transcription factor to promote the expression of APOC2. Lipoproteins including APOA1 and APOA4 are involved in transfer of lipid molecules and are regulated by *APOA1-AS* and *APOA4-AS*. ER, endoplasmic reticulum.

in promoters to regulate the expression of genes involved in lipid metabolism.[86] SREBPs are found as transmembrane proteins in the endoplasmic reticulum and Golgi; protein cleavage releases the DNA-binding domain which then acts as a transcription factor.[87] In the past few years, several lncRNAs that modulate the activity of SREBPs have been characterised. Overexpression of *MALAT1* in HepG2 cells, for example, increases the stability of the mRNA encoding SREBP-1c, resulting in increased intracellular lipid droplets.[88] siRNA-mediated knockdown of *MALAT1* in the *ob/ob* mouse model of obesity results in a decrease in hepatic lipid content. These experiments also revealed that depletion of *MALAT1* in the *ob/ob* mouse results in improved insulin sensitivity and glucose tolerance.

In a similar mechanism, *H19* – through interaction with PTBP1 – stabilises the *SREBP-1c* transcript and may also promote nuclear localisation of the SREBP-1c protein.[89] Both *H19* and its binding protein are increased by fatty acids in hepatocytes and mouse fatty liver models. Data also suggest that *H19* is induced in patients with type 2 diabetes.[90] Interestingly, *H19* is increased in mouse models of diabetes and temporary fasting.[91] Mechanistically, it is proposed that *H19* also interacts with S-adenosylhomocysteine hydrolase to suppress DNA methylation[92] of the gene encoding hepatocyte nuclear factor-4α (HNF4α), resulting in increased expression of this transcriptional regulator of gluconeogenesis.

Liver X receptors (LXRs) are key transcription factors that control cholesterol homeostasis.[93] Activation of LXRs results in decreased cholesterol content in the liver.[94] *LeXis* (liver-expressed LXR-induced sequence) was originally characterised in cells treated with the LXR agonist GW3965.[95] Detailed analysis of the *LeXis* interactome suggests that *LeXis* regulates cholesterol anabolic pathways through interaction with the transcriptional coactivator RALY. In adenovirus-transduced mouse models, the induction of *LeXis* is associated with reduced serum cholesterol levels.

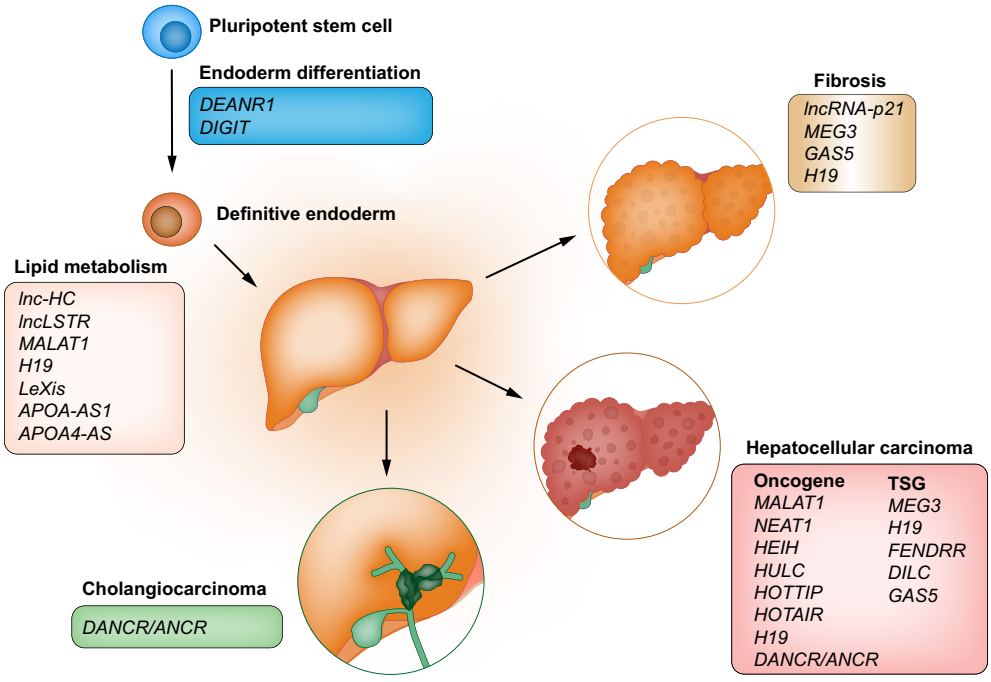

Fig. 4. An overview of lncRNAs involved in liver development, metabolism and disease. LncRNAs implicated in hepatocellular carcinoma are classified based on whether they act as oncogenes or tumour suppressor genes (TSG).

LncRNAs that modulate the transfer of lipid molecules

Lipoproteins are a class of lipid-binding proteins that are involved in transfer of lipid molecules in the body. Apolipoprotein A1 (APOA1) is synthesised in both the liver and intestine and is the primary protein found in HDL complexes,[96] which are responsible for the transport of excess cholesterol to the liver (as previously reviewed[97]). Apolipoprotein A4 (APOA4), is produced in the intestinal epithelium upon lipid absorption and is involved in a number of different physiological functions including lipid absorption, metabolism, and platelet aggregation.[98]

APOA1-AS is a transcript antisense to the gene encoding APOA1, which negatively regulates APOA1 transcription in both *in vivo* and *in vitro* liver models.[99] Although not much is known about the functional mechanisms of this lncRNA, it is proposed that *APOA1-AS* recruits various histone-modifying enzymes to the *APOA1* locus, which in turn regulate APOA1 expression.

Similar to *APOA1-AS*, *APOA4-AS* belongs to the class of NAT lncRNAs.[100] Expression of *APOA4-AS* is elevated in fatty liver disease, and depletion of this lncRNA in the *ob/ob* mouse model results in reduced expression of APOA4 and decreased serum triglyceride levels. *APOA4-AS* interacts with an RNA-stabilising protein named human antigen R (HuR) in a sequence-dependent manner. The depletion of HuR protein is associated with reduced *APOA4-AS* and *APOA4* transcript levels and suggests that interaction with HuR may help promote stability of both *APOA4-AS* and *APOA4* mRNA.

LncRNAs in liver injury and fibrosis

Microarray profiling of lncRNAs in the whole mouse liver uncovered multiple transcripts whose expression changed significantly as a result of liver injury induced by 3 weeks of carbon tetrachloride (CCl_4) treatment.[101] Among these, *Gm2199* was shown to be repressed with CCl_4-induced damage. Treatment of hepatocytes with CCl_4 caused a significant reduction in proliferation, which was restored by ectopic expression of *Gm2199*. As for the mechanism, the authors provide evidence that *Gm2199* achieves these effects through increased expression and activation of mitogen-activated protein kinase ERK1/2. *In vivo* studies corroborated these results by showing that increased expression of *Gm2199* protects cells from the adverse effects of CCl_4 treatment. Although a human lncRNA orthologous to *Gm2199* has not yet been described and thus *Gm2199* may not have relevance for human disease, this study demonstrates how an lncRNA can affect intricate molecular pathways involved in liver injury.

The list of lncRNAs identified that modulate fibrosis, in general, continues to grow and was recently reviewed elsewhere.[102] We will focus here on lncRNAs most relevant to liver fibrosis (Fig. 4). Hepatic stellate cells (HSCs) are the primary cell type responsible for liver fibrosis,[103–105] and lncRNAs expressed in human HSCs were recently described, many of which are co-expressed with extracellular matrix genes.[106]

In mouse models of CCl_4-induced liver fibrosis, *lincRNA-p21* was found to be significantly decreased. Overexpression of *lincRNA-p21* in primary HSCs resulted in increased expression of the p21 protein and reduced expression of α-smooth muscle actin (α-SMA [*ACTA2*]) and collagen type I α 1 (COL1A1), suggesting a role for *lncRNA-p21* in suppressing HSC activation and transformation to myofibroblasts.[107]

MEG3 (maternally expressed gene 3) is a maternally imprinted gene whose non-coding product has been described in multiple cancers.[108,109] *MEG3* expression is decreased in fibrotic liver tissue and activated in primary HSCs. Overexpression of this lncRNA through a viral induction system also showed antifibrotic activity in murine CCl_4-induced liver fibrosis.[110,111] In the immortalised human HSC line LX-2, *MEG3* transcript levels are reduced in response to TGF-β treatment.[111] The mechanism by which *MEG3* confers antifibrotic activity is still largely unknown.

Table 1. LncRNAs associated with liver cancer.

