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REVIEW PAPER

Characterization of anti-steatogenic long noncoding RNAs and their epigenetic influence on the development of metabolic fatty liver disease – a systematic review

Oleksandr Abaturov , Anna Nikulina

Department of Pediatrics 1 and Medical Genetics, Dnipro State Medical University, Dnipro, Ukraine

ABSTRACT

Introduction and aim. Numerous transcriptomic studies have demonstrated that the development of metabolically associated fatty liver disease is accompanied by changes in the expression level of long noncoding RNAs (lncRs). The aim: to present a brief description of the role of anti-steatogenic lncRs in the epigenetic influence on the development of metabolically associated fatty liver disease, analyzing the data of modern scientific literature.

Material and methods. An analysis of 64 reports over the past 10 years was conducted from the databases PubMed; MEDLINE; EMBASE; Cochrane Systematic Reviews Database; BIOSIS which were selected using the indicated keywords. Quality aspects were assessed using the adapted Newcastle–Ottawa Scale, PROSPERO CRD420250652980.

Analysis of the literature. Hypoexpression of AC012668 – increased representation of miR-380-5p, activation of *LRP2*; B4GALT1-AS1/lncSHGL, MEG3 – activation of lipogenesis, gluconeogenesis in hepatocytes; FLRL2 – inhibition of *BMAL1* and *SIRT1*; Gm16551 – increased expression of *ACC1, SCD1*; HR1 – activation of *SREBP1c*. LncLSTR – activation of cytochrome Cyp8b1 transcription; MRAK052686 reduction of *FABP7* expression.

Conclusion. The formation of hepatosteatosis is supported by a decrease in the expression level of anti-steatogenic lncRs, such as AC012668, B4GALT1-AS1/lncSHGL, MEG3, FLRL2, Gm16551, lncHR1, lncLSTR, MRAK052686. LncRs overexpression is compensatory in escalating inflammation, hyperglycemia.

Keywords. anti-steatogenic long noncoding RNAs, epigenetic regulation, metabolically associated fatty liver disease, obesity

Introduction

Long noncoding RNAs (lncRs) actively participate in the regulation of the functioning of various biological processes. Aberrant lncR expression may be a key molecular driver of the development of metabolic diseases, in particular, obesity, type 2 diabetes mellitus (T2DM), arterial hypertension, dyslipidemia, and metabolic-associated fatty liver disease (MAFLD).[1-5] Numerous transcriptomic studies have demonstrated that the development of MAFLD is accompanied by changes in lncR expression levels.[6–8] A genome-wide screening performed in key metabolic organs of mice identified 359 metabolically responsive lncRs. Unlike previous studies that have demonstrated the role of lncR-mediated epigenetic regulation in the development of MAFLD, our review contains a systematic analysis of only statistically verified reports on this issue. It is believed that lncRs, which act as regulators of subtle metabolic reprogramming of cells, and their transcription mechanisms in the near future may become a target for drugs in the implementation of drug treatment of metabolic, inflammatory, autoimmune, neurodegenerative, oncological and other diseases.[9-12]

Corresponding author: Anna Nikulina, e-mail: anna.nikulina.201381@gmail.com

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Brief description of long noncoding RNAs

Currently, at least 28,000 lncRs have been identified in humans, with transcript lengths exceeding 200 nucleotides, hence the name long lncRs. lncR genes are transcribed by RNA polymerases I, II, and III.[13-17] Due to the different location in the genome of lncR coding sequences relative to protein-coding genes, lncRs are grouped into five different classes: long intergenic noncoding RNAs, antisense RNAs, sense RNAs, sense intronic RNAs, and enhancer lncRs. Depending on the functions they perform, there are: 1) signaling lncRs, which are specifically associated with intracellular molecular signaling pathways; 2) lncR traps, which interact with transcription factors and remove them from chromatin; 3) targeting lncRs, which bind to protein complexes and direct them to gene promoters or specific sites in the genome; 4) scaffolding lncRs, which connect various protein complexes, control gene expression. Notably, antisense transcription, which occurs through interaction with complementary sense transcripts encoded by the opposite gene, prevents transcription altogether.[18] Currently, there are several databases that provide updated information on lncRs (Table 1).

