Regulation of hepatic microRNA expression by hepatocyte nuclear factor 4 alpha

Hong Lu, Xiaohong Lei, Jerry Liu, Curtis Klaassen

Hong Lu, Xiaohong Lei, Department of Pharmacology, SUNY Upstate Medical University, Syracuse, NY 13210, United States
Jerry Liu, Curtis Klaassen, Department of Medicine, University of Kansas Medical Center, Kansas City, KS 66160, United States

Author contributions: Lu H wrote the paper; Lu H, Lei X and Liu J performed the experiments and analyzed the data; Lu H and Klaassen C conceived and designed the experiments.

Supported by NIH grant ES019487 in part.

Institutional animal care and use committee statement: All animal procedures in the study were approved by the Institutional Animal Care and Use Committee of the University of Kansas Medical Center. The animal protocol was designed to minimize the pain or distress to the mice. Aged-matched young-adult Hnf4α Liv-KO mice and their wild-type control littermates were fed rodent chow (#8606, Teklad; Harlan, Indianapolis, IN). Mice were housed at an ambient temperature of 22 °C with alternating 12-h light/dark cycles and allowed water and feed ad libitum.

Conflict-of-interest statement: The authors have no conflict of interest to declare.

Data sharing statement: No additional data are available.

Open-Access: This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

Manuscript source: Invited manuscript

Correspondence to: Hong Lu, PhD, Assistant Professor, Department of Pharmacology, SUNY Upstate Medical University, 750 E Adams ST, Syracuse, NY 13210, United States. luh@upstate.edu
Telephone: +1-315-4647978

Abstract

AIM
To uncover the role of hepatocyte nuclear factor 4 alpha (HNF4α) in regulating hepatic expression of microRNAs.

METHODS
Microarray and real-time PCR were used to determine hepatic expression of microRNAs in young-adult mice lacking Hnf4α expression in liver (Hnf4α-LivKO). Integrative genomics viewer software was used to analyze the public chromatin immunoprecipitation-sequencing datasets for DNA-binding of HNF4α, RNA polymerase- II, and histone modifications to loci of microRNAs in mouse liver and human hepatoma cells. Dual-luciferase reporter assay was conducted to determine effects of HNF4α on the promoters of mouse and human microRNAs as well as effects of microRNAs on the untranslated regions (3′ UTR) of two genes in human hepatoma cells.

RESULTS
Microarray data indicated that most microRNAs remained unaltered by Hnf4α deficiency in Hnf4α-LivKO mice. However, certain liver-predominant microRNAs were down-regulated similarly in young-adult male and female Hnf4α-LivKO mice. The down-regulation of miR-101, miR-192, miR-193a, miR-194, miR-215, miR-802, and miR-122 as well as induction of miR-34 and miR-29 in male Hnf4α-LivKO mice were confirmed by real-time
INTRODUCTION

Hepatocyte nuclear factor 4 alpha (HNF4α) is a master regulator of liver development and function[2-4]. HNF4α is essential for hepatocyte differentiation in fetal liver[12-14], maintenance of liver function in adult[5,6], and protection against liver cirrhosis and liver cancer[7,8]. HNF4α is critical in regulating hepatic metabolism of fatty acids, bile acids, and ureagenesis[9-11]. Moreover, HNF4α is essential in regulating hepatic expression of drug processing genes, namely cytochrome P450s, phase-II conjugation enzymes, and transporters[1,12,13].

There are very large individual variations in hepatic basal expression of HNF4α in humans[24], and mutation of HNF4α causes maturity onset diabetes of young humans[25]. The expression and/or transcriptional activity of HNF4α is decreased markedly in severe cirrhotic livers, alcoholic liver disease, tumor necrosis factor-α-induced hepatotoxicity, and hepatoma progression[16-19]. Thus, it is important to understand how HNF4α deficiency affects hepatic gene expression and its underlying mechanism.

Interestingly, overexpression of HNF4α in hepatocellular carcinoma (HCC) markedly decreases the stemness of gene expression and the percentage of cancer stem cells in HCC[7]; however, the underlying mechanism is unknown. Epigenetic modifications play key roles in regulating gene expression and stem cell differentiation. Our recent study demonstrates that Hnf4α deficiency in young-adult mouse livers causes marked alteration in histone methylation and acetylation, which is associated with induction of certain key epigenetic enzymes, including enhancer of zeste homolog 2 (EZH2), G9a and DNA methyltransferase (cytosine-5) 1 (Dnmt1)[20]. EZH2 plays a key role in maintaining the stemness of stem cells[21]. Therefore, establishment and maintenance of the epigenome of differentiated hepatocytes may be a key mechanism in the regulation of gene expression and cell differentiation by HNF4α.

The importance of HNF4α in regulating hepatic expression of mRNAs has been well established, however the underlying mechanism remains less clear. HNF4α directly binds to a large number of gene promoters in human and mouse liver[22-24]. Hnf4α deficiency in young-adult mouse liver caused induction of certain key epigenetic modifiers[20]. However, our analysis of published data of chromatin immunoprecipitation-sequencing (ChIP-seq) of Hnf4α in adult mouse liver[25] revealed no binding of Hnf4α to these epigenetic modifiers, suggesting indirect regulation of these epigenetic modifiers by Hnf4α in liver. microRNAs are important post-transcriptional regulators of gene expression, and deregulation of microRNAs is common in human hepatocarcinogenesis[26]. Through binding to the untranslated regions (UTRs, usually the 3′UTR) of mRNAs, microRNAs affect the stability/translation of mRNAs and thus the mRNA and/or protein levels of their target genes. We hypothesized that HNF4α can indirectly regulate hepatic gene expression through directly regulating hepatic expression of certain microRNAs. Thus, the purpose of this study was to uncover the role of HNF4α in regulating hepatic expression of microRNAs. We used microarray and real-time PCR to determine hepatic expression of microRNAs in young-adult mice lacking Hnf4α expression in liver (Hnf4α-LivKO). We used integrative genomics viewer (IGV) software to analyze the public ChIP-seq datasets.
MATERIALS AND METHODS

Preparation of liver samples

The livers of male and female young-adult mice with liver-specific knockout of Hnf4α (Hnf4α-LivKO) (Hnfα-flox/flox, Alb-cre/+), and age-matched wild-type (Hnf4α-flox/flox, Alb-cre/-) littermates at the age of 45 d were collected in the previous study[27] and stored at -80 °C until use. All animal procedures in the study were approved by the Institutional Animal Care and Use Committee of the University of Kansas Medical Center[27].

Microarray profiling of microRNA expression in Hnf4α-LivKO mice

Pooled total RNAs from livers of young-adult (42-45 d old) male and female Hnf4α-LivKO and their age-matched wild-type littermates (n = 5-6) were used for microarray analysis of microRNAs, utilizing mirCURY™ LNA array version 11.0 (Exiqon, Denmark), which contains probes targeting all mouse miRNAs registered in the miRBASE version 13.0. Background correction was conducted utilizing normexp plus offset method with offset value 10[28]. The non-linear regression method was used for data normalization to remove certain systematic biases from microarray data, such as dye effects or intensity dependence.

Heat map and unsupervised hierarchical clustering of microRNAs

The heat map diagram shows the result of the 2-way hierarchical clustering of microRNAs and samples[29]. Each row represents a microRNA and each column represents a pooled liver sample. The microRNA clustering tree is shown on the left, and the sample clustering tree appears at the top. The color scale shown at the bottom illustrates the relative expression level of a microRNA across all samples: Red color represents an expression level above mean, blue color represents expression lower than the mean. The clustering is performed on log2(5s rRNA/Hy5) ratios which passed the filtering criteria on variation across samples; LogMedianDifferences > 0.58, corresponding to 50% differential expression.

Quantification of microRNAs using real-time PCR

mirCURY LNA™ Universal RT microRNA PCR (Exiqon) was used to quantify microRNAs in individual RNA samples from livers of male Hnf4α-LivKO mice. All PCR reagents and specific LNA-modified PCR primer sets were purchased from Exiqon. The PCR primer sets for mmu-miR-19b, 26a, 29b, 34a, 122, 192, 193a-3p, 194 and 195 target both human and mouse microRNA homologs, whereas PCR primer sets for mmu-miR-101b, 215, and 802 were specific for mouse microRNAs. The relative expression of each microRNA was normalized by 5s rRNA and U6 RNA with values of wild-type mice set at 100.

Use of public database to analyze DNA-binding of HNF4α and the chromatin status of microRNAs in mouse liver, intestine, and human hepatoma HepG2 cells

Actively transcribed genes typically remain in loosely-packed euchromatin, where DNA is more accessible to the transcriptional machinery. DNAse-I hypersensitive sites (DHSs), determined by DNAse-sequencing (DNAseq), is a key determining factor of the chromatin accessibility of transcription factors. DNA-binding of RNA polymerase 2 (Pol2) is widely used as a marker of active transcription. Histone H3 trimethylation at lysine-4 (H3K4me3) is enriched around the transcription start sites (TSS) and correlates tightly with active gene transcription[30,31], whereas H3 trimethylation at lysine-36 (H3K36me3) along the gene coding regions after TSSs correlated highly with transcription elongation[32]. Our previous study shows that alterations of H3K4me3 correlate bi-directionally with mRNA expression in HNF4α-null livers[20]. Conversely, Histone H3 trimethylation at lysine-27 (H3K27me3) and at lysine-9 (H3K9me3) are well-established epigenetic signatures of gene silencing[31,32]. The public genome-wide datasets of DNAseq (GSM1003818) as well as ChIP-seq of H3K4me3 (GSM769014), H3K36me3 (GSM1000151), H3K9me3 (GSM1087075), H3K27me3 (GSM1087069), Pol2 (GSM722763) and HNF4α (GSM1390711) in wild-type mouse liver were retrieved from GEO DataSets and uploaded into the IGV software[34] to visualize the DNA-binding of HNF4α, Pol2 and these epigenetic signatures in each microRNA locus in mouse liver. Similarly, the public genome-wide datasets of DNAseq (GSM816662) as well as ChIP-seq of H3K4me3 (GSM945182), H3K36me3 (GSM945211), H3K9me3 (GSM1003519), H3K27me3 (GSM945231), Pol2 (GSM935543), and HNF4α (GSM935619) in HepG2 cells were retrieved from GEO DataSets for their visualization in the IGV software. Additionally, to determine the role of tissue-specific binding of HNF4α in the tissue-specific regulation of miRs, ChIP-seq data for DNA-binding of HNF4α in the mouse liver (GSM130711) and small intestinal villus cells (GSM851120) were compared using the IGV software.