LncRNA	Role	Mechanism	Ref
Hepatocellular carcinoma (HCC)			
MALAT1	Oncogene	Activation of oncogenic splicing factor SRSF1	118
NEAT1	Oncogene	Acts through the sponge activity to inhibit miR-129-5p.	121
HEIH	Oncogene	Interacts with PRC2 component EZH2	122
HULC	Oncogene	Inhibition of PTEN tumour suppressor gene through the sponge-activity on miR-15a	131
HOTTIP	Oncogene	Regulates gene expression from the *HOXA* region	134
HOTAIR	Oncogene	Repressively alters the chromatin landscape of promoters through recruitment of PRC2	137
DANCR/ANCR	Oncogene	Increased stability of HNRNPA1	143
H19	Mixed	Conflicting studies suggest both oncogenic and tumour suppressor roles in HCC	138–140
MEG3	Tumour suppressor	Increases expression of miR-122 through which pyruvate kinase muscle isozyme M2 (PKM2) is down-regulated	153
FENDRR	Tumour suppressor	Acts as a miR-423-5p sponge to regulate GADD45B expression	155
DILC	Tumour suppressor	Interacts with the *IL6* promoter to suppress its expression. Inhibits IL6/STAT3 autocrine signalling pathway in LCSCs	157
GAS5	Tumour suppressor	Functions as a sponge to bind miRNAs. Binds to miR-126-3p, whose ectopic expression has been shown to decrease cell proliferation in HCC. Binds to miR-21 to derepress the PTEN protein levels	161
Cholangiocarcinoma (CCA)			
DANCR/ANCR	Oncogene	Associated with increased cellular proliferation and migration through interaction with EZH2	145

In HCC, lncRNAs have been described to function as both oncogenes and tumour suppressors. In CCA, *DANCR/ANCR* has been shown to function as an oncogene.

MEG3 is suggested to inhibit HSC activation by suppressing epithelial-mesenchymal transition through interactions with smoothened (SMO) protein. *MEG3* has also been shown to induce cholestatic liver damage by accelerating the decay of small heterodimer partner (*SHP*) mRNA through interactions with PTBP1.[112] In turn, attenuation of SHP protein, which is a key repressor in the bile acid biosynthesis pathway, results in liver injury.

The role of *GAS5* (growth arrest-specific transcript 5) in liver fibrosis has also been described.[113] Expression of *GAS5* is decreased in human and mouse fibrotic liver tissues. Similarly, *in vitro* experiments employing activated HSCs also suggest that *GAS5* expression is reduced compared to quiescent HSCs. Overexpression of this lncRNA results in decreased expression of *COL1A1* and *ACTA2* in HSCs *in vitro* and decreased collagen levels in CCl_4-induced murine liver fibrosis models, as determined by measurement of hepatic hydroxyproline content. Mechanistically, *GAS5* is considered to function as a ceRNA through interaction with miR-233. The authors demonstrated that sequestration of miR-233 by *GAS5* results in increased expression of the miRNA target transcripts including the p27 gene, whose protein product is directly involved in HSC proliferation and activation.

H19 is induced in cholestatic liver fibrosis.[114] Ectopic expression of *H19* in the liver is associated with increased necrosis and fibrosis in the setting of bile duct ligation (BDL), and *H19*-deficient mice show reduced cholestatic liver injury and fibrosis in response to BDL. *H19* exerts its function in this setting by binding and inhibiting the zinc finger E-box-binding homeobox 1 (ZEB1), whose activity suppresses activation of epithelial cell adhesion molecule (EpCAM). Ectopic expression of ZEB1 or depletion of EpCAM in *H19*-deficient mice reduced fibrosis, leading to a model where bile acids induce *H19* expression in hepatocytes and cholangiocytes. *H19* then interacts with ZEB1, preventing binding to the EpCAM promoter, leading to increased EpCAM expression and increased susceptibility to cholestatic liver fibrosis.

LncRNAs in liver cancer

LncRNAs are increasingly recognised as mediators of human cancers.[115] In this regard, depending on the context, lncRNAs can function as either oncogenes or tumour suppressors. In the liver, lncRNAs have been identified that are associated with hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA) (Table 1), which comprise the majority of liver cancer cases, and were reviewed recently.[116] Below, we describe oncogenic lncRNAs that are involved in promoting HCC and CCA; we then look at lncRNAs that function as tumour suppressors to hinder malignant growth.

Oncogenic lncRNAs

As the name suggests, *MALAT1*, also known as *NEAT2* (nuclear-enriched abundant transcript 2), is an oncogenic lncRNA in human cancer.[117] This lncRNA is highly conserved in mammals, and its role in numerous biological processes has been extensively studied. In HCC, *MALAT1* is upregulated, and its depletion results in lower tumorigenicity, which is largely driven by reduced expression of the oncogenic splicing factor SRSF1.[118] Furthermore, *MALAT1* activates the mammalian target of rapamycin (mTOR) pathway through SRSF1-mediated splicing of S6 kinase 1 (S6K1) to an alternative isoform named Iso-2. The Iso-2 protein product has oncogenic potential through direct binding and activation of mTOR complex 1 (mTORC1).

We discussed the role of *NEAT1* as a structural lncRNA previously. However, *NEAT1* has also been implicated in multiple cancers,[119,120] and its role and expression status in HCC have recently been described.[121] Expression profiling of *NEAT1* transcripts in normal liver and HCC tumour tissues and cell lines revealed that *NEAT1* is induced in neoplastic cells. Fang *et al.*

suggested that, mechanistically, *NEAT1* exerts its oncogenic property as a ceRNA through binding and inhibition of miR-129-5p, whose main cellular targets include valosin-containing protein (VCP) and the NF-κB inhibitor protein (IκB).

HEIH (high expression in HCC) was originally identified in HBV-associated HCC tissues.[122] RNA pulldown assays indicated that *HEIH* interacted with the PRC2 complex protein EZH2. shRNA-mediated depletion of *HEIH* caused increased expression of genes that are normally repressed by the PRC2 complex. *HEIH* promotes cell cycle progression and tumour growth via a pathway that is at least in part dependent on EZH2. This study also found that the expression level of *HEIH* in tumour tissue has potential as a prognostic marker in patients with HCC

HULC (highly upregulated in liver cancer) is an oncogenic lncRNA whose role, among others, has been described in hepatic, gastric, and colorectal cancers.[123] Abnormal expression of *HULC* is associated with poor prognosis of pancreatic cancers and hepatomas in the clinic.[124,125] Measuring serum levels of this lncRNA has also been proposed as a biomarker in liver cancer.[126,127] *HULC* influences many cell traits in cancer through multi-pronged metabolic and cellular signalling pathways that are all relevant in hepatic biology.

The transcription factor CREB and the HBV X protein (HBx) have been well-studied in the context of liver cancer[128] and are upstream regulators of *HULC*. *HULC* can act as a miRNA sponge. It contains binding sites for miR-372, which usually targets translation of the CREB-kinase PRKACB. PRKACB-mediated phosphorylation of CREB results in chromatin modifications that promote gene expression including that of *HULC*.[129] Additionally, infection with HBV is associated with increased levels of *HULC* in HCC samples. Detailed analysis of HBV-induced HCCs has demonstrated that HBV promotes the expression of *HULC*.[130] Interestingly, *HULC* has also been shown to accelerate liver cancer via inhibition of the *PTEN* tumour suppressor gene through sponge-activity on miR-15a.[131]

HULC also promotes lipogenesis in HCC. Cholesterol molecules upregulate the expression of *HULC* by activating the retinoic receptor RXR-α.[125] Increased expression of *HULC* is associated with increased methylation of the gene encoding miR-9. This results in decreased expression of miR-9 and increased expression of its target, the nuclear receptor peroxisome proliferator-activated receptor-α (PPAR-α). Increased levels of PPAR-α then induce transcription of long-chain-fatty-acid-CoA ligase 1 (ACSL1), to promote fatty acid synthesis.

HOTTIP is induced in HCC.[132,133] Studies have shown that the *HOTTIP* gene is located near to the *HOXA13* gene and controls the gene expression of the *HOXA* loci. The *HOXA* locus codes for a multitude of transcription factors that are involved in embryogenesis, with aberrant expression of these factors implicated in cancers.[134]

HOTAIR was originally identified through microarray tiling of the *HOXC* locus on chromosome 12.[28] *HOTAIR* repressively alters the chromatin landscape of promoters through recruitment of PRC2. In a separate but related mechanism, *HOTAIR* recruits the lysine-specific demethylase 1 (LSD1) complex, which is composed of LSD1, REST and CoREST proteins, and mediates demethylation of activating histone marks (H3K4me3).[135,136] The combined action of PRC2 and LSD1 complexes suppresses target gene expression. The role of *HOTAIR* in HCC has been extensively studied and reviewed elsewhere.[137] Aberrant expression of *HOTAIR* is correlated with worse outcomes in patients with HCC.

H19 is discussed above as a factor that regulates lipid metabolism and liver fibrosis; its role in HCC is less clear. In one study, increased expression of *H19* was identified in the setting of hypoxia and metastatic disease, suggesting possible oncogenic activity,[138] while in HCC cell lines ectopic expression of *H19* was shown to induce cell apoptosis and inhibit cell proliferation.[139] Further data from mouse models found that expression of *H19* from a modified *H19* locus negatively correlates with tumour emergence in SV40-induced HCCs.[140]

DANCR (differentiation antagonising non-protein coding RNA), also known as *ANCR*, has been implicated in many cancers.[141,142] *DANCR* is highly expressed in HCC and promotes cellular proliferation and metastasis.[143] In HepG2-induced xenograft mouse models of HCC, depletion of *DANCR* results in reduced tumour burden and decreased metastasis. The main mechanism through which *DANCR* exerts its oncogenic role is through interaction with the heterogenous nuclear protein ribonucleoprotein A1 (HNRNPA1), which regulates epithelial-mesenchymal transition in HCC. *DANCR* directly binds to the HNRNPA1 protein resulting in its increased stability. *DANCR* has also been shown to have sponge activity, binding to miR-140-3p to inhibit its ability to affect HNRNPA1 translation.