Table 1. lncR databases

Databases	Website	Website characteristics
CLS FL	–	Contains annotations for 807 lncRs
Lnc2Cancer database v3.0	http://www.bio-bigdata.net/lnc2cancer or http://bio-bigdata.hrbmu.edu.cn/lnc2cancer	Provides information on 2659 lncRs associated with 216 human cancer subtypes
lncATLAS	https://lncatlas.crg.eu/	LncATLAS displays subcellular localization for user-selected lncRs. Only GENCODE annotated lncR genes are present, which can be accessed using their identifier or official gene name
LncExpDB	https://ngdc.cncb.ac.cn/lncexpdb/	Covers expression profiles of 101,293 high-quality human lncR genes, predicts potential functional lncRs and their interacting partners
LncRNADisease v3.0	http://www.rnanut.net/lncrnadisease	Provides information on 2297 lncR interactions
Mammalian ncRNA-Disease Repository v3.1	http://rna-society.org/mndr/	Presents 40,000 human lncR and disease associations
MiTranscriptome (v2)	https://www.mitranscriptome.org	The full catalog includes over 91,000 genes. Contains annotations for 63,615 ncRs
NONCODE (v5)	http://v5.noncode.org/	Contains annotations for 96,308 human ncRs. The most comprehensive resource

lncR genes, compared to protein-coding genes, are characterized by a high level of tissue specificity. Thus, it has been shown that tissue specificity is characteristic of 78% of lncRs and only 19% of mRNAs. The lncR pool contains a larger number of organ-specific and clone-specific RNAs. The activity of lncR expression depends on the state of tissues and the presence of pathological processes. The biogenesis of most lncRs is similar to mRNA synthesis. LncRs are transcribed by RNA polymerase II, after which the lncR transcript undergoes capping, polyadenylation, and splicing, resulting in mRNA-like transcripts. Unlike mRNAs, which are localized in the cytoplasm, lncRs are predominantly located in the cell nucleus. A certain portion of lncRs is exported to the cell cytoplasm, where they are distributed to various intracellular organelles.[19-22]

LncRs have the ability to bind to DNA, RNA, and proteins, and therefore they participate in the regulation of: 1) gene expression at the level of transcription and posttranscriptional processes; 2) alternative splicing, and 3) the formation of protein complexes. Some lncRs bind to target mRNA transcripts and can enhance or repress their subsequent translation, provided that these lncRs are expressed from a gene silencer or enhancer. LncRs can regulate the expression of genes distant from the location of the lncR, they promote the formation of DNA loops, bringing the enhancer and the target site of the gene promoter closer to each other.[23]

It has been demonstrated that lncRs function as "sponges" for microRNAs (miRs) in the cytoplasm of the cell or as host genes for miR transcription in the cell nucleus. In addition, lncRs mediate DNA methylation and act as modular scaffolds for chromatin-modifying complexes.[24,25] lncRs are also involved in chromosome inactivation, induction of pluripotency, and cell differentiation.[26,27]

Recently, data have been obtained that some lncRs with open reading frames can be translated into small proteins or micropeptides that play a significant role in the body's immune response and carcinogenesis.[28,29]

It has been demonstrated that in liver biopsies of patients with MAFLD, 1735 lncRs and 1485 mRNAs are differentially expressed. Increased expression is characteristic of 535 lncRs and decreased expression is characteristic of 1200 lncRs.[30]

The role of anti-steatogenic lncRs in the development of hepatic steatosis in MAFLD

The development of hepatic steatosis in MAFLD is supported by a decrease in the expression level of anti-steatosis lncRs, such as AC012668, FLRL2, Gm16551, LncHR1, B4GALT1-AS1/lncSHGL, MEG3, and others (Table 2).

Aim

To present a brief description of the role of anti-steatogenic long noncoding RNAs in the epigenetic influence on the development of metabolically associated fatty liver disease, analyzing the data of modern scientific literature.