Generation of expression vectors for wildtype and mutant mouse Hnf4α

The mouse Hnf4α cDNA was synthesized by Integrated DNA Technologies, Inc (IDT, Coralville, IA) and cloned into the pcDNA3 backbone to generate the expression vector for wildtype Hnf4α, which was named as pcDNA3-Hnf4α. The expression vector for the 304 serine to aspartic acid (S304D) mutant of Hnf4α was generated using pcDNA3-Hnf4α and the Q5® Site-Directed Muta-
Lu H et al. Regulation of hepatic microRNA by HNF4α

genesis Kit (New England Biolabs), and verified by sequencing.

Generation of reporter constructs for the promoters of human miR-101-2 and mouse miR-101b

miR-101 is mainly transcribed from the human miR-101-2 and mouse miR-101b loci, which are located in the intron9-9 of RNA terminal phosphate cyclase-like 1 (RCL1) gene. The first base of the pre-miR-101-2 was assigned as chr9:4840297[36], located within intron5-6 of RCL1, around where prominent peaks of H3K4me3 and DNA-binding of Pol2 and HNF4α were identified. Thus, we PCR cloned a 739-bp fragment of miR-101-2 proximal promoter (-926 to -190 bp), located within the intron5-6 of RCL1, into the KpnI/MluI sites of pGL3-Basic reporter vector, which was named as pGL3-miR-101b. In mice, miR-101b is predominantly expressed in the liver[37]. Similar to its human ortholog miR-101-2, we found prominent peaks of HNF4α, H3K4me3 and Pol2 that start at the intron5-6 of Rcl1 and extend to intron7-8 and intron9-9 of Rcl1. Thus, we PCR cloned a 933-bp fragment of the miR-101b promoter, located within intron5-6 of Rcl1 that contains the peaks of HNF4α and Pol2, into the KpnI/MluI sites of pGL3-Basic reporter vector, which was named as pGL3-miR-101b.

Generation of reporter constructs for the proximal and/or distal promoters of human and mouse miR-194-2/miR-192 cluster

Mouse miR-194-1/miR-215 and miR-194-2/miR-192 forms gene clusters in chromosome 1 and 19, respectively. The miR-194-1/miR-215 loci is expressed lowly in mouse liver[38]. In mouse liver, we found prominent peaks of DHSs, HNF4α, Pol2 and H3K4me3 located approximately 1.6 kb upstream of the miR-194-2. Thus, we PCR cloned a 1973 bp fragment (-1694 to +279 bp) of the promoter of the mouse miR-194-2/miR-192 cluster into the MluI/XhoI sites of pGL3-Basic reporter vector, which was named as pGL3-mmiR-194-2. The sequences of all promoters used for PCR cloning of miR promoters are listed in Supplemental Materials.

A previous study indicates that a single approximately 2.4 kb transcript contains the human pri-miR-194-2 transcript and a 5’ AK092802 CDNA. In the human colon cancer Caco-2 cells, HNF1α binds to a HNF1 site located between -70 and -52 bp upstream of the transcription start site (TSS) of AK092802 to activate the promoter of pri-miR-194-2[39]. The upstream genomic region close to the TSS of pri-miR-194-2 contains some highly conserved regions between humans and mice[40]. We found prominent peaks of DHSs, HNF4α, Pol2 and H3K4me3 within a 350 bp fragment from -329 to +21 bp upstream of the TSS of AK092802, which was PCR cloned into the KpnI/MluI sites of pGL3-Basic reporter vector and named as pGL3-hmiR-194-2-Dist. Genomic DNA prepared from C57BL/6 mouse liver and human embryonic kidney 293 cells were used as the PCR templates. In addition to the prominent peaks of HNF4α and Pol2 identified in approximately 2 kb upstream of the human miR-194-2 loci, smaller peaks of HNF4α and Pol2 were also found in the proximal promoter of human miR-194-2. A DNA fragment of 417 bp that contains 5’ Kpn1 and 3’ Hind III restriction sites as well as a wild-type and mutant 405 bp human miR-194-2 promoter (from -405 to +1) were synthesized and verified by sequencing (GenScript United States Inc., Piscataway, NJ), and ligated into the KpnI/HindIII site in the pGL3-basic vector, which was named as pGL3-hmiR194-2-Pro and pGL3-hmiR194-2-TriM. The mutant 405-bp human miR-194-2 promoter had mutations of 3 putative HNF4-binding sites predicted by software of NHR-scan[38] and HNF4 Binding Site Scanner[39] (for DNA sequences see Supplemental Materials).

Generation of reporter construct for the mouse miR-802 promoter

We PCR cloned a 2 kb fragment of the mouse miR-802 promoter (-2004 to -1 bp) into the MluI/XhoI sites of pGL3-Basic to generate the reporter vector for mouse miR-802 promoter, which was named as pGL3-mmIR-802 Pro.

Determination of effect of HNF4α on the promoter activities of human and mouse miRs

Human hepatocellular adenoma HepG2 cells were maintained in D-MEM with 5% FBS. Cells were added to 96-well plates and grown to approximately 80% confluence. Plasmid DNA including pGL3 reporter vectors, the pRL-CMV luciferase (as control for transfection efficiency), pCDNA3-HNF4α and pCMV-CCAAT/enhancer-binding protein α (C/EBPα) (gift from Dr. Magnus Nord, Karolinska Institute), or pCDNA3 were complexed with Lipofectamine 3000 reagent (Invitrogen, Carlsbad, CA) and applied to individual wells, according to the manufacturer’s protocol. Transfected cells were lysed with passive lysis buffer (Promega) 24 h after transfection. Promoter activities of cell lysates were quantified by Dual-Glo™ luciferase assay (Promega) with the control values of pGL3-Basic vs pRL-CMV set at 1.0. To study the role of SP1 in mediating the transactivation of human miR-194-2 proximal promoter by HNF4α, the SP1 inhibitor mithramycin was added 1 h after transfection and cells were lysed 24 h after transfection for dual-luciferase assay.

Generation of reporter construct for the 3'UTR of mouse chromodomain helicase DNA binding protein 1 (Chd1) and H3f3 mRNAs

The chromatin remodeling factor Chd1 is required to maintain the open chromatin and pluripotency of mouse embryonic stem cells[40]. DNA sequence containing 48 bp of the 3'UTR of mouse Chd1 mRNA (NM_007690.3, 6708-6756, in bold), namely CTATGGTATGGCCTTT AATATAAAAACGTTACGTACACACTGTGATATA CGCGTA, and its antisense sequence AGCTTACGCG TATATACATCAGTGACTGTAACACGTTTTATATAA
GCCAATCA were synthesized by IDT. DNA sequence containing 48 bp of the 3’UTR of mouse H3F3b mRNA (NM_008211.3, 1593-1639, in bold), namely CTAAGTTA GTATCTTGAAGTTTATGACGTTGTTGA CTAATAGCGTA, and its antisense sequence AGCT TACGGTGATTGTCAACATAATTCGCTAATAA CTGTTAAAGTACTA were synthesized by IDT. The two sense and antisense oligos were annealed and ligated into the Spe I/HindIII site between the luciferase cDNA and SV40 polyA in pmIR-REPORT™ microRNA Expression Reporter Vector (Applied Biosystems/Ambion, Austin, TX), which was named pMIR-Chd1 and pMIR-H3f3, respectively. The correctness of pMIR-Chd1 and pMIR-H3f3 was verified by the unique restriction site (ACCGGT) for MluI that was introduced into the synthetic oligo.

**Determination of effect of miR-194 and miR-192 on the stability of mouse Chd1 and H3f3 3’-UTR using dual-luciferase assay**

HepG2 human hepatocellular adenoma cells were maintained in D-MEM with 5% FBS. Cells were added to 96-well plates and grown to approximately 80% confluence. Plasmid DNA including pmiR-Chd1 (or pmiR-H3f3), the pRL-CMV luciferase, and a synthetic mimic of miR-194/miR-192 (miScript miR-194/miR-192, QIAGEN Inc, Valencia, CA), or AllStars Negative Control siRNA (QIAGEN, as negative control for microRNAs) were co-transfected using Lipofectamine 2000 (Invitrogen, Carlsbad, CA), according to the manufacturer’s protocol of DNA-RNAi co-transfection. Transfected cells were lysed with passive lysis buffer (Promega) 24 h after transfection. Promoter activities of cell lysates were quantified by Dual-Glo™ luciferase assay (Promega) with the control values of pmiR-Chd1/pmiR-H3f3 vs pRL-CMV set at 1.0.

**Animal care and use statement:** The animal protocol was designed to minimize the pain or distress to the mice. Age-matched young-adult HNF4α Liv-KO mice and their wild-type littermates were fed rodent chow (#8064, Teklad; Harlan, Indianapolis, IN). Mice were housed at an ambient temperature of 22 °C with alternating 12-h light/dark cycles and allowed water and feed ad libitum.

**Statistical analysis**

Data are presented as mean ± SE. Differences between two groups were determined using Student’s t-test. For multiple comparisons, analysis of variance was performed, followed by the Student-Newman-Keuls Method in SigmaPlot 12.5, with significance set at P < 0.05.