While the role of lncRNAs in liver cancers has mostly been described in HCCs, at least one lncRNA has been described in the context of CCA. CCA is an aggressive form of liver cancer whose malignant cells originate from bile ducts.[144] *DANCR* is induced in CCA in addition to HCC. *In vitro* analysis of *DANCR* in CCA models shows that this lncRNA is associated with increased cell proliferation and migration through interaction with EZH2, which leads to epigenetic suppression of the fructose-bisphosphatase 1 (*FBP1*) gene.[145] FBP1 is an enzyme that regulates gluconeogenesis and is implicated in multiple forms of cancer including HCCs.[146–148] While *DANCR* appears to function as an oncogene in both HCC and CCA, the described mechanisms of action for DANCR in these 2 liver malignancies are currently different. Future studies will be required to understand if interactions with HNRNPA1 also play a role in CCA and if interactions with EZH2 also play a role in HCC.

Tumour suppressor lncRNAs

In addition to having roles in liver fibrosis as discussed before, *MEG3* is also implicated in cervical, pancreatic, and many other cancers.[149,150] Whereas other lncRNAs appear to promote oncogenesis, *MEG3* is largely thought to function as a tumour suppressor gene.[108,151] Compared to normal cells, malignant hepatocytes show a significant reduction in the expression of this lncRNA.[152] Overexpression of *MEG3* in both cancer cell lines and mouse models inhibits growth of liver cancer cells.[153] The authors suggest that *MEG3* increases expression of miR-122 through which pyruvate kinase muscle isozyme M2 (PKM2) is downregulated. PKM2 is an important metabolic enzyme in glycolysis that influences oncogenesis.[154]

As a miRNA-sponge, *FENDRR* (foetal-lethal non-coding developmental regulatory RNA) has a unique role in HCC development. Compared to normal cells and tissues, *FENDRR* levels are drastically lower in HCC cell lines and tissues.[155] The sponge activity of *FENDRR* is exerted through binding to miR-423-5p, which targets and degrades growth arrest and DNA-damage-inducible beta protein gene (*GADD45B*) transcripts. Lower expression of the lncRNA in HCC allows the miRNA to attenuate GADD45B protein levels, which results in unchecked proliferation and immune evasion of tumour cells. Forced

expression of *FENDRR* in mice resulted in decreased burden and proliferation of cancer cells.

Progression of liver cancer is thought to be mediated through a class of stem-like cancer cells termed liver cancer stem cells (LCSCs), which are capable of sustaining proliferation within the tumour.[156] *DILC* (downregulated in liver cancer stem cells) has been identified to play crucial roles in these cells.[157] Depletion of this lncRNA resulted in expansion of the LCSC population and progression of disease, whereas ectopic expression led to a reduction in the size of the LCSC population in the tumour. Mechanistically, *DILC* is thought to interact with the *IL6* promoter leading to its inhibition. IL6 suppression disrupts the IL6/STAT3 autocrine signalling pathway, which is required for the maintenance of LCSCs.

GAS5 has been implicated in multiple forms of cancers,[158] and is a well-known tumour suppressor gene.[159] However, its multitude of roles in HCC have only recently been investigated.[160–162] Wang *et al.* showed that *GAS5* expression in HCC is lower than in normal cells and tissues, and depletion of *GAS5* increased resistance to the chemotherapeutic agent doxorubicin. The authors found that *GAS5* acts as a sponge to inhibit oncogenic miR-21, which targets the tumour suppressor gene *PTEN*. In contrast, Faranda *et al.* also reported that the *GAS5* transcript functions as a sponge to bind miR-126-3p, whose ectopic expression has been shown to decrease cell proliferation in HCC model cell lines. Although the authors did not elaborate on the mechanistic function of this miRNA in the context of HCC, studies in endothelial cells suggest that miR-126 acts through the downregulation of Spred-1, which is a negative regulator of the mitogen-activated protein kinase signalling pathway.[163]

Targeting lncRNAs?

Studies suggest that the expression of lncRNAs is more tissue specific than that of protein-coding mRNAs.[164] This observation makes lncRNAs attractive targets for therapeutic intervention in cases where tissue-specific modalities are highly desirable. As a major metabolic organ, the liver also has a remarkable ability to absorb external therapeutic agents.[165–167] Because of this property, the employment of agents to deplete lncRNAs could be an effective approach to affect the gene expression of specific cell types in the liver.

The best-studied class of molecules that through direct and specific interactions affect RNA transcript levels are antisense oligonucleotides (ASOs).[168] ASOs constitute a large family of chemically related molecules that exert their function through different cellular mechanisms.[169] For example, double-stranded ASOs such as small interfering RNAs (siRNA) form a complex with Argonaute protein that actively cleaves the target transcripts in the cytoplasm,[170] whereas single-stranded ASOs such as locked nucleic acids activate RNase H to cleave target transcripts in both the nucleus and cytoplasm.[171] The choice between siRNA or locked nucleic acid should, therefore, consider the cellular localisation of target transcripts. Irrespective of the mechanism, cleaved RNAs are quickly degraded by various exonucleases.[172]

Multiple ASOs are currently being employed clinically to target liver-based gene expression. Mipomersen, for example, targets apolipoprotein B-100, a component of LDL, to treat patients with familial hypercholesterolemia disorder.[173] However, the clinical use of ASOs, including mipomersen, is not without caveats and drawbacks, as ASOs may elicit an interferon response and tissue toxicity.[174] Patisiran is another successful example of an ASO approved for clinical use. Patisiran, a lipid nanoparticle-containing double-stranded RNA, is used to treat polyneuropathy of hereditary transthyretin (TTR)-mediated amyloidosis. Partisiran binds to *TTR* mRNA and causes its degradation resulting in a reduction in serum and tissue deposition of TTR proteins. We envision lncRNAs can be targeted using similar approaches.

Conjugation of chemical groups to ASOs, as a method to increase half-life and tropism to a specific tissue or cell type, has also been examined. For example, conjugation of cholesterol molecules to ASOs enhances their absorption by hepatocytes.[175] Hepatocytes express asialoglycoprotein receptors whose function is to clear aged circulating serum glycoproteins that have lost terminal sialic acid moieties in their glycan chain, resulting in exposure of N-acetylgalactosamine residues.[176] For this reason, N-acetylgalactosamine-conjugated molecules have been under active investigation for hepatocyte-specific delivery of siRNAs.[177,178]

The delivery of ASOs through lipid-containing vehicles is also under active consideration. Liposome-mediated delivery of ASOs also enhances the efficiency and tropism of liver cells. Sato *et al.* exploited vitamin A-coupled liposome systems to introduce ASOs (targeting the collagen chaperone gp46) to rat HSCs in order to inhibit cirrhosis.[179] They found that liposome-mediated delivery of the ASO caused a marked reduction in liver fibrosis and increased survival in rat models. As an alternative ASO delivery method, lipid-like nanoparticles have also been used to introduce ASOs into the liver.[180] Lipid-like nanoparticle-siRNAs against procollagen α-I were primarily localised to non-parenchymal cells in the liver and caused a significant reduction in collagen expression and deposition of collagen in mouse liver tissue. With increased efficiency in the delivery of ASOs to specific cell types in the liver, we anticipate seeing the development of ASO-based drugs that target disease-relevant lncRNAs in the not too distant future.

Although most of our discussion has focused on *in vivo* depletion of lncRNAs, delivery of lncRNAs to specific cell types in the liver could also have significant therapeutic value for managing chronic liver disease and cancer. The methods of RNA delivery in general and lncRNA in particular, as well as methods to induce their expression in tissues, are still in their infancy. Adeno-associated virus-based delivery methods have been acclaimed as the current gold standard for tissue delivery of nucleic acids in model organisms.[181] The rather obvious drawback of using this system is that it is largely limited by lncRNA size and concern over carcinogenicity of the virus-delivery method.[182] Due to the unstable nature of RNAs and their large size, methods to directly deliver lncRNA molecules (as nanoparticles or other vehicles) to human tissues have not been well developed. With the development of safer and more effective delivery methods, the introduction of lncRNAs may have great potential in liver-based gene therapy.

LncRNAs as biomarkers in diagnostic settings

Because of their tissue-specific expression, lncRNAs are being investigated as biomarkers in clinical settings.[183] In liver cancer, studies and meta-analyses to identify lncRNAs that could be used as biomarkers have already begun.[184] Through analysis of data generated from the cancer genome atlas (TCGA) consortium, Li *et al.* constructed mRNA-lncRNA coexpression networks. The

expression pattern of coding and non-coding transcripts in HCC led to the identification of several lncRNAs that have potential as biomarkers for HCC.[185] Although these lncRNAs were not able to predict patient outcomes, they hold promise in the diagnostic setting.