Table 2. Hypoexpression of long noncoding RNAs associated with the development of hepatic steatosis in MAFLD[31,32]

lncR	Effect	Molecular target	Signaling cascade
AC012668	Promotes lipid accumulation	miR-380-5p	miR-380-5p/LRP2
B4GALT1-AS1/ lncSHGL	Activates lipogenesis and gluconeogenesis		PI3K/AKT; hnRNPA1/ CaM
FLRL2	Activates lipogenesis, inflammatory response		Arnt1/Sirt1
Gm16551	Activates lipogenesis de novo		ACC1 i SCD1
LncHR1	Promotes lipid accumulation		SREBP1c, PDK1/AKT/ FoxO1
lncLSTR	Promotes lipid accumulation		Cyp8b1/FXR/apoC2
MEG3	Stimulation of lipogenesis	miR-21	LRP6/AKT/ mTORC1
MRAK052686	Promotes lipid accumulation		ZBTB20

Material and methods

An analysis of 64 literature sources over the past 10 years was conducted from the databases PubMed, MEDLINE; EMBASE; PreMedline In-Process & Other Non-Indexed Citations; The Cochrane Systematic Reviews Database, DARE, NHS EED and HTA databases; Web of Knowledge Science Citation Index; Web of Knowledge ISI Proceedings; CRD databases; BIOSIS, which were selected using the following keywords: long non-coding anti-steatogenic RNAs, epigenetic regulation, metabolically associated fatty liver disease, obesity. Inclusion criteria were based on the PECOS approach, depending on the sample types:[33]

Type 1 (n=2) – MAFLD patients (P (Population): people over 18 years of age; E (Exposure): diagnostic criteria for MAFLD;[34] C (Comparison): MAFLD patients vs. healthy individuals; O (Outcome): decreased expression of lncRs, assessed by RT-qPCR with escalation of MAFLD factors; S (Study design): Cross-sectional study (CSS)/Dynamic prospective study of the "case-control" type (DPCC)).

Type 2 (n=12) – MAFLD modeling *in vivo* (P: 8-week-old mouse line with an average body weight of 18-20 g; E: High-fat diet (HFD) according to 12 weeks, assessed by analysis of biological samples from participants; C: MAFLD mice vs. healthy mice; O: decreased expression of lncR, assessed by RT-qPCR with escalation of MAFLD factors; S: Dynamic prospective experimental studies (DPES).

Type 3 (n=10) – MAFLD modeling *in vitro* (P: human hepatocytes of cell lines or mice; E: FFA treating followed by transfection of lncRs activators/inhibitors, cell culture analysis, and assessment of human homologs using the Basic Local Alignment Search (BLAST) tool of the National Center for Biotechnology Information; C: FFA-treating of cells versus intact cells; O: decreased expression of lncRs, assessed by RT-qPCR with escalation of MAFLD factors; S: DPES.

Studies were excluded if the determination of lnsR expression was carried out by methods other than the analysis of biological samples of participants, if the effect in MAFLD modeling was assessed over a shorter period of time, and the criteria for diagnosing MAFLD were not considered the main outcome. Quality aspects were assessed using the adapted Newcastle–Ottawa Scale. Studies that received a score of 7 were considered to have a low risk of bias; 6 - medium risk of bias; 5 or less - high risk of bias.[35]

Analysis of the literature

Of the 64 reports highlighting the contribution of lncRs to MAFLD development, we focused on 35 reports associated with lncRs expression as a biomarker for MAFLD development, namely: 2 (AC012668); 3 (B4GALT1-AS1/lncSHGL); 7 (FLRL2); 1 (Gm16551); 2 (LncHR1); 2 (LncLSTR); 15 (MEG3); 4 (MRAK052686). Most of them had a moderate risk of bias. Ultimately, only 16 (24 non-randomized clinical trials (nRCT)) out of 35 reports contained reliable information and met the standardized criteria for inclusion in the study. The bias for low risk was 54.2%, for intermediate risk 29.2%, and for high risk 16.6%. The quality of the nRCTs on the contribution of lncRs to the development of MAFLD using NOS is presented in Table 3.