**RESULTS**

**Results of microarray analysis of microRNAs in pooled young-adult male and female Hnf4α-LivKO mouse livers**

Generally, there were few gender differences in hepatic expression of microRNAs in mice (Figure 1), which is similar to that in rats[41]. Hepatic expression of most microRNAs remained unchanged (< 50% differential expression among the four pooled samples) in Hnf4α-LivKO mice (data not shown). However, Hnf4α-LivKO mouse livers had up- or down-regulation of a small portion of microRNAs that are important in regulating cell proliferation, differentiation, and apoptosis (Figure 1). Thirty microRNAs were found to have ≥ 50% differential expression among the four pooled samples, namely male WT and Hnf4α-LivKO as well as female WT and Hnf4α-LivKO mice. Fourteen microRNAs had > 50% lower expression in Hnf4α-LivKO mice than in WT mice (Figure 1A). Among them, the 4 liver-predominant microRNAs miR-194, miR-192, miR-215 and miR-193 were 71%, 72%, 70% and 70% lower, respectively, in Hnf4α-LivKO male mouse livers than WT males (WTM). miR-101a and 101b, which are expressed moderately in liver, also decreased > 50% in male Hnf4α-LivKO mice. Female Hnf4α-LivKO mouse livers had very similar lower expression of these microRNAs than WT females (Figure 1A). In contrast, two microRNAs that are expressed highly in liver, namely miR-122 and miR-26a[42,43], had less than 50% differential expression in all the groups (Supplemental Table 1).

In contrast to the down-regulation of certain liver-predominant microRNAs, hepatic expression of 16 microRNAs were > 50% higher in Hnf4α-LivKO mice than in WT mice (Figure 1B). The tumor-suppressor miR-34a[44] was expressed at relatively low levels in wild-type mouse liver, but was induced 2.6 fold in male Hnf4α-LivKO mouse livers. Tumor-suppressor miR-29b and miR-195[45] were highly and modestly expressed in WT mouse livers, respectively, and were 90% and 70% higher, respectively, in male Hnf4α-LivKO mouse livers than WTM (Figure 1B).

The oncogenic miR-17-92 locus encodes a cluster of 7 microRNAs transcribed as a single primary transcript[46]. Four miR-17-92 members, namely miR-17, 19a, 19b and 20 tended to be higher in Hnf4α-LivKO mouse liver (Figure 1B).

**Verification of changes in hepatic microRNAs in male Hnf4α-LivKO mice by real-time PCR**

To verify the changes in microRNAs detected by microarray in the pooled liver samples, real-time PCR was used to quantify 12 microRNAs in individual samples from Hnf4α-LivKO mice (Figure 2). Because similar alterations of these microRNAs were found in male and female Hnf4α-LivKO mice (Figure 1), only individual male Hnf4α-LivKO liver samples were used in this study. The selection of these 12 microRNAs for verification was based on their relative expression levels (Supplemental Table 1) and their reported importance in cellular pathophysiology.

Compared to male WT mice, male Hnf4α-LivKO mice had markedly lower levels of miR-101b (7% of WT values), miR-192 (24%), miR-193a (24%), miR-194 (16%), miR-215 (59%) and miR-802 (33%) (Figure 2A-B), but higher levels of miR-29b (190%) and miR-34a (244%) (Figure 2C). In contrast, hepatic levels of miR-26a and miR-195 were similar between male WT and Hnf4α-LivKO mice (Figure 2C-D). Hepatic miR-122 was
modestly (30%) lower in male Hnf4α-LivKO mice than male WT mice (Figure 2D).

**DNA-binding of HNF4α in mouse liver and small intestine as well as the chromatin status of microRNAs in mouse liver**

To understand the mechanism of regulation of microRNA expression by HNF4α in mouse liver, we used IGV software to analyze the published genome-wide DNase-seq and ChIP-seq data on DNA-binding of HNF4α as well as the presence of DHSs, Pol2 and active (H3K4me3) and suppressing (H3K9me3 and H3K27me3) epigenetic signatures, in the loci of several microRNAs in mouse liver and/or small intestine. Consistent with their high expression in mouse liver and/or small intestine, consistent with their high expression in mouse liver, miR-192/miR-194-2 cluster, consistent with their high expression in mouse intestine[37].

Similarly, peaks of DHSs, HNF4α, Pol2 and H3K4me3 were also found in the gene loci of miR-193 and miR-802 (Figure 3D and E); however, the peaks were smaller and less sequential compared to those in the gene loci of miR-122, miR-194-2/miR-192 and miR-101b. In contrast, no clear peaks of H3K36me3 were found in regions that encode the mature transcripts of miR-193 and miR-802 (Figure 3D and E). Interestingly, the silencing mark H3K27me3 was found to span the whole locus of miR-802, whereas a peak of H3K9me3 was found 3' downstream of the miR-802 (Figure 3E). In summary, the data suggest that these five microRNAs might be directly regulated by HNF4α in mouse liver.

Much smaller peaks of HNF4α were found in the gene loci of miR-194-1/miR-215, miR26a-1 and miR26a-2, and DNA-binding of HNF4α was not associated with prominent peaks of Pol2 or H3K4me3 in mouse liver (Figure 4A-C). Conversely, although prominent peaks of DHSs, HNF4α, Pol2 and H3K4me3 were found in the miR-26b locus, the direction of HNF4α, Pol2 and H3K4me3 peaks was toward the upstream of miR-26b, rather than the transcription initiation of miR-26b (Figure 4D). These data suggest that HNF4α may not have a direct and/or important role in regulating hepatic expression of miR-194-1/miR-215, miR26a and miR26b. In contrast, large peaks of HNF4α were found in the distal and proximal promoter of the miR-194-1/miR-215 cluster in mouse small intestine (Figure 4A), suggesting that HNF4α may be important in regulating the high expression of the miR-194-1/miR-215 cluster in mouse small intestine[38].

It was reported that HNF4α binds to the proximal...
promoter of miR-29 a-b cluster in cultured mouse hepatocytes, and acute loss of HNF4α decreased the levels of miR-29a and miR-29b in isolated hepatocytes and livers from mice on a mixed background of Sv129/FVB [47]. However, only a small peak of HNF4α was found within 10 kb of the mouse miR-29 a-b loci in adult liver from C57BL/6 mice, and the small HNF4α peak was not associated with peaks of Pol2 or H3K4me3 (Figure 5A). In contrast, a larger peak of HNF4α was found in the promoter of the miR-29 a-b loci in the small intestine (Figure 5A). Thus, the role of HNF4α in regulating hepatic expression of miR-29 a-b cluster in mice may be strain and/or cell-context dependent.

Recent studies indicate that HNF4α directly regulates miR-124 and miR-134 in human liver, and down-regulation of HNF4α is associated with reduction of miR-124 and miR-134 in human HCC [48,49]. However, our microarray data showed that miR-124 and miR-134 were expressed very lowly in mouse liver, and Hnf4α deficiency had no effect on hepatic expression of miR-124 and miR-134 in mice (Supplemental Table 1). Consistently, there were no clear peaks of HNF4α, Pol2, or the activating signatures H3K4me3, H3K36me3 in the loci of the 3 mouse miR-124 genes, namely miR-124a-1, 124a-2 and 124a-3, in livers of C57BL/6 mice (Figure 5B-D). In contrast, large peaks of the silencing mark H3K27me3 were found in the whole loci of miR-124a-1, 124a-2 and 124a-3, and a large peak of H3K9me3 was found in the miR-124a-1 locus (Figure 5B-D). Similarly, there were no prominent peaks of DHSs, Hnf4α, Pol2, H3K4me3, or H3K36me3 detected in the locus of mouse miR-134 gene, where the silencing mark H3K9me3 was found (Figure 5E). Taken together, the very low signal of miR-124s and miR-134 in the microarray data (Supplemental Table 1) and the lack of activating epigenetic signatures but enrichment of silencing epigenetic signatures in the loci of miR-124s and miR-134 strongly indicate that miR-124 and miR-134 are expressed very lowly in adult mouse liver, and they are not HNF4α-targe genes in mouse liver. Thus, there appear to be species differences between humans and mice in hepatic basal expression and regulation of miR-124 and miR-134 by HNF4α.

Because our data of microRNA expression and analysis of public database for ChIP-seq strongly suggest that HNF4α has a critical direct role in maintaining hepatic expression of miR-194/miR-192 and miR-101b in mice, we further examined DNA-binding of HNF4α and chromatin status in the gene loci of miR-194/miR-192 and miR-101-2 in the human hepatoma HepG2 cells using the data from public database (Figure 6). Very similar to the mouse miR-194-2/miR-192 cluster (Figure 3B), starting from approximately 2 kb upstream of the
human pri-miR-194-2, prominent sequential peaks of DHSs, HNF4α, Pol2, H3K4me3 and H3K36me3 were identified in the human miR-194-2/miR-192 gene cluster in HepG2 cells (Figure 6A). Very similar to the mouse miR-101b, the human miR-101-2 gene body is located in the intron8-9 of the RCL1 gene, and clear (but weaker than miR-194-2) sequential peaks of HNF4α, Pol2, H3K4me3 and H3K36me3 were identified in the intron5-6 of RCL1 (Figure 6B). These data strongly suggest that HNF4α may also have a direct critical role in regulating hepatic expression of miR-194-2/miR-192 and miR-101-2 in humans. In contrast, there were no clear peaks of HNF4α, Pol2, or H3K4me3 (Figure 6C) in the miR-122 locus which is known to be silenced in HepG2 cells\[42\].