As described earlier, perhaps the most promising lncRNA biomarker in HCC is *HULC*. *HULC* transcripts are significantly elevated in the plasma of patients with HCC.[126,127] Additionally, in comparison to serum derived from healthy individuals, higher levels of *HULC* transcripts are found in the serum of patients with positive HBV status. Detection of lncRNAs such as *HULC* in plasma suggests that lncRNAs have the potential to serve as blood-based biomarkers in the clinical setting.

Conclusion

LncRNAs play essential roles in early liver development, the metabolic function of the liver, liver fibrosis, and cancer. While lncRNAs have been identified that regulate each of these stages, these transcripts likely represent only a small fraction of the lncRNAs that control key processes from liver development to homeostasis to disease. As we gain a deeper understanding of the diversity and function of lncRNAs across the different cell types of the liver, we anticipate this knowledge will lead to the establishment of lncRNAs as therapeutic targets to treat diseases of, or regulated by, the liver; this knowledge could also be used to identify new biomarkers to track or predict disease progression.

Abbreviations

ABCA1, ATP-binding cassette transporter A1; ACTA2/α-SMA, α-smooth muscle actin; APO, apolipoprotein; ASO, antisense oligonucleotides; BDL, bile duct ligation; CCA, cholangiocarcinoma; CCl_4, carbon tetrachloride; ceRNA, competing endogenous RNA; COL1A1, collagen type I α 1; CYP, cytochrome P450; DE, definitive endoderm; *DANCR*, differentiation antagonising non-protein coding RNA; *DEANR1*, definitive endoderm-associated lncRNA1; *DIGIT*, divergent to goosecoid, induced by TGF-β family signalling; *DILC*, downregulated in liver cancer stem cells; EpCAM, epithelial cell adhesion molecule; eRNA, enhancer RNAs; EST, expression sequence tag; FBP1, fructose-bisphosphatase 1; *FENDRR*, foetal-lethal non-coding developmental regulatory RNA; FXR, farnesoid X receptor; *GAS5*, growth arrest-specific transcript 5; H3K4me3, histone 3 lysine 4 trimethylation; H3K18ac, histone 3 lysine 18 acetylation; H3K36me3, histone 3 lysine 36 trimethylation; HCC, hepatocellular carcinoma; *HEIH*, high expression In HCC; HNRNPA1, heterogenous nuclear protein ribonucleoprotein A1; *HOTAIR*, HOX transcript antisense RNA; *HOTTIP*, HOXA transcript at the distal tip; HSC, hepatic stellate cells; HuR, human antigen R; *HULC*, highly upregulated in liver cancer; LCSC, liver cancer stem cell; LeXis, liver-expressed LXR-induced sequence; lincRNA, long intervening non-coding RNA; lncRNA, long non-coding RNA; *LncLSTR*, lncRNA liver-specific triglyceride regulator; LSD1, lysine-specific demethylase 1; LXR, liver X receptors; *MALAT1*, metastasis-associated lung adenocarcinoma transcript 1; *MEG3*, maternally expressed gene 3; mTOR, mammalian target of rapamycin; NAT, natural antisense transcript; *NEAT1*, nuclear enriched abundant transcript 1; ORF, open reading frame; PKM2, pyruvate kinase muscle isozyme M2; PPAR-α, peroxisome proliferator-activated receptor-α; PRC, polycomb repressive complex; RACE, rapid amplification of cDNA ends; RNA Pol, RNA polymerase; S6K1, S6 kinase 1; SHP, small heterodimer partner; siRNA, small interfering RNA; SREBPs, steroid response binding proteins; SREs, sterol response elements; TGF-β, transforming growth factor-β; TTR, transthyretin; XIST, X-inactive specific transcript; ZEB1, zinc finger E-box-binding homeobox 1.

Financial support

ACM is supported by NIH/NIDDK grant R01DK116999, NIH/NICHD grant R01HD09277302 and a Pew Biomedical Scholars Award. Dr. Mullen also receives research support from the Chan Zuckerberg Initiative and has received research funding from Boehringer Ingelheim, Bristol-Myers Squibb, Takeda Pharmaceuticals and Roche Pharmaceuticals in the last 36 months.

Conflict of interest

The authors declare no conflicts of interest that pertain to this work.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

The authors contributed equally to the production of this manuscript.

Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jhepr.2020.100177.

References

Author names in bold designate shared co-first authorship

[1] Clamp M, Fry B, Kamal M, Xie X, Cuff J, Lin MF, et al. Distinguishing protein-coding and noncoding genes in the human genome. Proc Natl Acad Sci U S A 2007;104:19428–19433.
[2] Matera AG, Terns RM, Terns MP. Non-coding RNAs: lessons from the small nuclear and small nucleolar RNAs. Nat Rev Mol Cell Biol 2007;8:209–220.
[3] Kapranov P, Cawley SE, Drenkow J, Bekiranov S, Strausberg RL, Fodor SPA, et al. Large-scale transcriptional activity in chromosomes 21 and 22. Science 2002;296:916–919.
[4] Ulitsky I, Bartel DP. lincRNAs: genomics, evolution, and mechanisms. Cell 2013;154:26–46.
[5] Brown JA, Valenstein ML, Yario TA, Tycowski KT, Steitz JA. Formation of triple-helical structures by the 3′-end sequences of MALAT1 and MEN noncoding RNAs. Proc Natl Acad Sci U S A 2012;109:19202–19207.
[6] **Derrien T**, **Johnson R**, Bussotti G, Tanzer A, Djebali S, Tilgner H, et al. The GENCODE v7 catalog of human long noncoding RNAs: analysis of their gene structure, evolution, and expression. Genome Res 2012;22:1775–1789.
[7] Clark MB, Johnston RL, Inostroza-Ponta M, Fox AH, Fortini E, Moscato P, et al. Genome-wide analysis of long noncoding RNA stability. Genome Res 2012;22:885–898.
[8] Tani H, Imamachi N, Mizutani R, Imamura K, Kwon Y, Miyazaki S, et al. Genome-wide analysis of long noncoding RNA turnover. Methods Mol Biol 2015;1262:305–320.
[9] Sigova AA, Mullen AC, Molinie B, Gupta S, Orlando DA, Guenther MG, et al. Divergent transcription of long noncoding RNA/mRNA gene pairs in embryonic stem cells. Proc Natl Acad Sci U S A 2013;110:2876–2881.
[10] Dinger ME, Pang KC, Mercer TR, Mattick JS. Differentiating protein-coding and noncoding RNA: challenges and ambiguities. Plos Comput Biol 2008;4:e1000176.
[11] Consortium TF, The FANTOM Consortium. The transcriptional landscape of the mammalian genome. Science 2005;309:1559–1563.
[12] Guttman M, Amit I, Garber M, French C, Lin MF, Feldser D, et al. Chromatin signature reveals over a thousand highly conserved large non-coding RNAs in mammals. Nature 2009;458:223–227.
[13] Trapnell C, Williams BA, Pertea G, Mortazavi A, Kwan G, van Baren MJ, et al. Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat Biotechnol 2010;28:511–515.
[14] Housman G, Ulitsky I. Methods for distinguishing between protein-coding and long noncoding RNAs and the elusive biological purpose of translation of long noncoding RNAs. Biochim Biophys Acta 2016;1859:31–40.
[15] Frohman MA, Dush MK, Martin GR. Rapid production of full-length cDNAs from rare transcripts: amplification using a single gene-specific oligonucleotide primer. Proc Natl Acad Sci U S A 1988;85:8998–9002.