At the same time, a compensatory overexpression of MEG3 was noted under the additional influence of metabolically unfavorable factors associated with persistent meta-inflammation (the presence of concomitant diabetes type 2, obesity) or the cultivation of hepatocytes in a medium with TNF-α.[46;50]

AC012668. It was found that in MAFLD, the expression activity of lncR AC012668 (1.642 nt URS-00004FA763_9606) is suppressed, while overexpression of lncR AC012668 is accompanied by suppression of the expression of genes associated with lipogenesis (*SREBP1c, FASN, SCD1*) and inhibition of triglyceride accumulation processes in hepatocytes. AC012668 sequesters miR-380-5p, whose molecular target is low density lipoprotein receptor-related protein 2 (LRP2). Decreased expression of lncR AC012668 leads to increased expression of miR-380-5p, which suppresses expression of the *LRP2* gene, which promotes lipid accumulation in liver tissue. Administration of exogenous AC012668 inhibits the progression of hepatic steatosis.[36]

B4GALT1-AS1/lncSHGL. In liver biopsies of people with MAFLD, a decrease in the expression level of homo sapiens (human) beta-1,4-galactosyltransferase 1 antisense RNA 1 (B4GALT1-AS1; 1,185 nt URS000075AF86_9606; in mice, designated as lncSHGL) is noted. B4GALT1-AS1/lncSHGL is a non-secretory lncR, which under physiological conditions is highly expressed in liver tissue and has the ability to inhibit lipogenesis and gluconeogenesis.[37,52] Overexpression of lncR lncSHGL is accompanied by phosphorylation of AKT, which leads to inhibition of gluconeogenesis and

Table 3. Analysis of the quality of nRCTs associated with the contribution of AC012668, B4GALT1-AS1/lncSHGL, FLRL2, Gm16551, LncHR1, LncLSTR, MEG3, MRAK052686 to the development of MAFLD using NOS*

Author, year	Individual participant data, as a model of the MAFLD	Lnc Rs expression levelin hepatocytes (h) or serum (s), RE[a] (M±m)		S[b]	C[c]	E[d]/O[e]	Risk of study bias
		MAFLD$^+$	MAFLD$^-$				
	AC012668						
Chen et al., 2021[36]	MAFLD modeling in C57BL/6J mice (n=10/10) [DPES]	0.3 ±0.04 (h)	1 ±0.07 (h)	3	2	3	8
	MAFLD modeling in human hepatocyte cell culture LO_2 [f, DPES]	0.4 ±0.02	1.1 ±0.03	3	2	3	8
	B4GALT1-AS1/lncSHGL						
Wang et al., 2018[37]	MAFLD modeling in C57BL/6J mice (n=6/8) [DPES]	0.6±0.01 (h)	1 ±0.1 (h)	3	2	3	8
	MAFLD patients [CSS] (n=6/6)	0.7 ±0.13 (h)	1.1 ±0.2 (h)	3	2	2	7
	FLRL2						
Chen et al., 2017[38]	MAFLD modeling in C57BL/6J mice (n=4/4) [DPES]	0.2 ±0.01 (h)	0.96 ±0.02(h)	2	2	2	6
Chen et al., 2019[39]	MAFLD modeling in C57BL/6J mice (n=6/6) [DPES]	0.46 ±0.05(h)	0.9 ±0.03 (h)	3	2	2	7
	MAFLD modeling in human hepatocyte cell culture [DPES]	0.25 ±0.02	0.82 ±0.01	3	2	2	7
	Gm16551						
Di Mauro et al., 2021[40]	MAFLD modeling in C57BL/6J mice (n=8/8) [DPES]	↓(h)	N (h)	3	1	2	6
	LncHR1						
Li et al., 2017[41]	MAFLD (HCV) modeling in C57BL/6J mice (n=6/6) [DPES]	1.12 ±0.07(h)	2.93 ±0.09(h)	3	1	2	6
Li et al., 2018[42]	MAFLD modeling in human hepatocyte cell culture Huh7 [g, DPES]	↓	↓/N (p>0.05)	2	2	2	6
	LncLSTR						
Li et al., 2015[43]	MAFLD modeling in C57BL/6J mice (n=7/8) [DPES]	0.08 ±0.001	0.93 ±0.003	3	2	3	8
	MEG3						
Zhu et al., 2016[44]	MAFLD modeling in C57BL/6J mice (n=?) [DPES]	↓ (h)	N (h)	1	1	3	5
	MAFLD modeling in mouse hepatocyte cell culture [DPES]	↓	N	1	1	3	5
Wang et al., 2018[45]	MAFLD modeling in C57BL/6J mice C57BL/6J (n=?) [DPES]	↓(h)	N (h)	1	2	3	6
	MAFLD modeling in mouse hepatocyte cell culture [DPES]	↓	N	1	2	3	6
Zhu et al., 2019[46]	MAFLD (T2DM/ob/ob[i]) modeling in C57BL/6J mice (n=10/10[i] [DPES]S	2.8 ±0.08 (h)	1.1 ±0.03 (h)	3	1	3	7
	MAFLD (T2DM/ob/ob) modeling in cell culture of primary mouse hepatocytes [DPES]	2.3 ±0.1	1.1 ±0.08	3	1	3	7
Huang et al., 2019[47]	MAFLD modeling in C57BL/6J mice C57BL/6J (n=?) [DPES]	↓(h)	N (h)	1	1	3	5
	MAFLD modeling in human hepatocyte cell culture $HepG_2$ [j, DPES]	↓	N	1	1	3	5
Zou et al., 2022[48]	MAFLD (MASH[k]) modeling in C57BL/6J mice (n=10/15/15) [DPES]	0.6±0.06 (0.3±) h	1.12 ±0.09 (h)	3	2	3	8
	MAFLD modeling in mouse hepatocyte cell culture [DPES]	0.4 ±0.01	0.9 ±0.05	3	2	3	8
Erdem et al., 2023[49]	MAFLD patients (n=180/60) [DPCC]	0.41 ±0.02(s)	1.68 ±0.03(s)	3	2	3	8
Meng et al., 2024[50]	MAFLD (TNF-α[l]) modeling in human hepatocyte cell culture HepG[2DPES]S	4.1 ±0.1	0.9 ±0.07	3	1	2	6
	MRAK052686						
Yuan et al., 2015[51]	MAFLD modeling in human hepatocyte cell culture Huh7[DPES]	0.64±0.04	1.7±0.08	2	2	3	7