**Regulation of the mouse and human miR-194-2/miR-192 gene cluster by HNF4α.**

Hepatic expression of miR-194 is markedly downregulated in mice null for Hnf1α\[36\], a down-stream target
of HNF4α. In small intestine, miR-194 is transcriptionally up-regulated by Hnf1α [37]. Hepatic mRNA expression of Hnf1α decreased modestly in Hnf4αα-LivKO mice [1]. We found that HNF1α and HNF4α modestly activated the reporter for the mouse miR-194-2/miR-192 gene cluster 1.5 and 2.8 fold, respectively, and they synergistically activated mouse miR-194-2/miR-192 promoter 7.5 fold (Figure 7A). ChIP-seq results showed that HNF4α bound strongly to the distal promoter but weakly to the proximal promoter of human miR-194-2/miR-192 cluster (Figure 6A). To determine the role of HNF4α in regulating the miR-194-2/miR-192 gene cluster in humans, we generated reporter vectors for the distal and proximal promoters of human miR-194-2/miR-192 cluster. Surprisingly, HNF4α only modestly activated the distal promoter 3 fold, but very strongly activated the proximal promoter of human miR-194-2/miR-192 cluster by 200 fold (Figure 7B). To identify the critical cis-elements responsible for the very strong transactivation of this proximal promoter by HNF4α, we engineered luciferase reporter constructs for the mutated 400 bp proximal promoter of human miR-194-2. Surprisingly, mutations of the 3 putative HNF4-binding sites (HNF4-RE) within the 400 bp miR-194-2 promoter had little effects on the transactivation of this promoter by HNF4α (Figure 7C). HNF4α can transactivate the human p21 promoter via physically interacting with the general transcription factor SP1, independent of DNA-binding of HNF4α, because the S304D mutant of HNF4α which has markedly decreased DNA-binding activity [50], is equally active as the WT HNF4α in transactivating p21 [51]. Thus, we tested the hypothesis that HNF4α can DNA-binding-independently transactivate the proximal human miR-194-2 promoter via interacting with SP1. We found that mithramycin, a widely used SP1 inhibitor [52], dramatically suppressed the HNF4α-transactivation of both the WT and HNF4RE-mutant miR-194-2 promoter by 94% and 95%, respectively (Figure 7C). Moreover,
The S304D-mutant of HNF4α was equally active as the WT HNF4α in transactivating the proximal human miR-194-2 promoter (Figure 7D). Taken together, these data strongly indicate that HNF4α can DNA-binding-independently transactivate the proximal human miR-194-2 promoter via interacting with SP1.

**Regulation of mouse miR-101b and human miR-101-2 promoters by HNF4α**

The mouse miR-101b promoter was moderately active in HepG2 cells (Figure 8A), whereas the human miR-101-2 promoter was largely inactive in HepG2 cells (Figure 8B). C/EBPα, a liver-enriched transcription factor, plays a key role in regulating liver-specific gene expression. The expression of C/EBPα is low in HepG2 cells, and re-expression of C/EBPα in HepG2 cells can reactivate certain liver-specific genes[53]. Our analysis of published ChIP-seq data for C/EBPα in mouse liver (GSM1037657) showed that C/EBPα bound to the miR-101b promoter, located in the Intron5-6 of Rd1, in close proximity to the proximal human miR-194-2 promoter.
Figure 6 Analysis of DNAse-I hypersensitive sites as well as DNA-binding of HNF4α, RNA polymerase II (Pol2), and methylated histones to loci of miR-194-2/miR-192 (A), miR-101-2 (B) and miR-122 (C) in human hepatoma HepG2 cells. Data of DHSs (determined by DNAse-seq) and DNA-binding of proteins (determined by ChIP-seq) were retrieved from the public database of GEO DataSets and visualized in the IGV software. The peak values/ranges for each mark were shown in square brackets or under the line mark. DHSs: DNAse-I hypersensitive sites; H3K36me3: H3 trimethylation at lysine-36; H3K4me3: H3 trimethylation at lysine-4; H3K27me3: H3 trimethylation at lysine-27; H3K9me3: H3 trimethylation at lysine-9; Pol2: Polymerase 2; HNF4α: Hepatocyte nuclear factor 4 alpha; ChIP-seq: Chromatin immunoprecipitation-sequencing; IGV: Integrative genomics viewer.

Figure 7 Activation of mouse (A) and human (B-D) miR-194-2/miR-192 promoter by HNF4α. Human hepatoma HepG2 cells were transfected with firefly luciferase vectors containing wild-type and mutant miR-194-2 promoter, pRL-CMV, and an expression vector for HNF4α/HNF1α. Dual-luciferase reporter assay was conducted 24 h after transfection. The y-axis represents relative luciferase activity for microRNA promoter normalized by the renilla luciferase. \( n = 4 \), Mean ± SE. \(^a_p < 0.05\) compared to vector control; \(^c_p < 0.05\) compared to HNF4α alone group. HNF4α: Hepatocyte nuclear factor 4 alpha.
Regulation of mouse miR-802 promoter by HNF4α

Different from miR-101, HNF4α had no effect on the 2 kb mouse miR-802 promoter, and HNF4α did not enhance the transactivation of the miR-802 promoter by C/EBPα in HepG2 cells (Figure 8C).

Regulation of mouse Chd1 and H3f3 by miR-194 and miR-192

TargetScan was used to identify potential targets of liver-predominant microRNAs down-regulated in Hnf4α-LivKO livers. miR-192/215 and miR-194 have a perfect match (8 mer) and very high context score percentile of 96%-99% with human and mouse histone H3F3b (H3.3b) and Chd1, respectively, indicating a very high likelihood of inhibition (Table 1). Therefore, we generated luciferase reporters for the 3'UTR of H3.3 and Chd1. Results of dual luciferase assay showed that miR-194 and miR-192 significantly decreased the luciferase activity for the 3' UTR of Chd1 (Figure 9A) and H3.3 (Figure 9B) by 37% and 36%, respectively, in HepG2 cells.

DISCUSSION

The present study demonstrates that Hnf4α is essential for hepatic expression of certain liver-predominant microRNAs, namely miR-101, miR-192, miR-193 and miR-194. HNF4α transactivates these miRs via direct DNA-binding to the promoters and/or interacting with the general transcription factor SP1. These miRs target essential epigenetic modifiers, such as EZH2 (by miR-101), histone H3.3 (by miR-192) and Chd1 (by miR-194) (Figure 10).

The present data provide the first evidence that HNF4α is essential for hepatic expression of miR-194 in mice, and likely in humans. In both mice and humans, miR-194 is expressed highly in kidney and GI tract including liver and small intestine[37]. The tissue distribution of miR-194 parallels that of HNF4α. In liver, miR-194 signals are detected in hepatocytes but not in non-parenchymal cells, and miR-194 is down-regulated during dedifferentiation of hepatocytes[54]. miR-194 inhibits the metastasis of mesenchymal-like liver cancer cells. Moreover, ChIP-seq results demonstrate direct binding of HNF4α to the distal and proximal promoters of mouse and human miR-194-2 (Figure 3B and 6A). Furthermore, results of reporter assays indicate that HNF4α potently activates the promoter of mouse and human miR-194-2/miR-192 gene cluster (Figure 7). Taken together, these data strongly indicate that HNF4α plays a key role in maintaining hepatic expression of miR-194 in mice and humans.

Two recent studies of mice with inducible knockout of Hnf4α demonstrate that acute loss of Hnf4α in adult mouse liver triggers extensive hepatocyte proliferation, hepatomegaly, and increased HCC[55-57]. The increased intestinal cell proliferation in mice with specific loss of Hnf4α in the adult intestinal epithelium is ascribed to the activation of the Wnt/beta-catenin system[58], miR-194 negatively control expression of frizzled-6, which activates the beta-catenin pathway[36]. Therefore, Hnf4α may

HNF4α. Moreover, putative C/EBP binding sites are highly enriched in the human miR-101-2 promoter, predicted by the Alibaba2 software. We found that HNF4α and C/EBPα activated the mouse miR-101b promoter 6.2 and 8.9 fold, respectively, and they synergistically activated the miR-101b promoter 19 fold in HepG2 cells (Figure 8A). Similarly, HNF4α and C/EBPα activated the human miR-101-2 promoter 11 and 33 fold, respectively, and they synergistically activated the miR-101-2 promoter 65 fold in HepG2 cells (Figure 8B).

Figure 8 Activation of (A) mouse miR-101b, (B) human miR-101-2, and (C) mouse miR-802 promoter by HNF4α. Human hepatoma HepG2 cells were transfected with firefly luciferase vectors containing microRNA promoter, pRL-CMV, and an expression vector for HNF4α and/or C/EBPα. Dual-luciferase reporter assay was conducted 24 h after transfection. The Y-axis represents relative luciferase activity for microRNA promoter normalized by the renilla luciferase. n = 4, Mean ± SE. "a" P < 0.05 compared to vector control; "c" P < 0.05 compared to HNF4α alone group. HNF4α: Hepatocyte nuclear factor 4 alpha; C/EBPα: CCAAT/enhancer-binding protein α.
The chromatin remodeling factor CHD1 is required to maintain the open chromatin and pluripotency of mouse embryonic stem cells\(^\text{(40)}\). CHD1 is required for chromatin incorporation of the histone variant H3.3, which is gene-rally associated with active genes\(^\text{(59)}\). However, CHD1 may also repress gene expression via association with HDACs\(^\text{(60)}\). Overexpression of HNF4\(\alpha\) in hepatoma cells dramatically decreased the "stemness" gene expression and the percentage of cancer stem cells in HCC\(^\text{(8)}\); however, the underlying mechanism is unknown. HNF4\(\alpha\), via regulating miR-194, might inhibit stemness gene expression by targeting the chromatin remodeling factor CHD1, which deposits the unmodified or altered histone H3.3 into chromatin and increases the stemness of Hnf4\(\alpha\)-LivKO hepatocytes.

The present data indicate that Hnf4\(\alpha\) is essential for hepatic expression of miR-192, and the histone variant H3.3 is a direct target of miR-192. Thus, down-regulation of miR-192 may be the underlying mechanism of hepatic induction of H3.3 in young-adult Hnf4\(\alpha\)-LivKO mice\(^\text{(21)}\).