[16] Lagarde J, Uszczynska-Ratajczak B, Carbonell S, Pérez-Lluch S, Abad A, Davis C, et al. High-throughput annotation of full-length long noncoding RNAs with capture long-read sequencing. Nat Genet 2017;49:1731–1740.
[17] **Ulitsky I**, **Shkumatava A**, Jan CH, Sive H, Bartel DP. Conserved function of lincRNAs in vertebrate embryonic development despite rapid sequence evolution. Cell 2011;147:1537–1550.
[18] Diederichs S. The four dimensions of noncoding RNA conservation. Trends Genet 2014;30:121–123.
[19] Washietl S, Kellis M, Garber M. Evolutionary dynamics and tissue specificity of human long noncoding RNAs in six mammals. Genome Res 2014;24:616–628.
[20] Zhao Y, Li H, Fang S, Kang Y, Wu W, Hao Y, et al. Noncode 2016: an informative and valuable data source of long non-coding RNAs. Nucleic Acids Res 2016;44:D203–D208.
[21] Harrow J, Frankish A, Gonzalez JM, Tapanari E, Diekhans M, Kokocinski F, et al. GENCODE: the reference human genome annotation for the ENCODE Project. Genome Res 2012;22:1760–1774.
[22] RNAcentral. A hub of information for non-coding RNA sequences. Nucleic Acids Res 2019;47:D221–D229.
[23] Volders P-J, Anckaert J, Verheggen K, Nuytens J, Martens L, Mestdagh P, et al. LNCipedia 5: towards a reference set of human long non-coding RNAs. Nucleic Acids Res 2019;47:D135–D139.
[24] Seal RL, Chen L, Griffiths-Jones S, Lowe TM, Mathews MB, O'Reilly D, et al. A guide to naming human non-coding RNA genes. EMBO J 2020;39:e103777.
[25] Brannan CI, Dees EC, Ingram RS, Tilghman SM. The product of the H19 gene may function as an RNA. Mol Cell Biol 1990;10:28–36.
[26] Brown CJ, Ballabio A, Rupert JL, Lafreniere RG, Grompe M, Tonlorenzi R, et al. A gene from the region of the human X inactivation centre is expressed exclusively from the inactive X chromosome. Nature 1991;349:38–44.
[27] Brockdorff N, Ashworth A, Kay GF, Cooper P, Smith S, McCabe VM, et al. Conservation of position and exclusive expression of mouse Xist from the inactive X chromosome. Nature 1991;351:329–331.
[28] Rinn JL, Kertesz M, Wang JK, Squazzo SL, Xu X, Brugmann SA, et al. Functional demarcation of active and silent chromatin domains in human HOX loci by noncoding RNAs. Cell 2007;129:1311–1323.
[29] He Y, Vogelstein B, Velculescu VE, Papadopoulos N, Kinzler KW. The antisense transcriptomes of human cells. Science 2008;322:1855–1857.
[30] Wight M, Werner A. The functions of natural antisense transcripts. Essays Biochem 2013;54:91–101.
[31] Beiter T, Reich E, Williams RW, Simon P. Antisense transcription: a critical look in both directions. Cell Mol Life Sci 2009;66:94–112.
[32] Neeman Y, Dahary D, Levanon E, Sorek R, Eisenberg E. Is there any sense in antisense editing? Trends Genet 2005;21:544–547.
[33] Watanabe T, Totoki Y, Toyoda A, Kaneda M, Kuramochi-Miyagawa S, Obata Y, et al. Endogenous siRNAs from naturally formed dsRNAs regulate transcripts in mouse oocytes. Nature 2008;453:539–543.
[34] Andersson R, Chen Y, Core L, Lis JT, Sandelin A, Jensen TH. Human gene promoters are intrinsically bidirectional. Mol Cell 2015;60:346–347.
[35] Kim T-K, Hemberg M, Gray JM, Costa AM, Bear DM, Wu J, et al. Widespread transcription at neuronal activity-regulated enhancers. Nature 2010;465:182–187.
[36] Natoli G, Andrau J-C. Noncoding transcription at enhancers: general principles and functional models. Annu Rev Genet 2012;46:1–19.
[37] Melgar MF, Collins FS, Sethupathy P. Discovery of active enhancers through bidirectional expression of short transcripts. Genome Biol 2011;12:R113.
[38] Zhao Y, Zhou J, He L, Li Y, Yuan J, Sun K, et al. MyoD induced enhancer RNA interacts with hnRNPL to activate target gene transcription during myogenic differentiation. Nat Commun 2019;10:5787.
[39] Rahnamoun H, Lee J, Sun Z, Lu H, Ramsey KM, Komives EA, et al. RNAs interact with BRD4 to promote enhanced chromatin engagement and transcription activation. Nat Struct Mol Biol 2018;25:687–697.
[40] Goudarzi M, Berg K, Pieper LM, Schier AF. Individual long non-coding RNAs have no overt functions in zebrafish embryogenesis, viability and fertility. Elife 2019;8:e40815.
[41] Koch F, Fenouil R, Gut M, Cauchy P, Albert TK, Zacarias-Cabeza J, et al. Transcription initiation platforms and GTF recruitment at tissue-specific enhancers and promoters. Nat Struct Mol Biol 2011;18:956–963.
[42] Engreitz JM, Haines JE, Perez EM, Munson G, Chen J, Kane M, et al. Local regulation of gene expression by lncRNA promoters, transcription and splicing. Nature 2016;539:452–455.
[43] Brown CJ, Hendrich BD, Rupert JL, Lafrenière RG, Xing Y, Lawrence J, et al. The human XIST gene: analysis of a 17 kb inactive X-specific RNA that contains conserved repeats and is highly localized within the nucleus. Cell 1992;71:527–542.
[44] Galupa R, Heard E. X-chromosome inactivation: a crossroads between chromosome architecture and gene regulation. Annu Rev Genet 2018;52:535–566.
[45] Colognori D, Sunwoo H, Kriz AJ, Wang C-Y, Lee JT. Xist deletional analysis reveals an interdependency between xist RNA and polycomb complexes for spreading along the inactive X. Mol Cell 2019;74:101–117.e10.
[46] Wang KC, Yang YW, Liu B, Sanyal A, Corces-Zimmerman R, Chen Y, et al. A long noncoding RNA maintains active chromatin to coordinate homeotic gene expression. Nature 2011;472:120–124.
[47] Rinn JL. lncRNAs: linking RNA to chromatin. Cold Spring Harb Perspect Biol 2014;6:a018614.
[48] Goff LA, Rinn JL. Linking RNA biology to lncRNAs. Genome Res 2015;25:1456–1465.
[49] Mohammad F, Mondal T, Guseva N, Pandey GK, Kanduri C. Kcnq1ot1 noncoding RNA mediates transcriptional gene silencing by interacting with Dnmt1. Development 2010;137:2493–2499.
[50] Chen L-L, Carmichael GG. Altered nuclear retention of mRNAs containing inverted repeats in human embryonic stem cells: functional role of a nuclear noncoding RNA. Mol Cell 2009;35:467–478.
[51] Sasaki YTF, Ideue T, Sano M, Mituyama T, Hirose T. MENε/β noncoding RNAs are essential for structural integrity of nuclear paraspeckles. Proc Natl Acad Sci U S A 2009;106:2525–2530.
[52] Clemson CM, Hutchinson JN, Sara SA, Ensminger AW, Fox AH, Chess A, et al. An architectural role for a nuclear noncoding RNA: NEAT1 RNA is essential for the structure of paraspeckles. Mol Cell 2009;33:717–726.
[53] Bond CS, Fox AH. Paraspeckles: nuclear bodies built on long noncoding RNA. J Cell Biol 2009;186:637–644.
[54] Tripathi V, Ellis JD, Shen Z, Song DY, Pan Q, Watt AT, et al. The nuclear-retained noncoding RNA MALAT1 regulates alternative splicing by modulating SR splicing factor phosphorylation. Mol Cell 2010;39:925–938.
[55] Galganski L, Urbanek MO, Krzyzosiak WJ. Nuclear speckles: molecular organization, biological function and role in disease. Nucleic Acids Res 2017;45:10350–10368.
[56] West JA, Davis CP, Sunwoo H, Simon MD, Sadreyev RI, Wang PI, et al. The long noncoding RNAs NEAT1 and MALAT1 bind active chromatin sites. Mol Cell 2014;55:791–802.
[57] Wang Y, Hu S-B, Wang M-R, Yao R-W, Wu D, Yang L, et al. Genome-wide screening of NEAT1 regulators reveals cross-regulation between paraspeckles and mitochondria. Nat Cell Biol 2018;20:1145–1158.
[58] Ebert MS, Sharp PA. MicroRNA sponges: progress and possibilities. RNA 2010;16:2043–2050.
[59] Liu Y, Xue M, Du S, Feng W, Zhang K, Zhang L, et al. Competitive endogenous RNA is an intrinsic component of EMT regulatory circuits and modulates EMT. Nat Commun 2019;10:1637.
[60] Ulitsky I. Interactions between short and long noncoding RNAs. FEBS Lett 2018;592:2874–2883.
[61] Cardoso-Moreira M, Halbert J, Valloton D, Velten B, Chen C, Shao Y, et al. Gene expression across mammalian organ development. Nature 2019;571:505–509.
[62] Grote P, Herrmann BG. Long noncoding RNAs in organogenesis: making the difference. Trends Genet 2015;31:329–335.
[63] Gordillo M, Evans T, Gouon-Evans V. Orchestrating liver development. Development 2015;142:2094–2108.
[64] Tremblay KD, Zaret KS. Distinct populations of endoderm cells converge to generate the embryonic liver bud and ventral foregut tissues. Dev Biol 2005;280:87–99.
[65] Jiang W, Liu Y, Liu R, Zhang K, Zhang Y. The lncRNA DEANR1 facilitates human endoderm differentiation by activating FOXA2 expression. Cell Rep 2015;11:137–148.
[66] Daneshvar K, Pondick JV, Kim B-M, Zhou C, York SR, Macklin JA, et al. DIGIT is a conserved long noncoding RNA that regulates GSC expression to control definitive endoderm differentiation of embryonic stem cells. Cell Rep 2016;17:353–365.
[67] Daneshvar K, Ardehali MB, Klein IA, Fu-Kai H, Kratkiewicz AJ, Mahpour A, et al. lncRNA DIGIT and BRD3 protein form phase-separated condensates to regulate endoderm differentiation. Nat Cell Biol 2020;22:1211–1222.
[68] Gluchowski NL, Becuwe M, Walther TC, Farese Jr RV. Lipid droplets and liver disease: from basic biology to clinical implications. Nat Rev Gastroenterol Hepatol 2017;14:343–355.
[69] van Solingen C, Scacalossi KR, Moore KJ. Long noncoding RNAs in lipid metabolism. Curr Opin Lipidol 2018;29:224–232.