* RE[a] – relative expression by real time PCR (qPCR) of differentially expressed genes in the microarray analysis; S[b] – selection; C[c] – comparability; E[d] – exposure; O[e] – outcome; LO_2[f] – the human normal hepatocyte; Huh7[g] – an immortal cell line composed of epithelial-like, tumorigenic cells; ob/ob[i] – mice are hyperphagic, obese, hyperinsulinemic, and hyperglycemic, so they are used as a model for diabetes and obesity; $HepG_2$[j] – a hepatoblastoma cell line; MASH[k] – metabolic dysfunction-associated steatohepatitis; TNF-α[l]– Tumor necrosis factor α

lipogenesis gene expression (*PEPCK, G6Pase, FAS*). It is believed that lncR lncSHGL recruits heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1), which increases the translation efficiency of calmodulin (*CALM*) gene mRNA. In turn, calmodulin independently of insulin and calcium stimulates the PI3K/AKT-associated signaling pathway in hepatocytes. It is believed that drug activation of the lncR B4GALT1-AS1/hnRNPA1 signaling pathway is a potential new direction for the treatment of both hepatic steatosis and type 2 diabetes.[37] It is possible that lncR B4GALT1-AS1 is involved in the development of liver fibrosis.[53]

FLRL2. Fatty liver-related lncR (FLRL): FLRL1, FLRL2, FLRL6 – are involved in the regulation of circadian rhythm.[36,38] It has been established that in experimental animals with a model of MAFLD, the expression activity of lncR FLRL2, which is highly associated with the development of hepatic steatosis, is significantly reduced.[38] Overexpression of FLRL2 inhibits lipogenesis activity, contributes to the reduction of steatosis and liver inflammation in MAFLD. Given that the sequence encoding FLRL2 is located in the intronic region of the AHR nuclear translocator-like protein gene (basic helix-loop-helix ARNT like 1 – BMAL1), its expression