The replacement H3 variant H3.3 is encoded by two genes termed H3.3A and H3.3B, both code for the same amino acid sequence, but differ in nucleotide sequences\(^\text{(61)}\). H3.3 is the exclusive substrate for replication-independent deposition, which provides a mechanism for the immediate activation of genes that are silenced by histone modification\(^\text{(62,63)}\). H3.3 is important in epigenetic memory\(^\text{(64)}\). H3.3/H2A.Z double variant-containing nucleosomes mark "nucleosome-free regions" of active promoters and other regulatory

---

**Table 1  Targeting of human and mouse genes by liver-predominant microRNAs predicted by TargetScan**

| Predicted pairing of target region (top) and microRNA (bottom) | Seed match | Context score percentile |
|---------------------------------------------------------------|------------|------------------------|
| Position 1085-1091 of human H3F3B 3′UTR miR-192/215 | 5′...AUUUACUGAGUUUAGGUUCAA... | 8 mer | 96 |
| Position 1064-1070 of mouse H3F3b 3′UTR miR-192/215 | 5′...UCCUUAUGGANUUAGGUUCAA... | 8 mer | 99 |
| Position 1109-1115 of mouse CHD1 3′UTR miR-194 | 5′...GACUUUAAUAAACUGUCAA... | 8 mer | 99 |
| Position 1100-1106 of mouse CHD1 3′UTR miR-194 | 5′...GCUUUAAUAAACUGUCAA... | 8 mer | 99 |

3′UTR: Untranslated regions.

---

**Figure 9** Effects of miR-194 and miR-192 on the activities of luciferase reporter vectors for the 3′UTR of mouse Chd1 and H3f3. Human hepatoma HepG2 cells were transfected with plasmid DNA including pmiR-Chd1 (or pmiR-H3f3), the pRL-CMV luciferase, and a synthetic mimic of miR-194/miR-192, or AllStars Negative Control siRNA (as negative control for microRNAs) using Lipofectamine 2000. Dual-luciferase reporter assay was conducted 24 h after transfection. The Y-axis represents relative luciferase activity for the 3′UTR of Chd1 or H3f3 normalized by the renilla luciferase. \(n=4\), Mean ± SE. *P < 0.05 compared to control (AllStars Negative Control siRNA). 3′UTR: Untranslated regions.

**Figure 10** Diagram that illustrates the regulation of hepatic microRNA expression by Hnf4\(\alpha\) in mouse liver. HNF4\(\alpha\): Hepatocyte nuclear factor 4 alpha; C/EBP\(\alpha\): CCAAT/enhancer-binding protein \(\alpha\).

---

Inhibit cell proliferation through the miR-194→frizzled-6→beta-catenin signaling pathway.

The chromatin remodeling factor CHD1 is required to maintain the open chromatin and pluripotency of mouse embryonic stem cells\(^\text{(40)}\). CHD1 is required for chromatin incorporation of the histone variant H3.3, which is generally associated with active genes\(^\text{(59)}\). However, CHD1 may also repress gene expression via association with HDACs\(^\text{(60)}\). Overexpression of HNF4\(\alpha\) in hepatoma cells dramatically decreased the "stemness" gene expression and the percentage of cancer stem cells in HCC\(^\text{(8)}\); however, the underlying mechanism is unknown. HNF4\(\alpha\), via regulating miR-194, might inhibit stemness gene expression by targeting the chromatin remodeling factor CHD1, which deposits the unmodified or altered histone H3.3 into chromatin and increases the stemness of Hnf4\(\alpha\)-LivKO hepatocytes.

The present data indicate that Hnf4\(\alpha\) is essential for hepatic expression of miR-192, and the histone variant H3.3 is a direct target of miR-192. Thus, down-regulation of miR-192 may be the underlying mechanism of hepatic induction of H3.3 in young-adult Hnf4\(\alpha\)-LivKO mice\(^\text{(21)}\).

The replacement H3 variant H3.3 is encoded by two genes termed H3.3A and H3.3B, both code for the same amino acid sequence, but differ in nucleotide sequences and gene organization\(^\text{(61)}\). H3.3 is the exclusive substrate for replication-independent deposition, which provides a mechanism for the immediate activation of genes that are silenced by histone modification\(^\text{(62,63)}\). H3.3 is important in epigenetic memory\(^\text{(64)}\). H3.3/H2A.Z double variant-containing nucleosomes mark "nucleosome-free regions" of active promoters and other regulatory events.
regions. Deposition of H3.3 can rapidly derepress gene silencing. Taken together, Hnf4α directly regulates miR-192, and the down-regulation of miR-192 in Hnf4α-LivKO livers may be the underlying mechanism of hepatic induction of H3.3, which contributes to the marked alteration of epigenome and transcriptome in Hnf4α-LivKO livers.

The present study indicates that Hnf4α is required for hepatic expression of the tumor-suppressor mir-101. mir-101 is predominantly expressed in the liver, and mir-101 is down-regulated in HCC and mir-101 directly represses EZH2, a protooncogene that silences the expression of tumor-suppressors via H3K27me3. Down-regulation of mir-101 in Hnf4α-LivKO mouse livers might be the underlying mechanism of induction of EZH2 and increased H3K27me3 observed previously.

The present data indicate that Hnf4α is important for hepatic basal expression of the tumor-suppressor mir-193a. mir-193a and miR-365 closely cluster in chromosome 11 in mice. The tumor-suppressor mir-193a is down-regulated in the majority of HCC in humans and mir-193a prevents the resistance of HCC to 5-fluorouracil via repressing the expression of serine/arginine-rich splicing factor 2 (SRSPF2). Through maintaining hepatic expression of mir-193a, Hnf4α might regulate expression of SRSPF2 and the splicing of transcripts in liver. Interestingly, mir-193a also targets directly Wilms’ tumor protein 1 (WT1). WT1 is overexpressed in cirrhotic liver and HCC, and induction of WT1 down-regulates Hnf4α expression in liver. The putative feedback regulatory loop of Hnf4α-mir-193a-WT1 and its significance in liver cirrhosis and carcinogenesis warrant further investigation.

The present data provide the first evidence that Hnf4α is important for hepatic expression of mir-802 (Figure 2). Results of reporter assay (Figure 8C) suggest that Hnf4α may indirectly regulate hepatic miR-802 expression via C/EBPα, whose DNA-binding activity decreased in Hnf4α-LivKO mice and human hepatoma cells. Interestingly, the miR-802 locus is marked with both the activating signature H3K4me3 and the silencing signature H3K27me3, a feature of bivalent chromatin which allows a low basal expression but timely activation of developmentally-regulated genes. Hnf1β is a direct target of mir-802, and Hnf1β is overexpressed in adult Hnf4α-LivKO mouse livers. In mouse liver, mir-802 is expressed at 10-fold higher levels in hepatocytes than non-hepatocytes. In contrast, Hnf1β is strongly expressed in cholangiocytes but weakly in hepatocytes, and Hnf1β plays a key role in bile-duct morphogenesis and glucose homeostasis. Thus, the putative Hnf4α-C/EBPα-mir-802-Hnf1β pathway might play a role in controlling cell-specific expression of HNF1β and liver morphogenesis during liver development.

The tumor-suppressor microRNAs miR-34a, miR-192, miR-215 and miR-194 are all p53-inducible microRNAs. The induction of the p53-target gene p21 in Hnf4α-null mouse livers suggests that p53 is activated by Hnf4α deficiency, which may contribute to the induction of the p53-target mir-34a and miR-29b (Figure 2B). However, hepatic expression of other p53-target microRNAs miR-192, miR-215 and miR-194 are markedly down-regulated in Hnf4α-LivKO mice. It is interesting that Hnf4α can transactivate two p53-target genes, p21 and miR-194 (Figure 7), independent of DNA-binding of Hnf4α to the promoter. The AMP-activated protein kinase (AMPK) phosphorylates Hnf4α at S304, resulting in a marked decrease in the DNA-binding activity and decreased transactivation of apolipoprotein C3. AMPK suppresses lipogenesis and carcinogenesis in liver and may indirectly regulate hepatic miR-802. Regulation of hepatic microRNA by HNF4α suggests that p53 is activated by HNF1β plays a key role in bile-duct morphogenesis and glucose homeostasis.

The liver-specific miR-122 is important in regulating hepatic cholesterol and lipid metabolism, and down-regulation of miR-122 contributes to HCC malignancy. Hnf4α can directly activate the expression of miR-122 in mouse liver. However, knockdown of Hnf4α does not affect the high expression of miR-122 in a HCC cell line, although miR-122 expression correlates strongly with Hnf4α. In contrast, hepatic miR-122 expression is regulated by Hnf1α. The moderate down-regulation of miR-122 in Hnf4α-LivKO mouse livers parallels the moderate decrease of Hnf1α in these mice. Taken together, these data suggest that HNF4α has a positive but limited role in regulating hepatic expression of miR-122.

The present study demonstrates species differences between humans and mice regarding the interaction of HNF4α with miR-regulated inflammatory and carcinogenic pathways in the liver.

Our previous study found that Hnf4α deficiency in...
young-adult mice causes marked alteration of histone modifications, which is associated with induction of epigenetic modifiers such as EzH2 and histone H3.3.\textsuperscript{20}

However, ChIP-seq data reveal no direct binding of Hnf4\(\alpha\) to these epigenetic modifiers in adult mouse livers, suggesting that these epigenetic modifiers may not be directly regulated by Hnf4\(\alpha\). The present study provides the first evidence of the essential role of Hnf4\(\alpha\) in maintaining hepatic expression of certain microRNAs, including miR-101, miR-192, miR-193a, miR-194 and miR-802. These microRNAs target certain key proteins in gene regulation and epigenetic modifications, such as WT1 (by miR-193a),\textsuperscript{7,19} HNF1\(\beta\) (by miR-802),\textsuperscript{19} CHD1 (by miR-194) (Figure 9), Ezh2 (by miR-101),\textsuperscript{19} SRF2 (by miR-193a),\textsuperscript{19} and histone H3.3 (by miR-192) (Figure 9). Establishment and maintenance of hepatic expression of these microRNAs by Hnf4\(\alpha\) may play a key role in the indirect regulation of hepatic transcriptome and epigenome by Hnf4\(\alpha\) (Figure 10).

**ACKNOWLEDGMENTS**

The authors would like to thank the members of Sequencing Core at SUNY Upstate Medical University for their technical support.