[70] Muret K, Désert C, Lagoutte L, Boutin M, Gondret F, Zerjal T, et al. Long noncoding RNAs in lipid metabolism: literature review and conservation analysis across species. BMC Genomics 2019;20:882.
[71] Phillips MC. Molecular mechanisms of cellular cholesterol efflux. J Biol Chem 2014;289:24020–24029.
[72] Pullinger CR, Eng C, Salen G, Shefer S, Batta AK, Erickson SK, et al. Human cholesterol 7α-hydroxylase (CYP7A1) deficiency has a hypercholesterolemic phenotype. J Clin Invest 2002;110:109–117.
[73] Wang J, Greene S, Eriksson LC, Rozell B, Reihnér E, Einarsson C, et al. Human sterol 12α-hydroxylase (CYP8B1) is mainly expressed in hepatocytes in a homogenous pattern. Histochem Cell Biol 2005;123:441–446.
[74] Hofmann AF, Hagey LR. Key discoveries in bile acid chemistry and biology and their clinical applications: history of the last eight decades. J Lipid Res 2014;55:1553–1595.
[75] Claudel T, Staels B, Kuipers F. The farnesoid X receptor: a molecular link between bile acid and lipid and glucose metabolism. Arterioscler Thromb Vasc Biol 2005;25:2020–2030.
[76] Forman BM, Goode E, Chen J, Oro AE, Bradley DJ, Perlmann T, et al. Identification of a nuclear receptor that is activated by farnesol metabolites. Cell 1995;81:687–693.
[77] Wang H, Chen J, Hollister K, Sowers LC, Forman BM. Endogenous bile acids are ligands for the nuclear receptor FXR/BAR. Mol Cell 1999;3:543–553.
[78] **Makishima M**, **Okamoto AY**, Repa JJ, Tu H, Learned RM, Luk A, et al. Identification of a nuclear receptor for bile acids. Science 1999;284:1362–1365.
[79] Parks DJ, Blanchard SG, Bledsoe RK, Chandra G, Consler TG, Kliewer SA, et al. Bile acids: natural ligands for an orphan nuclear receptor. Science 1999;284:1365–1368.
[80] Lan X, Yan J, Ren J, Zhong B, Li J, Li Y, et al. A novel long noncoding RNA Lnc-HC binds hnRNPA2B1 to regulate expressions of Cyp7a1 and Abca1 in hepatocytic cholesterol metabolism: Hepatology, Vol. XX, No. X, 2015 Lan et al. Hepatology 2016;64:58–72.
[81] Oram JF, Lawn RM. ABCA1. The gatekeeper for eliminating excess tissue cholesterol. J Lipid Res 2001;42:1173–1179.
[82] Li P, Ruan X, Yang L, Kiesewetter K, Zhao Y, Luo H, et al. A liver-enriched long non-coding RNA, lncLSTR, regulates systemic lipid metabolism in mice. Cell Metab 2015;21:455–467.
[83] Pandak WM, Bohdan P, Franklund C, Mallonee DH, Eggertsen G, Björkhem I, et al. Expression of sterol 12alpha-hydroxylase alters bile acid pool composition in primary rat hepatocytes and in vivo. Gastroenterology 2001;120:1801–1809.
[84] Li-Hawkins J, Gåfvels M, Olin M, Lund EG, Andersson U, Schuster G, et al. Cholic acid mediates negative feedback regulation of bile acid synthesis in mice. J Clin Invest 2002;110:1191–1200.
[85] Kast HR, Nguyen CM, Sinal CJ, Jones SA, Laffitte BA, Reue K, et al. Farnesoid X-activated receptor induces apolipoprotein C-II transcription: a molecular mechanism linking plasma triglyceride levels to bile acids. Mol Endocrinol 2001;15:1720–1728.
[86] Shimano H, Sato R. SREBP-regulated lipid metabolism: convergent physiology — divergent pathophysiology. Nat Rev Endocrinol 2017;13:710–730.
[87] Ferré P, Foufelle F. SREBP-1c transcription factor and lipid homeostasis: clinical perspective. Horm Res Paediatr 2007;68:72–82.
[88] Yan C, Chen J, Chen N. Long noncoding RNA MALAT1 promotes hepatic steatosis and insulin resistance by increasing nuclear SREBP-1c protein stability. Sci Rep 2016;6:22640.
[89] Liu C, Yang Z, Wu J, Zhang L, Lee S, Shin D-J, et al. Long noncoding RNA H19 interacts with polypyrimidine tract-binding protein 1 to reprogram hepatic lipid homeostasis: metabolic liver disease. Hepatology 2018;67:1768–1783.
[90] Nilsson E, Matte A, Perfilyev A, de Mello VD, Käkelä P, Pihlajamäki J, et al. Epigenetic alterations in human liver from subjects with type 2 diabetes in parallel with reduced folate levels. J Clin Endocrinol Metab 2015;100:E1491–E1501.
[91] Zhang N, Geng T, Wang Z, Zhang R, Cao T, Camporez JP, et al. Elevated hepatic expression of H19 long noncoding RNA contributes to diabetic hyperglycemia. JCI Insight 2018;3:e120304.
[92] Zhou J, Yang L, Zhong T, Mueller M, Men Y, Zhang N, et al. H19 lncRNA alters DNA methylation genome wide by regulating S-adenosylhomocysteine hydrolase. Nat Commun 2015;6:10221.
[93] Tontonoz P. Transcriptional and posttranscriptional control of cholesterol homeostasis by liver X receptors. Cold Spring Harb Symp Quant Biol 2011;76:129–137.
[94] Zhang Y, Breevoort SR, Angdisen J, Fu M, Schmidt DR, Holmstrom SR, et al. Liver LXRα expression is crucial for whole body cholesterol homeostasis and reverse cholesterol transport in mice. J Clin Invest 2012;122:1688–1699.
[95] Sallam T, Jones MC, Gilliland T, Zhang L, Wu X, Eskin A, et al. Feedback modulation of cholesterol metabolism by the lipid-responsive noncoding RNA LeXis. Nature 2016;534:124–128.
[96] Cheung MC, Albers JJ. Distribution of high density lipoprotein particles with different apoprotein composition: particles with A-I and A-II and particles with A-I but no A-II. J Lipid Res 1982;23:747–753.
[97] Toth PP. Reverse cholesterol transport: high-density lipoprotein's magnificent mile. Curr Atheroscler Rep 2003;5:386–393.
[98] Qu J, Ko C-W, Tso P, Bhargava A. Apolipoprotein A-IV: a multifunctional protein involved in protection against atherosclerosis and diabetes. Cells 2019;8:319.
[99] Halley P, Kadakkuzha BM, Faghihi MA, Magistri M, Zeier Z, Khorkova O, et al. Regulation of the apolipoprotein gene cluster by a long noncoding RNA. Cell Rep 2014;6:222–230.
[100] Qin W, Li X, Xie L, Li S, Liu J, Jia L, et al. A long non-coding RNA, APOA4-AS, regulates APOA4 expression depending on HuR in mice. Nucleic Acids Res 2016;44:6423–6433.
[101] Gao Q, Gu Y, Jiang Y, Fan L, Wei Z, Jin H, et al. Long non-coding RNA Gm2199 rescues liver injury and promotes hepatocyte proliferation through the upregulation of ERK1/2. Cell Death Dis 2018;9:602.
[102] **Zhang Y**, **Luo G**, Zhang Y, Zhang M, Zhou J, Gao W, et al. Critical effects of long non-coding RNA on fibrosis diseases. Exp Mol Med 2018;50:e428.
[103] Friedman SL, Roll FJ, Boyles J, Bissell DM. Hepatic lipocytes: the principal collagen-producing cells of normal rat liver. Proc Natl Acad Sci U S A 1985;82:8681–8685.
[104] Maher JJ, McGuire RF. Extracellular matrix gene expression increases preferentially in rat lipocytes and sinusoidal endothelial cells during hepatic fibrosis in vivo. J Clin Invest 1990;86:1641–1648.
[105] Mederacke I, Hsu CC, Troeger JS, Huebener P, Mu X, Dapito DH, et al. Fate tracing reveals hepatic stellate cells as dominant contributors to liver fibrosis independent of its aetiology. Nat Commun 2013;4:2823.
[106] Zhou C, York SR, Chen JY, Pondick JV, Motola DL, Chung RT, et al. Long noncoding RNAs expressed in human hepatic stellate cells form networks with extracellular matrix proteins. Genome Med 2016;8:31.
[107] Zheng J, Dong P, Mao Y, Chen S, Wu X, Li G, et al. lincRNA-p21 inhibits hepatic stellate cell activation and liver fibrogenesis via p21. FEBS J 2015;282:4810–4821.
[108] Zhang J, Yao T, Wang Y, Yu J, Liu Y, Lin Z. Long noncoding RNA MEG3 is downregulated in cervical cancer and affects cell proliferation and apoptosis by regulating miR-21. Cancer Biol Ther 2016;17:104–113.
[109] Zhang Y, Wu J, Jing H, Huang G, Sun Z, Xu S. Long noncoding RNA MEG3 inhibits breast cancer growth via upregulating endoplasmic reticulum stress and activating NF-κB and p53. J Cell Biochem 2018;39:131.
[110] Yu F, Geng W, Dong P, Huang Z, Zheng J. LncRNA-MEG3 inhibits activation of hepatic stellate cells through SMO protein and miR-212. Cell Death Dis 2018;9:1014.
[111] He Y, Wu Y-T, Huang C, Meng X-M, Ma T-T, Wu B-M, et al. Inhibitory effects of long noncoding RNA MEG3 on hepatic stellate cells activation and liver fibrogenesis. Biochim Biophys Acta 2014;1842:2204–2215.
[112] Zhang L, Yang Z, Trottier J, Barbier O, Wang L. Long noncoding RNA MEG3 induces cholestatic liver injury by interaction with PTBP1 to facilitate shp mRNA decay. Hepatology 2017;65:604–615.
[113] Yu F, Zheng J, Mao Y, Dong P, Lu Z, Li G, et al. Long non-coding RNA growth arrest-specific transcript 5 (GAS5) inhibits liver fibrogenesis through a mechanism of competing endogenous RNA. J Biol Chem 2015;290:28286–28298.
[114] Song Y, Liu C, Liu X, Trottier J, Beaudoin M, Zhang L, et al. H19 promotes cholestatic liver fibrosis by preventing ZEB1-mediated inhibition of epithelial cell adhesion molecule. Hepatology 2017;66:1183–1196.
[115] Schmitt AM, Chang HY. Long noncoding RNAs in cancer pathways. Cancer Cell 2016;29:452–463.
[116] Lim LJ, Wong SYS, Huang F, Lim S, Chong SS, Ooi LL, et al. Roles and regulation of long noncoding RNAs in hepatocellular carcinoma. Cancer Res 2019;79:5131–5139.
[117] Ji P, Diederichs S, Wang W, Böing S, Metzger R, Schneider PM, et al. MALAT-1, a novel noncoding RNA, and thymosin β4 predict metastasis and survival in early-stage non-small cell lung cancer. Oncogene 2003;22:8031–8041.
[118] Malakar P, Shilo A, Mogilevsky A, Stein I, Pikarsky E, Nevo Y, et al. Long noncoding RNA MALAT1 promotes hepatocellular carcinoma