level corresponds to the expression of both the *BMAL1* gene and the closest sirtuin 1 (*SIRT1*) gene. FLRL2 promotes Bmal1 transcription and accumulation of Bmal1 protein in the cytoplasm, which translocates into the cell nucleus and binds to the *Sirt1* gene promoter, enhancing its transcription. Decreased FLRL2 expression in MAFLD leads to inhibition of *BMAL1* and *SIRT1*. Thus, the lack of expression of lncR FLRL2 is associated with a low level of *SIRT1* gene expression, which leads to: 1) increased activity of NR1H3/LXRα and SREBP1c with increased lipogenesis and patatin-like phospholipase domain-containing protein 3 (PNPLA3);[5] 2) inhibition of peroxisome proliferator activated receptor alpha (PPARα), reducing the efficiency of lipid β-oxidation; 3) disruption of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kappaB), which induces inflammation.[36,37,52-55] It is known that overexpression of the *SIRT1* gene is accompanied by recovery from MAFLD, while deficiency of SIRT1 production contributes to the progression of the manifestations of this disease. Cheng Tian and colleagues believe that insufficient SIRT1 activity contributes to the development of MAFLD by increasing lipid accumulation in hepatocytes, reducing the efficiency of autophagy, developing oxidative stress in hepatocytes, and inflaming the liver tissue.[56]

Gm16551. Decreased expression of the anti-steatogenic lncR Gm16551 (mus musculus (mouse) predicted gene 16551; 3.162 nt URS0000AAF6F6_10090), which is observed in MAFLD, promotes increased expression of the *ACC1* and *SCD1* genes, and induces the development of hepatic steatosis. Interestingly, administration of coffee to mice fed a HFD restores Gm16551 expression levels to values observed in lean mice.[40]

LncHR1. Decreased expression of the transcript 1 regulated by the hepatitis C virus (homo sapiens (human) lncR induced by HCV, regulator of SREBF1 – lncHR1; 420 nt URS000038DCB1_9606), which causes the development of hepatic steatosis, is pathognomonic for MAFLD. Anti-steatogenic lncHR1 is a potent negative regulator of SREBP1c. LncHR1 inhibits SREBP1c activity by phosphorylating components of the PDK1/AKT/FoxO1 signaling cascade.[42] Overexpression of lncHR1 is accompanied by a decrease in the expression level of the SREBP1c factor, which is induced by oleic acid, and inhibition of the process of triglyceride accumulation in lipid droplets of hepatocytes.[41] Decreased lncHR1 expression promotes activation of the SREBP1c factor, which induces lipogenesis and the development of hepatic steatosis.

LncLSTR. With the development of MAFLD, the level of expression of long intergenic RNA liver-specific triglyceride regulator (LSTR) in blood serum significantly decreases. Mice with liver-specific depletion of lncLSTR show a marked reduction in serum triglyceride levels. lncLSTR has been shown to directly bind to TAR DNA binding protein (TARDBP), which represses the transcription of cytochrome p450 family 8 subfamily b member 1 (Cyp8b1). Inhibition of Cyp8b1 activity leads to significant changes in the composition of bile acids. The altered composition of bile activates the expression of farnesoid X receptor (FXR). Farnesoid X receptor is a bile acid-activated receptor that is mainly expressed in liver and intestinal tissues and has 2 forms in mammals: FXRα and FXRβ. FXR is known to modulate several metabolic pathways, including bile acid synthesis and lipid metabolism. For example, FXR activation leads to the induction of apolipoprotein C2 (apoC2). Patients with homozygous apoC2 deficiency have elevated serum triglyceride levels and significantly reduced LPL activity, suggesting that apoC2 is an activator of LPL. An increase in the activity of apoC2 leads to a powerful activation of LPL and leads to an increase in the clearance of triglycerides from the peripheral blood stream. Suppression of the increased expression of apoC2 in lncLSTR KD mice induces restoration of the reduced serum triglyceride levels caused by lncLSTR knockout. Decreased lncLSTR expression leads to increased lipid content in hepatocytes.[43,57]