**COMMENTS**

**Background**

Hepatocyte nuclear factor 4 alpha (HNF4\(\alpha\)) is a liver-enriched master regulator of liver development and function. HNF4\(\alpha\) plays a key role in regulating hepatic transcriptome and epigenome. However, little was known about the role of HNF4\(\alpha\) in regulating hepatic expression of microRNAs, essential modulators of the transcriptome and epigenome. Additionally, HNF4\(\alpha\) deficiency causes marked induction of a large number of genes in mouse liver; however, the mechanism of suppression of hepatic gene expression by HNF4\(\alpha\) remains poorly understood.

**Research frontiers**

Previous studies demonstrate that HNF4\(\alpha\) regulates hepatic expression of miR-122, miR-124 and miR-29.

**Innovations and breakthroughs**

This is the first study to use microarray and liver-specific knockout mice to determine the genome-wide role of HNF4\(\alpha\) in the regulation of hepatic expression of microRNAs in mice. The key changes in hepatic microRNA expression induced by HNF4\(\alpha\) deficiency were verified by real-time polymerase chain reaction. Moreover, hepatic microRNA expression were correlated with chromatin accessibility as well as DNA-binding of HNF4\(\alpha\), RNA polymerase II, and activating/silencing epigenetic signatures to determine the role of HNF4\(\alpha\) in regulating hepatic expression of these microRNAs. The novel key role of HNF4\(\alpha\) in regulating liver-predominant expression of miR-101-2/miR-101b and the miR-194-2/miR-192 cluster was confirmed by lucerase reporter assay.

**Applications**

Results from this study uncover species differences and similarities between humans and mice in the role of HNF4\(\alpha\) in regulating hepatic expression of certain important microRNAs. Such novel knowledge will help understand the role of HNF4\(\alpha\) in post-transcriptional regulation of gene expression and maintenance of the normal epigenome and physiology in mouse and human liver.

**Terminology**

Epigenetic signatures/marks are modifications of the genome that do not change the underlying DNA sequence but can switch genes on and off and thus affect how cells express genes. Typical epigenetic signatures/marks include DNA methylation and histone modifications.

**Peer-review**

The study appears to be properly conducted and written. No major criticisms and/or weaknesses were noted.

**REFERENCES**

1. Lu H, Gonzalez FJ, Klaassen C. Alterations in hepatic miRNA expression of phase II enzymes and xenobiotic transporters after targeted disruption of hepatocyte nuclear factor 4 alpha. *Toxicol Sci* 2010; 118: 380-390 [PMID: 20935164 DOI: 10.1093/toxsci/kfq280]

2. Lu H. Crosstalk of HNF4\(\alpha\) with extracellular and intracellular signaling pathways in the regulation of hepatic metabolism of drugs and lipids. *Acta Pharm Sin B* 2016; 6: 393-408 [PMID: 27709085 DOI: 10.1016/j.apsb.2016.07.003]

3. Battle MA, Konopka G, Parviz F, Gaggel AL, Yang C, Sladek FM, Duncan SA. Hepatocyte nuclear factor 4alpha orchestrates expression of cell adhesion proteins during the epithelial transformation of the developing liver. *Proc Natl Acad Sci USA* 2006; 103: 8419-8424 [PMID: 16714383 DOI: 10.1073/pnas.0602461103]

4. Li J, Ning G, Duncan SA. Mammalian hepatocyte differentiation requires the transcription factor Hnf4\(\alpha\). *Genes Dev* 2000; 14: 464-474 [PMID: 10691738]

5. Kyrmizi I, Hatzis P, Katrakili N, Tronche F, Gonzalez FJ, Talalaimudi I. Plasticity and expanding complexity of the hepatic transcription factor network during liver development. *Genes Dev* 2006; 20: 2293-2305 [PMID: 16912278 DOI: 10.1101/gad.390096]

6. Hayhurst GP, Lee YH, Lambert G, Ward JM, Gonzalez FJ. Hepatocyte nuclear factor 4alpha (nuclear receptor 2A1) is essential for maintenance of hepatic gene expression and lipid homeostasis. *Mol Cell Biol* 2001; 21: 1393-1403 [PMID: 11158324 DOI: 10.1128/MCB.21.4.1393-1403.2001]

7. Gonzalez FJ. Regulation of hepatocyte nuclear factor 4 alpha-mediated transcription. *Drug Metab Pharmacokinet* 2008; 23: 2-7 [PMID: 18305369 DOI: 10.2133/dmpk.23.2]

8. Yin C, Lin Y, Zhang X, Chen XY, Zeng X, Yue HY, Hou JL, Deng X, Zhang JP, Han ZG, Xie WF. Differentiation therapy of hepatocellular carcinoma in mice with recombinant adenovirus carrying hepatocyte nuclear factor 4alpha gene. *Hepatology* 2008; 48: 1528-1539 [PMID: 18925631 DOI: 10.1002/hep.22210]

9. Ning BF, Ding J, Yin C, Zhong W, Wu K, Zeng X, Yang W, Chen XY, Zhang JP, Xiang CC, Deng X, Zhang JP, Han ZG, Xie WF. Hepatocyte nuclear factor 4 alpha suppresses the development of hepatocellular carcinoma. *Cancer Res* 2010; 70: 7640-7651 [PMID: 20876809 DOI: 10.1158/0008-5472.CAN-10-0824]

10. Inoue Y, Yu AM, Yim SH, MA X, Krausz KW, Inoue J, Xiang CC, Brownstein MJ, Eggertsen G, Björkhem I, Gonzalez FJ. Regulation of bile acid biosynthesis by hepatocyte nuclear factor 4alpha. *J Lipid Res* 2006; 47: 215-227 [PMID: 16264197 DOI: 10.1194/jlr.M500430-JLR200]

11. Inoue Y, Hayhurst GP, Inoue J, Mori M, Gonzalez FJ. Defective ureagenesis in mice carrying a liver-specific disruption of hepatocyte nuclear factor 4alpha (HNF4\(\alpha\)). HNF4\(\alpha\) regulates ornithine transcarbamylase in vivo. *J Biol Chem* 2002; 277: 25257-25265 [PMID: 11994307 DOI: 10.1074/jbc.M203126200]

12. Inoue Y, Yu AM, Inoue J, Gonzalez FJ. Hepatocyte nuclear factor 4alpha is a central regulator of bile acid conjugation. *J Biol Chem* 2004; 279: 2480-2489 [PMID: 14583614 DOI: 10.1074/jbc.M311015200]

13. Hwang-Verslues WW, Sladek FM. HNF4\(\alpha\)-role in drug metabolism and potential drug target? *Curr Opin Pharmacol* 2010; 10: 698-705 [PMID: 20833107 DOI: 10.1016/j.coph.2010.08.010]

14. Wiwi CA, Gute M, Waxman DJ. Sexually dimorphic P450 gene expression in liver-specific hepatocyte nuclear factor 4alpha-deficient mice. *Mol Endocrinol* 2004; 18: 1975-1987 [PMID: 15155787 DOI: 10.1210/me.2004-0129]
15 Wortham M, Czerwinski M, He L, Parkinson A, Wan YJ. Expression of constitutive androstane receptor, hepatic nuclear factor 4 alpha, and P450 oxidoreductase genes determines interindividual variability in basal expression and activity of a broad scope of xenobiotic metabolism genes in the human liver. Drug Metab Dispos 2007; 35: 1700-1710 [PMID: 17576804 DOI: 10.1124/dmd.107.016436]

16 Ryffel GU. Mutations in the human genes encoding the transcription factors of the hepatocyte nuclear factor (HNF)1 and HNF4 families: functional and pathological consequences. J Mol Endocrinol 2001; 27: 11-29 [PMID: 11463573 DOI: 10.1677/002700011]

17 Zhou Z, Kang X, Jiang Y, Song Z, Feng W, McClain CJ, Kang YJ. Preservation of hepatocyte nuclear factor-4alpha is associated with zinc protection against TNF-alpha hepatotoxicity in mice. Exp Biol Med (Maywood) 2007; 232: 622-628 [PMID: 17463158]

18 Lazarevich NL, Cheremnova OA, Varga EV, Ovchinnikov DA, Kudrjavtseva EI, Morozova OV, Fleishman DI, Engelhardt NV, Duncan SA. Progression of HCC in mice is associated with a downregulation in the expression of hepatocyte nuclear factors. Hepatology 2004; 39: 1038-1047 [PMID: 15057908 DOI: 10.1002/hep.20155]

19 Berasain C, Herrero JL, Garcia-Trevijano ER, Avila MA, Esteban JI, Mato JM, Prieto J. Expression of Wilms’ tumor suppressor in the liver with cirrhosis: relation to hepatocytic nuclear factor 4 and hepatocellular function. Hepatology 2003; 38: 148-157 [PMID: 12829997 DOI: 10.1053/hep.2003.50269]

20 Kang X, Zhong W, Liu J, Song Z, McClain CJ, Kang YJ, Zhou Z. Zinc supplementation reverses alcohol-induced steatosis in mice through reactivating hepatocyte nuclear factor-4alpha and peroxisome proliferator-activated receptor-alpha. Hepatology 2009; 50: 1241-1250 [PMID: 19637192 DOI: 10.1002/hep.20390]

21 Zhang Q, Lei X, Lu H. Alterations of epigenetic signatures in hepatocyte nuclear factor 4a. Methods for two-colour microarrays. J Cell Physiol 2007; 205: 472-480 [PMID: 16366746 DOI: 10.1002/jcp.20086]

22 Schmidt D, Wilson MD, Ballester B, Schwalie PC, Brown GD, Marshall A, Kutter C, Watt S, Martinez-Jimenez CP, Mackay S, Krützfeldt J, Liu Y, Xu D, Bateup SJ, Bolotin E, Kudrjavtseva EI, Morozova OV, Fleishman DI, Engelhardt NV, Lonjo S, Perozzi F, Sridharan R, Mason JS, Atkinson N, Oshlack A, Holmes M, Diyagama D, Holloway A, Smyth GK. A comparison of background correction methods for two-colour microarrays. Bioinformatics 2007; 23: 2700-2707 [PMID: 17720982 DOI: 10.1093/bioinformatics/btm412]