development by SRSF1 upregulation and mTOR activation. Cancer Res 2017;77:1155–1167.
[119] Shin VY, Chen J, Cheuk IW-Y, Siu M-T, Ho C-W, Wang X, et al. Long non-coding RNA NEAT1 confers oncogenic role in triple-negative breast cancer through modulating chemoresistance and cancer stemness. Cell Death Dis 2019;10:270.
[120] Li X, Wang X, Song W, Xu H, Huang R, Wang Y, et al. Oncogenic properties of NEAT1 in prostate cancer cells depend on the CDC5L–AGRN transcriptional regulation circuit. Cancer Res 2018;78:4138–4149.
[121] Fang L, Sun J, Pan Z, Song Y, Zhong L, Zhang Y, et al. Long non-coding RNA NEAT1 promotes hepatocellular carcinoma cell proliferation through the regulation of miR-129-5p-VCP-IκB. Am J Physiol Gastrointest Liver Physiol 2017;313:G150–G156.
[122] Yang F, Zhang L, Huo X-S, Yuan J-H, Xu D, Yuan S-X, et al. Long noncoding RNA high expression in hepatocellular carcinoma facilitates tumor growth through enhancer of zeste homolog 2 in humans. Hepatology 2011;54:1679–1689.
[123] Panzitt K, Tschernatsch MMO, Guelly C, Moustafa T, Stradner M, Strohmaier HM, et al. Characterization of HULC, a novel gene with striking up-regulation in hepatocellular carcinoma, as noncoding RNA. Gastroenterology 2007;132:330–342.
[124] Peng W, Gao W, Feng J. Long noncoding RNA HULC is a novel biomarker of poor prognosis in patients with pancreatic cancer. Med Oncol 2014;31:346.
[125] Cui M, Xiao Z, Wang Y, Zheng M, Song T, Cai X, et al. Long noncoding RNA HULC modulates abnormal lipid metabolism in hepatoma cells through an miR-9-mediated RXRA signaling pathway. Cancer Res 2015;75:846–857.
[126] Xie H, Ma H, Zhou D. Plasma HULC as a promising novel biomarker for the detection of hepatocellular carcinoma. Biomed Res Int 2013;2013:136106.
[127] Li J, Wang X, Tang J, Jiang R, Zhang W, Ji J, et al. HULC and Linc00152 act as novel biomarkers in predicting diagnosis of hepatocellular carcinoma. Cell Physiol Biochem 2015;37:687–696.
[128] Yu X, Zheng H, Chan MTV, Wu WKK. HULC: an oncogenic long non-coding RNA in human cancer. J Cell Mol Med 2017;21:410–417.
[129] Wang J, Liu X, Wu H, Ni P, Gu Z, Qiao Y, et al. CREB up-regulates long non-coding RNA, HULC expression through interaction with microRNA-372 in liver cancer. Nucleic Acids Res 2010;38:5366–5383.
[130] Du Y, Kong G, You X, Zhang S, Zhang T, Gao Y, et al. Elevation of highly up-regulated in liver cancer (HULC) by hepatitis B virus X protein promotes hepatoma cell proliferation via down-regulating p18. J Biol Chem 2012;287:26302–26311.
[131] Xin X, Wu M, Meng Q, Wang C, Lu Y, Yang Y, et al. Long noncoding RNA HULC accelerates liver cancer by inhibiting PTEN via autophagy cooperation to miR15a. Mol Cancer 2018;17:94.
[132] Quagliata L, Matter MS, Piscuoglio S, Arabi L, Ruiz C, Procino A, et al. Long noncoding RNA HOTTIP/HOXA13 expression is associated with disease progression and predicts outcome in hepatocellular carcinoma patients. Hepatology 2014;59:911–923.
[133] Wu L, Yang Z, Zhang J, Xie H, Zhou L, Zheng S. Long noncoding RNA HOTTIP expression predicts tumor recurrence in hepatocellular carcinoma patients following liver transplantation. HepatoBiliary Surg Nutr 2018;7:429–439.
[134] Lin C, Wang Y, Wang Y, Zhang S, Yu L, Guo C, et al. Transcriptional and posttranscriptional regulation of HOXA13 by lncRNA HOTTIP facilitates tumorigenesis and metastasis in esophageal squamous carcinoma cells. Oncogene 2017;36:5392–5406.
[135] Shi Y, Lan F, Matson C, Mulligan P, Whetstine JR, Cole PA, et al. Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. Cell 2004;119:941–953.
[136] Lee MG, Wynder C, Cooch N, Shiekhattar R. An essential role for CoREST in nucleosomal histone 3 lysine 4 demethylation. Nature 2005;437:432–435.
[137] Wu L, Zhang L, Zheng S. Role of the long non-coding RNA HOTAIR in hepatocellular carcinoma. Oncol Lett 2017;14:1233–1239.
[138] Matouk IJ, DeGroot N, Mezan S, Ayesh S, Abu-lail R, Hochberg A, et al. The H19 non-coding RNA is essential for human tumor growth. PLoS One 2007;2:e845.
[139] Schultheiss CS, Laggai S, Czepukojc B, Hussein UK, List M, Barghash A, et al. The long non-coding RNA H19 suppresses carcinogenesis and chemoresistance in hepatocellular carcinoma. Cell Stress Chaperones 2017;1:37–54.
[140] Yoshimizu T, Miroglio A, Ripoche M-A, Gabory A, Vernucci M, Riccio A, et al. The H19 locus acts in vivo as a tumor suppressor. Proc Natl Acad Sci U S A 2008;105:12417–12422.
[141] Malakootian M, Mirzadeh Azad F, Fouani Y, Taheri Bajgan E, Saberi H, Mowla SJ. Anti-differentiation non-coding RNA, ANCR, is differentially expressed in different types of brain tumors. J Neurooncol 2018;138:261–270.
[142] Liu B, Zhao H, Zhang L, Shi X. Silencing of long-non-coding RNA ANCR suppresses the migration and invasion of osteosarcoma cells by activating the p38MAPK signalling pathway. BMC Cancer 2019;19:1112.
[143] Wen Z, Lian L, Ding H, Hu Y, Xiao Z, Xiong K, et al. LncRNA ANCR promotes hepatocellular carcinoma metastasis through upregulating HNRNPA1 expression. RNA Biol 2020;17:381–394.
[144] Rizvi S, Khan SA, Hallemeier CL, Kelley RK, Gores GJ. Cholangiocarcinoma — evolving concepts and therapeutic strategies. Nat Rev Clin Oncol 2018;15:95–111.
[145] Wang N, Zhang C, Wang W, Liu J, Yu Y, Li Y, et al. Long noncoding RNA DANCR regulates proliferation and migration by epigenetically silencing FBP1 in tumorigenesis of cholangiocarcinoma. Cell Death Dis 2019;10:585.
[146] Li B, Qiu B, Lee DSM, Walton ZE, Ochocki JD, Mathew LK, et al. Fructose-1,6-bisphosphatase opposes renal carcinoma progression. Nature 2014;513:251–255.
[147] Dong C, Yuan T, Wu Y, Wang Y, Fan TWM, Miriyala S, et al. Loss of FBP1 by snail-mediated repression provides metabolic advantages in basal-like breast cancer. Cancer Cell 2013;23:316–331.
[148] Hirata H, Sugimachi K, Komatsu H, Ueda M, Masuda T, Uchi R, et al. Decreased expression of fructose-1,6-bisphosphatase associates with glucose metabolism and tumor progression in hepatocellular carcinoma. Cancer Res 2016;76:3265–3276.
[149] Zhang J, Gao Y. Long non-coding RNA MEG3 inhibits cervical cancer cell growth by promoting degradation of P-STAT3 protein via ubiquitination. Cancer Cell Int 2019;19:175.
[150] Ma L, Wang F, Du C, Zhang Z, Guo H, Xie X, et al. Long non-coding RNA MEG3 functions as a tumour suppressor and has prognostic predictive value in human pancreatic cancer. Oncol Rep 2018;39:1132–1140.
[151] Zhou Y, Yang H, Xia W, Cui L, Xu R, Lu H, et al. LncRNA MEG3 inhibits the progression of prostate cancer by facilitating H3K27 trimethylation of EN2 through binding to EZH2. J Biochem 2020;167:295–301.
[152] Braconi C, Kogure T, Valeri N, Huang N, Nuovo G, Costinean S, et al. microRNA-29 can regulate expression of the long non-coding RNA gene MEG3 in hepatocellular cancer. Oncogene 2011;30:4750–4756.
[153] Zheng Q, Lin Z, Xu J, Lu Y, Meng Q, Wang C, et al. Long noncoding RNA MEG3 suppresses liver cancer cells growth through inhibiting β-catenin by activating PKM2 and inactivating PTEN. Cell Death Dis 2018;9:253.
[154] Dong G, Mao Q, Xia W, Xu Y, Wang J, Xu L, et al. PKM2 and cancer: the function of PKM2 beyond glycolysis. Oncol Lett 2016;11:1980–1986.
[155] Yu Z, Zhao H, Feng X, Li H, Qiu C, Yi X, et al. Long non-coding RNA FENDRR acts as a miR-423-5p sponge to suppress the Treg-mediated immune escape of hepatocellular carcinoma cells. Mol Ther Nucleic Acids 2019;17:516–529.
[156] Magee JA, Piskounova E, Morrison SJ. Cancer stem cells: impact, heterogeneity, and uncertainty. Cancer Cell 2012;21:283–296.
[157] Wang X, Sun W, Shen W, Xia M, Chen C, Xiang D, et al. Long non-coding RNA DILC regulates liver cancer stem cells via IL-6/STAT3 axis. J Hepatol 2016;64:1283–1294.
[158] Ji J, Dai X, Yeung S-CJ, He X. The role of long non-coding RNA GAS5 in cancers. Cancer Manag Res 2019;11:2729–2737.
[159] Schneider C, King RM, Philipson L. Genes specifically expressed at growth arrest of mammalian cells. Cell 1988;54:787–793.
[160] Tao R, Hu S, Wang S, Zhou X, Zhang Q, Wang C, et al. Association between indel polymorphism in the promoter region of lncRNA GAS5 and the risk of hepatocellular carcinoma. Carcinogenesis 2015;36:1136–1143.
[161] Faranda T, Grossi I, Manganelli M, Marchina E, Baiocchi G, Portolani N, et al. Differential expression profiling of long non-coding RNA GAS5 and miR-126-3p in human cancer cells in response to sorafenib. Sci Rep 2019;9:9118.
[162] Wang C, Ke S, Li M, Lin C, Liu X, Pan Q. Downregulation of LncRNA GAS5 promotes liver cancer proliferation and drug resistance by decreasing PTEN expression. Mol Genet Genomics 2020;295:251–260.
[163] Wang S, Aurora AB, Johnson BA, Qi X, McAnally J, Hill JA, et al. The endothelial-specific microRNA miR-126 governs vascular integrity and angiogenesis. Dev Cell 2008;15:261–271.