MEG3. Homo sapiens (human) maternally expressed gene 3 (MEG3; 1,595 nt URS0000759EA9_9606), which is located in the imprinted locus DLK1-MEG3 in the 14q32.3 region of human chromosome, is highly generated in the physiological state and is reduced in the development of MAFLD. It has been demonstrated that activation of MEG3 expression inhibits lipogenesis. It is worth noting that the level of reduction in MEG3 expression is associated with the severity of MAFLD. The decrease in MEG3 expression is likely due to the increase in miR-136 expression.[29,45,47] Long non-coding RNA MEG3 is a competing endogenous RNA (ceRNA) for some miRNAs, among which miR-466i-5p, miR-574-5p, miR-770-5p are represented. The target genes of miR-466i-5p are integrin alpha M (*ITGAM*), prolyl 3-hydroxylase 2 (*P3H2*), serine/threonine kinase 10 (*STK10*); miR-574-5p – FAM20C Golgi associated secretory pathway kinase (*FAM20c*) gene; miR-770-5p – collagen type VI α chain 2 (*COL6A2*) gene. They are expressed in adipose tissue and regulate meta-inflammation, fibrosis and insulin resistance.[58,59] The MEG3 transcript has the ability to directly interact with miR-10b-5p, miR-21-5p, miR-27a-3p, miR-29a-3p, miR-33a-5p, miR-99a-3p, miR-99b-3p, miR-122-5p, miR-139-5p, miR-146b-3p, miR-214-3p, miR-222-5p, miR-296-3p, miR-302a-3p, miR-378a-5p.[49,60] Decreased MEG3 expression induces increased serum triglyceride, cholesterol, and ALT and AST concentrations, while MEG3 deficiency promotes increased expression of nuclear factor 2 of erythroid origin, similar to basic leucine zipper (bZIP) transcription factor 2 (NFE2L2). In hepatocytes, increased activity of the transcription factor

NFE2L2 enhances the expression of PPARγ and associated lipogenic genes, and, conversely, knockout of the *NFE2L2* gene in hepatocytes attenuates the expression of the *PPARG* gene. NFE2L2 activity induces increased VLDL levels and causes hepatic steatosis. NRF2-dependent expression of the PPARγ receptor gene in hepatocytes is believed to be a critical factor that initiates the development of MAFLD, and pharmacological inhibition of NFE2L2 in hepatocytes may be a key approach to prevent the development of hepatic steatosis.[45,50] It has been demonstrated that overexpression of MEG3 significantly reduces the expression of FoxO1, ACC1 and FASN and, as a result, inhibits lipid accumulation in HepG2 cells. It has also been found that MEG3 inhibits the translocation of the FoxO1 factor into the cell nucleus, inhibiting lipogenesis *de novo*. Whereas inhibition of MEG3 expression is accompanied by an increase in the levels of mRNA and proteins of FoxO1, ACC1, FASN, as well as the intensity of lipid accumulation in hepatocytes.[44] At the same time, it was shown that MEG3 sequesters miR-214-3p, promoting the activation of the expression of activating transcription factor-4 (ATF4), which has the ability to potentiate glucose synthesis *de novo* by stimulating FoxO1 transcriptional activity.[61] A decrease in the activity of the transcription factor ATF4 leads to a decrease in gene expression of the protein, CCAAT-enhancer-binding protein homologous protein (CHOP), and inhibition of cell apoptosis.[62] MEG3 sequesters miR-21, which targets low-density lipoprotein receptor-related protein 6 (LRP6), PPARα, and SMAD7. It has been found that miR-21 causes effects similar to those of the expression of genes involved in lipogenesis, including ACC1, SCD1, the SREBP1c factor, and the NR1H3/LXRα receptor. Overexpression of MEG3 is accompanied by an increase in the level of LRP6, which leads to a decrease in lipid accumulation in hepatocytes, an increase in the activity of PPARα receptors, which enhance lipid uptake and β-oxidation, and the inhibitory factor SMAD7, which suppresses the activity of the synthesis of profibrotic molecules.[60,63,64] While MEG3 deficiency is accompanied by overexpression of miR-21, which suppresses the expression of *LRP6, PPARA, SMAD7* genes, contributing to the development of both steatosis and liver fibrosis.[48] Experimental studies have shown that MEG3 activates the genes *SIRT6* and enhancer of zeste 2 polycomb repressive complex 2 subunit (*EZH2*), which are involved in the regulation of triglyceride accumulation and liver inflammation. Insufficient expression of MEG3 also causes the formation of hepatic fibrosis.[51]

LncR MRAK052686. The lncR gene MRAK052686 is located near the zinc finger and BTB domain containing 20 (ZBTB20) protein gene. The function of ZBTB20 is closely related to the antioxidant NFE2L2.[65] Decreased expression of lncR MRAK052686 is associated with suppression of NFE2L2 gene expression and increased expression of fatty acid binding protein 7 (*FABP7*) gene.[57] Decreased NFE2L2 activity is accompanied by increased endoplasmic reticulum stress and induction of inflammation. Fatty acid binding proteins are involved in intracellular lipid metabolism, such as fatty acid uptake, transport, oxidation, synthesis, and storage, and also play a role in regulating the activity of nuclear receptors.[66-68] An increase in the activity of FABP7 leads to the accumulation of lipids in the lipid droplets of the cytoplasm of hepatocytes.[64]