23 Eisen MB, Spellman PT, Brown PO, Botstein D. Cluster analysis and display of genome-wide expression patterns. Proc Natl Acad Sci U S A 1998; 95: 14863-14868 [PMID: 9843981 DOI: 10.1073/pnas.95.25.14863]

24 Vermeulen M, Mulder KW, Denissov S, Pijnappel WW, van Boven J, Varier CL, Balsevich MP, Stunnenberg HG, Mann M, Timmers HT. Selective anchoring of TFID to nucleosomes by trimethylation of histone H3 lysine 4. Cell 2007; 131: 58-69 [PMID: 17884155 DOI: 10.1016/j.cell.2007.08.016]

25 Kimura T, Rice KL, Castrillon DH, Li C, Auerbach J, Li T, Liu K. Histone modifications for human epigenome analysis. J Hum Genet 2013; 58: 439-445 [PMID: 23739122 DOI: 10.1038/jhg.2013.66]

26 Kolasisinska-Zwierz P, Down T, Latorre I, Liu T, Liu XS, Ahirger J. Differential chromatin marking of introns and expressed exons by H3K36me3. Nat Genet 2009; 41: 376-381 [PMID: 19182803 DOI: 10.1038/ng.322]

27 Pauler FM, Sloane MA, Huang R, Regha K, Koerner MV, Tamir I, Sommer A, Assodzi A, Jenuwein T, Barlow DP. H3K27me3 forms BLOCs over silent genes and intergenic regions and specifies a histone banding pattern on a mouse autosomal chromosome. Genome Res 2009; 19: 221-233 [PMID: 19047510 DOI: 10.1101/gr.080861.108]

28 Robinson JT, Thorvaldsdottir H, Winckler W, Guttman M, Landers ES, Getz G, Mestrov JP. Integrative genomics viewer. Nat Biotechnol 2011; 29: 24-26 [PMID: 21221095 DOI: 10.1126/nbt.1754]

29 Liu JI, Lin XJ, Yang XJ, Zhou L, He S, Zhuang SM, Yang J. A novel AP-1/miR-101 regulatory feedback loop and its implication in the migration and invasion of hepatoma cells. Nucleic Acids Res 2014; 42: 12041-12051 [PMID: 25260594 DOI: 10.1093/nat gku872]

30 Krützfeldt J, Rösch N, Haussler J, Manoharan M, Zavolan M, Stoffel M. MicroRNA-194 is a target of transcription factor 1 (Tcf1, HNF1a) in adult liver and controls expression of frizzled-6. Hepatology 2012; 55: 98-107 [PMID: 21887698 DOI: 10.1002/hep.24658]

31 Hino K, Tsuchiya K, Fukao T, Kiga K, Okamoto R, Kanai T, Watanabe M. Inducible expression of microRNA-194 is regulated by HNF-1alpha during intestinal epithelial cell differentiation. RNA 2008; 14: 1433-1442 [PMID: 18492795 DOI: 10.1261/rna.810208]

32 Sandelin A, Wasserman WW. Prediction of nuclear hormone receptor response elements. Mol Endocrinol 2005; 19: 595-606 [PMID: 15563547 DOI: 10.1210/me.2004-0101]

33 Bolotin E, Liao H, Ta TC, Yang C, Hwang-Verslues W, Evans JR, Jiang T, Sladek FM. Integrated approach for the identification of human hepatocyte nuclear factor 4alpha target genes using protein binding microarrays. Hepatology 2010; 51: 642-653 [PMID: 20054869 DOI: 10.1002/hep.23357]

34 Gaspar-Maia A, Alajem A, Poleso F, Sridharan R, Mason MJ, Heidersbach A, Ramalho-Santos J, McManus MT, Plath K, Mesheror E, Ramalho-Santos M. Chid regulates open chromatin and pluripotency of embryonic stem cells. Nature 2009; 460: 863-868 [PMID: 19587602 DOI: 10.1038/nature08212]

35 Cheung L, Gustavsson C, Norstedt G, Tollett-Egnell P. Sex-different and growth hormone-regulated expression of microRNA in rat liver. BMC Mol Biol 2009; 10: 13 [PMID: 19236699 DOI: 10.1186/1471-2199-10-13]

36 Jopling CL, Yi M, Lancaster AM, Lemon SM, Sarnow P. Modulation of hepatitis C virus RNA abundance by a liver-specific microRNA. Science 2005; 309: 1577-1581 [PMID: 16141076 DOI: 10.1126/science.1113290]

37 Kata J, Chivukula RR, O’Donnell KA, Wentzel EA, Montgomery CL, Hwang HW, Chang TC, Vivekandan P, Torbenson M, Clarke K, Mendell JR, Mendell JT. Therapeutic microRNA delivery suppresses tumorigenesis in a murine liver cancer model. Nat Genet 2009; 41: 1005-1017 [PMID: 19524505 DOI: 10.1038/ng.9401]
Lu H et al. Regulation of hepatic microRNA by HNF4α

Lowe SW, Cleave MA, Hannon GM. A microRNA component of the p53 tumour suppressor network. Nature 2007; 447: 1130-1134 [PMID: 17554337 DOI: 10.1038/nature09399]

Xu T, Zhu Y, Xiong Y, Ge YY, Yun JP, Zhang SM. MicroRNA-195 suppresses tumorigenesis and regulates G1/S transition of human hepatocellular carcinoma cells. Hepatology 2009; 50: 113-121 [PMID: 19441017 DOI: 10.1002/hep.22291]

Yin C, Wang PQ, Xu WP, Yang Z, Zhang Q, Ning BF, Zhang P, Zhou WP, Xie WF, Chen WS, Zhang X. Hepatocyte nuclear factor-4α reverses malignancy of hepatocellular carcinoma through regulating miR-134 in the DLK1-DIO3 region. Hepatology 2013; 58: 1964-1976 [PMID: 23775631 DOI: 10.1002/hep.26573]

Hwang-Verslues WW, Sladek FM. Nuclear receptor hepatocyte nuclear factor 4α competes with oncoprotein c-Myc for dimer formation and decreasing protein stability. J Mol Endocrinol 2009; 52: 2148-2157 [PMID: 20979124 DOI: 10.1530/JME.0b013e3181b707e0]

Su H, Yang JR, Xu T, Huang J, Xu L, Yuan Y, Zhang SM. MicroRNA-101, down-regulated in hepatocellular carcinoma, promotes apoptosis and suppresses tumorigenicity. Cancer Res 2009; 69: 1135-1142 [PMID: 19155302 DOI: 10.1158/0008-5472.CAN-08-2886]

Friedman JM, Liang G, Liu CC, Wolff EM, Tsai YC, Ye W, Zhou X, Jones PA. The putative tumor suppressor microRNA-101 modulates the cancer epigenome by repressing the polycomb group protein EZH2. Cancer Res 2009; 69: 6293-6299 [PMID: 19653671 DOI: 10.1158/0008-5472.CAN-08-0907]

Orsi GA, Couble P, Lopin B. Epigenetic and replacement roles of histone variant H3.3 in development and reproduction. Int J Dev Biol 2009; 53: 231-243 [PMID: 19412883 DOI: 10.1387/ijdb.082653go]

Ng RK, Gorden JB. Epigenetic memory of an active gene state depends on histone H3.3 incorporation into chromatin in the absence of transcription. Nat Cell Biol 2008; 10: 102-109 [PMID: 18066050 DOI: 10.1038/ncb1674]

Jin C, Cang Z, Wei G, Cui K, Peng W, Zhao K, Felsenfeld G. H3.3/H2A.Z double variant-containing nucleosomes mark ‘nucleosome-free’ regions of active promoters and other regulatory regions. Nat Genet 2009; 41: 941-945 [PMID: 19633671 DOI: 10.1038/ng.409]

Akdemir K, Hennikoff S. Histone H3 variants specify modes of chromatin assembly. Proc Natl Acad Sci USA 2002; 99 Suppl 4: 16477-16484 [PMID: 12177448 DOI: 10.1073/pnas.172403699]

Su H, Yang JR, Xu T, Huang J, Xu L, Yuan Y, Zhang SM. MicroRNA-101, down-regulated in hepatocellular carcinoma, promotes apoptosis and suppresses tumorigenicity. Cancer Res 2009; 69: 1135-1142 [PMID: 19155302 DOI: 10.1158/0008-5472.CAN-08-2886]

Huang VF, Varanasi US, Yang W, Leff T. AMP-activated protein kinase regulates HNF4α4phi alpha transcriptional activity by inhibiting dimer formation and decreasing protein stability. J Biol Chem 2003; 278: 27495-27501 [PMID: 12740371 DOI: 10.1074/jbc.M304112200]

Hwang-Verslues WW, Sladek FM. Nuclear receptor hepatocyte nuclear factor 4α competes with oncoprotein c-Myc for control of the p21/WAF1 promoter. Mol Endocrinol 2008; 22: 78-90 [PMID: 17885207 DOI: 10.1210/me.2007-0298]

Blume SW, Snyder RC, Ray R, Thomas S, Koller CA, Miller DM. Mithramycin inhibits SPI binding and selectively inhibits transcriptional activity of the dihydrofolate reductase gene in vitro and in vivo. J Clin Invest 1991; 88: 1613-1621 [PMID: 1834700 DOI: 10.1172/JCI115474]

Jover R, Fornal P, Gómez-Lechón MJ, Gröne HJ, Englert C, Martinez J, Kerjaschki D. Focal hepatocyte nuclear factor 4α target gene expression is lost in chemoresistance of human hepatocellular carcinoma cells. Hepatology 2012; 56: 278-286 [PMID: 22824738 DOI: 10.1002/hep.25497]

Su H, Yang JR, Xu T, Huang J, Xu L, Yuan Y, Zhang SM. MicroRNA-101, down-regulated in hepatocellular carcinoma, promotes apoptosis and suppresses tumorigenicity. Cancer Res 2009; 69: 1135-1142 [PMID: 19155302 DOI: 10.1158/0008-5472.CAN-08-2886]