[164] Mattioli K, Volders P-J, Gerhardinger C, Lee JC, Maass PG, Melé M, et al. High-throughput functional analysis of lncRNA core promoters elucidates rules governing tissue specificity. Genome Res 2019;29:344–355.
[165] Soni PN, Brown D, Saffie R, Savage K, Moore D, Gregoriadis G, et al. Biodistribution, stability, and antiviral efficacy of liposome-entrapped phosphorothioate antisense oligodeoxynucleotides in ducks for the treatment of chronic duck hepatitis B virus infection. Hepatology 1998;28:1402–1410.
[166] Zhang G, Budker V, Wolff JA. High levels of foreign gene expression in hepatocytes after tail vein injections of naked plasmid DNA. Hum Gene Ther 1999;10:1735–1737.
[167] Liu F, Song Y, Liu D. Hydrodynamics-based transfection in animals by systemic administration of plasmid DNA. Gene Ther 1999;6:1258–1266.
[168] Bennett CF, Frank Bennett C, Baker BF, Pham N, Swayze E, Geary RS. Pharmacology of antisense drugs. Annu Rev Pharmacol Toxicol 2017;57:81–105.
[169] Carthew RW, Sontheimer EJ. Origins and mechanisms of miRNAs and siRNAs. Cell 2009;136:642–655.
[170] Liu J, Valencia-Sanchez MA, Hannon GJ, Parker R. MicroRNA-dependent localization of targeted mRNAs to mammalian P-bodies. Nat Cell Biol 2005;7:719–723.
[171] Vickers TA, Crooke ST. The rates of the major steps in the molecular mechanism of RNase H1-dependent antisense oligonucleotide induced degradation of RNA. Nucleic Acids Res 2015;43:8955–8963.
[172] Lima WF, De Hoyos CL, Liang X-H, Crooke ST. RNA cleavage products generated by antisense oligonucleotides and siRNAs are processed by the RNA surveillance machinery. Nucleic Acids Res 2016;44:3351–3363.
[173] Rader DJ, Kastelein JJP. Lomitapide and mipomersen: two first-in-class drugs for reducing low-density lipoprotein cholesterol in patients with homozygous familial hypercholesterolemia. Circulation 2014;129:1022–1032.
[174] Hoy SM. Patisiran: first global approval. Drugs 2018;78:1625–1631.
[175] Soutschek J, Akinc A, Bramlage B, Charisse K, Constien R, Donoghue M, et al. Therapeutic silencing of an endogenous gene by systemic administration of modified siRNAs. Nature 2004;432:173–178.
[176] Schlesinger PH, Doebber TW, Mandell BF, White R, DeSchryver C, Rodman JS, et al. Plasma clearance of glycoproteins with terminal mannose and N-acetylglucosamine by liver non-parenchymal cells. Studies with β-glucuronidase, N-acetyl-β-d-glucosaminidase, ribonuclease B and agalacto-orosomucoid. Biochem J 1978;176:103–109.
[177] Springer AD, Dowdy SF. GalNAc-siRNA conjugates: leading the way for delivery of RNAi therapeutics. Nucleic Acid Ther 2018;28:109–118.
[178] Tanowitz M, Hettrick L, Revenko A, Kinberger GA, Prakash TP, Seth PP. Asialoglycoprotein receptor 1 mediates productive uptake of N-acetylgalactosamine-conjugated and unconjugated phosphorothioate antisense oligonucleotides into liver hepatocytes. Nucleic Acids Res 2017;45:12388–12400.
[179] Sato Y, Murase K, Kato J, Kobune M, Sato T, Kawano Y, et al. Resolution of liver cirrhosis using vitamin A–coupled liposomes to deliver siRNA against a collagen-specific chaperone. Nat Biotechnol 2008;26:431–442.
[180] Jiménez Calvente C, Sehgal A, Popov Y, Kim YO, Zevallos V, Sahin U, et al. Specific hepatic delivery of procollagen α1(I) small interfering RNA in lipid-like nanoparticles resolves liver fibrosis. Hepatology 2015;62:1285–1297.
[181] Wang D, Tai PWL, Gao G. Adeno-associated virus vector as a platform for gene therapy delivery. Nat Rev Drug Discov 2019;18:358–378.
[182] Tontonoz P, Wu X, Jones M, Zhang Z, Salisbury D, Sallam T. Long noncoding RNA facilitated gene therapy reduces atherosclerosis in a murine model of familial hypercholesterolemia. Circulation 2017;136:776–778.
[183] Slack FJ, Chinnaiyan AM. The role of non-coding RNAs in oncology. Cell 2019;179:1033–1055.
[184] Chen S, Zhang Y, Wu X, Zhang C, Li G. Diagnostic value of lncRNAs as biomarker in hepatocellular carcinoma: an updated meta-analysis. Can J Gastroenterol Hepatol 2018;2018:8410195.
[185] Li G, Shi H, Wang X, Wang B, Qu Q, Geng H, et al. Identification of diagnostic long non-coding RNA biomarkers in patients with hepatocellular carcinoma. Mol Med Rep 2019;20:1121–1130.
[186] Horton JD, Goldstein JL, Brown MS. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. J Clin Invest 2002;109:1125–1131.