Study limitations and strengths

This systematic review was performed in accordance with the Preferred Reporting Items for Systematic Reviews (PRISMA) guidelines using a validated method for quality analysis of stratified 64 reports.[69] All studies that examined lncRs expression in both MAFLD patients and in MAFLD animal models (*in vivo*) and hepatocyte cell cultures (*in vitro*) were included, primarily focusing on identifying anti-steatogenic lncRs. In MAFLD modeling, we used only representative results from studies. In vivo: experimental subject – 8-week-old male C57BL/6J mice, weighing 18-20 g (negative exposome factor: HFD 12 weeks); in vitro: the object of the experiment is a cell culture of human or mouse hepatocytes (negative exposome factor: cultivation with higher fatty acids for 24 hours, followed by transfection with small interfering RNA, miR mimic/inhibitor and small interfering target gene involved in the corresponding signaling pathway). This systematic review presented the relationship of a wide range of steatogenic signaling pathways associated with the development of MAFLD and suppressed by the physiological expression of anti-steatogenic lncRs. This review includes the rationale for target points of regulation of lncR physiological expression for future precision therapy of MAFLD. and data demonstrate the efficacy of MEG3 in protecting hepatocytes from FA-induced lipogenesis and inflammation. The systematic review was not based on the analysis of randomized clinical trials in patients with MAFLD due to their limitations and the high invasiveness of obtaining biopsy material in "blinded" conditions, as well as the ethical feasibility of collecting it in a control group.

Conclusion

Thus, in MAFLD, hypoexpression of lncRs is observed, which pathogenically affects the main mechanisms of hepatic steatosis development, enhancing lipogenesis *de novo*, inhibiting β-oxidation of fatty acids and lipid export from hepatocytes (Fig. 1).

The development of hepatic steatosis is supported by a decrease in the expression level of anti-steatogenic lncRs, such as AC012668, B4GALT1-AS1/lncSH-

GL, MEG3, FLRL2, Gm16551, lncHR1, lncLSTR, MRAK052686. LncRs overexpression is compensatory in escalating inflammation and hyperglycemia. Future research should aim to achieve consensus on the systematic presentation of results and facilitate the presentation of a core data set on the contribution of lncRs in MAFLD. The research group also aims to expand the current policy to encourage study authors to make RCT data available for meta-analysis purposes.

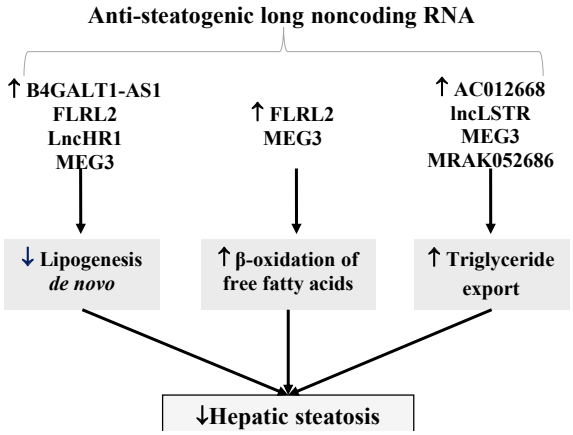

Fig. 1. Effect of anti-steatogenic lncR on the development of hepatic steatosis in MAFLD

Declarations

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Author contributions

Conceptualization, O.A. and A.N.; Methodology, O.A.; Validation, O.A. and A.N.; Formal Analysis, O.A. and A.N.; Investigation, O.A. and A.N.; Resources, O.A. and A.N.; Writing – Original Draft Preparation, O.A.; Writing – Review & Editing, O.A. and A.N.; Visualization, O.A. and A.N.; Supervision, O.A. and A.N.; Project Administration, O.A.

Conflicts of interest

All authors declare that they have no conflicts of interest.

Data availability

No datasets were generated or analyzed during the current study.

Ethics approval

Not applicable.

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