Friedman JM, Liang G, Liu CC, Wolff EM, Tsai YC, Ye W, Zhou X, Jones PA. The putative tumor suppressor microRNA-101 modulates the cancer epigenome by repressing the polycomb group protein EZH2. Cancer Res 2009; 69: 6293-6299 [PMID: 19258506 DOI: 10.1158/0008-5472.CAN-08-3114]

Varambally S, Rao Q, Mani RS, Shankar S, Wang X, Ateeg B, Laxman B, Cao X, Jang Y, Ramnathan V, Brenner JC, Yu J, Kim JH, Han B, Tan P, Kumar-Sinha C, Lonigro RJ, Palanisamy N, Maher CA, Chinnaiyan AM. Genomic loss of microRNA-101 leads to overexpression of histone methyltransferase EZH2 in cancer. Science 2008; 322: 1695-1699 [PMID: 19008416 DOI: 10.1126/science.1165395]

Xu C, Liu S, Fu H, Li S, Tie Y, Zhu J, Rong B, Yin J, Sun Z, Zheng X. MicroRNA-193b regulates proliferation, migration and invasion in human hepatocellular carcinoma cells. Eur J Cancer 2010; 46: 2828-2836 [PMID: 20655737 DOI: 10.1016/j.ejca.2010.06.127]

Ma K, He Y, Zhang H, Fei Q, Niu D, Wang D, Ding X, Xu H, Chen X, Zhu J. DNA methylation-regulated mir-193a-3p dictates resistance of hepatocellular carcinoma to 5-fluorouracil via repression of SRSF2 expression. J Biol Chem 2012; 287: 5639-5649 [PMID: 22171060 DOI: 10.1074/jbc.M111.291229]

Gebeshuber CA, Kornauth C, Dong L, Sieng R, Seibler J, Reiss M, Tauber S, Blüthmann H, Wang S, Kain T, Böhme GA, Mollet MJ, Gröne HJ, Englert C, Martinez J, Kerjaschki D. Focal segmental glomerulosclerosis is induced by microRNA-193a and its downregulation of WT1. Nat Med 2013; 19: 481-487 [PMID: 23502960 DOI: 10.1038/nm.3142]

Perugorria MJ, Castillo J, Latasa MU, Gohi S, Segura V, Sangro B, Prieto J, Avila MA, Berasain C. Wilms’ tumor 1 gene expression in hepatocellular carcinosomas promotes cell differentiation and resistance to chemotherapy. Cancer Res 2009; 69: 1538-1537
Lu H et al. Regulation of hepatic microRNA by HNF4α

[PMID: 19190340 DOI: 10.1185/0008-5472.CAN-08-2545]

74 Voigt P, Tee WW, Reindberg D. A double take on bivalent promoters. Genes Dev 2013; 27: 1318-1336 [PMID: 23786261 DOI: 10.1101/gad.219626.113]

75 Kornfeld JW, Baitzel C, Kämper AC, Nicholls HT, Voigt MC, Kornfeld JW. Evidence for a role of microRNA-29 in the regulation of hepatic metabolism. J Biol Chem 2008; 283: 80-87 [PMID: 18011613 DOI: 10.1074/jbc.M708254200]

76 Cofflin C, Gresh L, Fiette L, Tronche F, Schütz G, Babinet C, Pontoglio M, Yavin M, Barra J. Bile system morphogenesis defects and liver dysfunction upon targeted deletion of HNF1beta. Development 2002; 129: 1829-1838 [PMID: 11934849]

77 Braun CJ, Zhang X, Saveleva I, Wolfs S, Moll UM, Schepeker T, Ørntoft TF, Andersen CL, Dobbelstein M. p53-Responsive microRNAs 192 and 215 are capable of inducing cell cycle arrest. Cancer Res 2006; 66: 6133-6140 [PMID: 17045744 DOI: 10.1158/0008-5472.CAN-06-1569]

78 Fullerston MD. AMP-activated protein kinase and its multifaceted regulation of hepatic metabolism. Curr Opin Lipidol 2016; 27: 172-180 [PMID: 26906549 DOI: 10.1097/MOL.0000000000000273]

79 Motoshima H, Goldstein BJ, Igata M, Araki E. AMPK and cell proliferation--AMPK as a therapeutic target for atherosclerosis and cancer. J Physiol 2006; 574: 63-71 [PMID: 16613876 DOI: 10.1113/jphysiol.2006.108324]

80 Mott JL, Kobayashi S, Bront PK, Gores GJ. mir-29 regulates Mcl-1 protein expression and apoptosis. Oncogene 2007; 26: 6133-6140 [PMID: 17404574 DOI: 10.1097/MOL.0b013e318014d4e5]

81 Park SY, Lee JH, Ha M, Nam JW, Kim VN. miR-29 miRNAs activate p53 by targeting p85 alpha and CDC42. Proc Natl Acad Sci USA 2007; 104: 15805-15810 [PMID: 17890317 DOI: 10.1073/pnas.0707628104]

82 Fabbi M, Garzon R, Cinminio A, Liu Z, Zanesi N, Callegari E, Liu S, Alder H, Costinean S, Fernandez-Cymering C, Volinia S, Guler G, Morrison CD, Chan KK, Marcucci G, Calin GA, Huebner K, Croce CM. MicroRNA-29 family reverts aberrant methylation in lung cancer by targeting DNA methyltransferases 3A and 3B. Proc Natl Acad Sci USA 2007; 104: 15805-15810 [PMID: 17890317 DOI: 10.1073/pnas.0707628104]

83 Garzon R, Liu S, Fabbi M, Liu Z, Hepathy CE, Callegari E, Schwind S, Pang J, Yu J, Muthusamy N, Havelange V, Volinia S, Blum W, Rush LJ, Perrotti D, Andreff M, Bloomfield CD, Byrd JC, Chan K, Wu LC, Croce CM, Marcucci G. MicroRNA-29b induces global DNA hypomethylation and tumor suppressor gene reexpression in acute myeloid leukemia by targeting directly DNMT3A and 3B and indirectly DNMT1. Blood 2009; 113: 6411-6418 [PMID: 19211935 DOI: 10.1182/blood-2008-07-170589]

84 Takada S, Berezinkov E, Choi YL, Yamashita Y, Mano H. Potential role of miR-29b in modulation of Dnm3A and Dnm3b expression in primordial germ cells of female mouse embryos. RNA 2009; 15: 1507-1514 [PMID: 19509302 DOI: 10.1261/rna.1418309]

85 Ugalde AP, Ramsay AJ, de la Rosa J, Varela I, Mariño G, Cadiñas J, Lu J, Freije JM, López-Otín C. Aging and chronic DNA damage response activate a regulatory pathway involving miR-29 and p53. EMBO J 2011; 30: 2219-2232 [PMID: 2152233 DOI: 10.1038/emboj.2011.124]

86 Krützfeldt J, Rajewsky N, Braich R, Rajeev KG, Tuschi T, Manoharan M, Stoffel M. Silencing of microRNAs in vivo with ‘antagomirs’. Nature 2005; 438: 685-689 [PMID: 16258535 DOI: 10.1038/nature04303]

87 Esaú C, Davis S, Murray SF, Yu XX, Pandey SK, Pear M, Watts L, Booten SL, Grahman M, McKay R, Subramaniam A, Propp S, Lotto BA, Freier S, Bennett CF, Bhanot S, Monia BP. mir-122 regulation of lipid metabolism revealed by in vivo antisense targeting. Cell Metab 2006; 3: 87-98 [PMID: 16459310 DOI: 10.1016/j.cmet.2006.01.005]

88 Coulouarn C, Factor VM, Andersen JB, Durkin ME, Thorgeirsson SS. Loss of mir-122 expression in liver cancer correlates with suppression of the hepatic phenotype and gain of metastatic properties. Oncogene 2009; 28: 3526-3536 [PMID: 19617899 DOI: 10.1038/onc.2009.211]

89 Fornari F, Gramantieri L, Giovannini C, Veronese A, Ferracin M, Sabbioni S, Calin GA, Grazi GL, Croce CM, Tavolari S, Chieco P, Factor VM, Andersen JB, Durkin ME, Thorgeirsson SS. Loss of miR-122 expression in liver cancer correlates with suppression of the hepatic phenotype and gain of metastatic properties. Oncogene 2009; 28: 3526-3536 [PMID: 19617899 DOI: 10.1038/onc.2009.211]

90 Fornari F, Gramantieri L, Giovannini C, Veronese A, Ferracin M, Sabbioni S, Calin GA, Grazi GL, Croce CM, Tavolari S, Chieco P, Factor VM, Andersen JB, Durkin ME, Thorgeirsson SS. Loss of miR-122 expression in liver cancer correlates with suppression of the hepatic phenotype and gain of metastatic properties. Oncogene 2009; 28: 3526-3536 [PMID: 19617899 DOI: 10.1038/onc.2009.211]

91 Tsai WC, Hsu PW, Lai TC, Chau GY, Lin CW, Chen CM, Lin CD, Liao YL, Wang J, Chau YP, Hsu MT, Hsiao M, Huang HD, Tsou AP. MicroRNA-122, a tumor suppressor microRNA that regulates hepatic microRNA by HNF4α. J Hepatol 2009; 50: 761-767 [PMID: 19584283 DOI: 10.1016/S0168-8278(09)00467-0]

92 Li ZY, Li D. Positive regulation of hepatic miR-122 expression by HNF4α. J Hepatol 2009; 50: 761-767 [PMID: 19584283 DOI: 10.1016/S0168-8278(09)00467-0]

93 Cobb S, Niknejad N, Elsharkawy AM, Karin M. Gender disparity in liver cancer due to sex differences in MyD88-dependent IL-6 production. Science 2007; 317: 121-124 [PMID: 17613558 DOI: 10.1126/science.1140485]

P- Reviewer: Conti B, Romani A S- Editor: Kong JX L- Editor: A E- Editor: Li D
