MiRNA-Based Regulation of Hemostatic Factors through Hepatic Nuclear Factor-4 Alpha

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Abstract

MiRNAs have been reported as CIS-acting elements of several hemostatic factors, however, their mechanism as TRANS-acting elements mediated by a transcription factor is little known and could have important effects. HNF4α has a direct and important role in the regulation of multiple hepatic coagulation genes. Previous in vitro studies have demonstrated that miR-24-3p and miR-34a-5p regulate HNF4A expression. Here we aimed to investigate the molecular mechanisms of miR-24 and miR-34a on coagulation through HNF4A. Transfections with miR-24 and miR-34a in HepG2 cells decreased not only HNF4A but also F10, F12, SERPINC1, PROS1, PROC, and PROZ transcripts levels. Positive and significant correlations were observed between levels of HNF4A and several hemostatic factors (F5, F8, F9, F11, F12, SERPINC1, PROC, and PROS1) in human liver samples (N = 104). However, miR-24 and miR-34a levels of the low (10th) and high (90th) percentiles of those liver samples were inversely correlated with HNF4A and almost all hemostatic factors expression levels. These outcomes suggest that miR-24 and miR-34a might be two indirect elements of regulation of several hemostatic factors. Additionally, variations in miRNA expression profiles could justify, at least in part, changes in HNF4A expression levels and its downstream targets of coagulation.

Introduction

High levels of coagulation factors may disturb the fragile balance of the hemostatic system leading to thrombotic events. However, coagulation factors have a substantial interindividual variability in healthy human plasma [1,2] so that the threshold for the individual thrombotic risk will come defined by the joint action of genetic, environmental, and acquired factors [1,3–5]. Among the genetic elements that drive the synthesis of coagulation factors a hereditary component has been described for several of them although the heritable basis for high or low levels of factors remains unknown [1]. Interestingly, common regulatory genes coordinate simultaneously the expression of several clotting factors which would allow to categorize individuals in those with high or low levels of coagulation factors [2,4].
A good candidate among these common regulatory genes is Hepatocyte Nuclear Factor 4α (HNF4α, NR2A1, gene symbol HNF4A), a member of the nuclear receptor superfamily, essential for liver homeostasis and linked to several diseases including diabetes, hemophilia, atherosclerosis and hepatitis [6–8]. HNF4α has been linked to the expression of a large number of coagulation genes such as prothrombin [9], Factor (F) VII [10,11], FVIII [12], FIX [13,14], FX [15], FXI [16], FXII [17], protein S [18], protein Z [19], and antithrombin [20,21]. The full-body Hnf4a knock-out mouse is embryonic lethal [22] and gene targeting using short interfering RNA (siHNF4A) confirmed the impact of HNF4α in regulating hepatic coagulation factors transcription [23,24].

Several inductors and repressors, that weave a complex regulatory net, participate in the expression of HNF4A gene [25,26]. In addition, HNF4A is post-transcriptionally regulated by miRNA [27,28], small non-coding RNAs implicated in protein regulation in a large number of physiological and pathological processes [29]. Takagi et al. were the first describing in vitro the regulation of HNF4α by miR-24 and miR-34a [30].

The more intuitive and better described mechanism of action of miRNAs is as CIS-suppressive-regulatory elements. Thus, up to seven coagulation factor genes have been described to be targets of miRNAs [31]. However, the hypothesis that miRNAs repress common transcription factors then working as TRANS-regulatory elements for some genes remains to be explored. Here, we aimed to thoroughly gain a deeper insight into the physiological modulator role of miRNA in the expression of downstream coagulation targets of HNF4α.

Materials and Methods

Cell culture and tissue samples

HepG2 cells (American Type Culture Collection, Manassas, VA) were cultured in modified Eagle’s medium (MEM) (ThermoFisher Scientific, Madrid, Spain) at 37°C under 5% CO2. Medium was supplemented with 0.1 mM non-essential amino acids and with 10% fetal calf serum (ThermoFisher Scientific, Madrid, Spain). 104 liver samples from white donors were provided by the Research Center of Experimental Pathology Department of La Fe Hospital and CIBERehd (Valencia, Spain) and by St. Jude Children’s Research Hospital Liver Resource (Liver Tissue Procurement and Distribution System (NIH Contract #N01-DK-9-2310) and the Cooperative Human Tissue Network) [32]. None of the donors were from a vulnerable population and all donors provided written informed consent that was freely given. Human liver studies were approved by Ethics Committee for Clinical Investigation from Morales Meseguer Hospital in Murcia, Spain (#ESTU-19/12) and performed in accord with the declaration of Helsinki.

Transfection of miRNAs

Briefly, HepG2 cells were seeded twenty four hours before transfection in complete MEM medium supplemented with 10% fetal bovine serum at 37°C in a humidified incubator with 5% CO2 without antibiotics and transfected with 100 nM of chemically modified double-stranded RNAs that mimic endogenous miRNAs or SCR (Life Technologies, Madrid, Spain) using PepMute transfection reagent from SignaGen laboratories (Rockville, MD). After 48 h, cells were collected for subsequent mRNA and protein analyses.

RNA isolation and real-time RT-PCR

Total RNA was isolated using RNAzol® RT Reagent (Molecular Research Center, Inc. Cincinnati, OH). The RNA concentration and 260/280 ratio were determined by using NanoDrop
spectrophotometer (Thermo Scientific, Wilmington, DE). Reverse transcription reactions were performed using 100 ng of total RNA for each sample according to the manufacturer instructions (Life Technologies, Madrid, Spain).

Quantitative real-time PCR, using TaqMan® Gene Expression Assays (Applied Biosystems, Madrid, Spain) and gene-specific primers/probes (S1 Table), was performed on a LightCycler® 480 Real-Time PCR System (Roche Applied Science, Barcelona, Spain). F11 and F9 were previously quantified in our previous study [33] and their mRNA expression data were used for analyses in this study. Data were analyzed using the comparative threshold cycle method (2^ΔCt method) with β-actin as an endogenous reference control for quantification and normalization. To quantify expression levels of miRNAs, commercial RT-PCR assays for miR-24, miR-34a, and U6 snRNA (endogenous control) from Life Technologies were used.

Total protein extraction and western blotting
Transfected HepG2 cells were washed twice with phosphate-buffered saline (PBS). They were then lysed in RIPA buffer [150 mM NaCl, 1 mM ethylenediaminetetraacetic acid, 1% Nonidet P-40, 1% sodium deoxycholate, 0.1% sodium dodecyl sulfate (SDS), 20 mM 3-(N-morpholino)propanesulfonic acid (MOPS), 1 mM phenylmethylsulfonyl fluoride, pH 7.0] on ice for 20 min. The lysates were centrifuged at 12,000×g for 5 min at 4°C and the supernatants were collected for analysis of the protein concentration using a Bicinchoninic Acid (BCA) Protein Assay (Sigma-Aldrich, Madrid, Spain). These lysates (each 20 μg) were blotted and immunostained with different monoclonal antibodies: anti-HNF4α (ab92378; Abcam, Madrid, Spain) and anti-GAPDH (ab128915; Abcam, Madrid, Spain). HNF4α and GAPDH were immunodetected with the appropriate secondary antibody labeled with peroxidase (GE Healthcare, Barcelona, Spain). Western blotting detection, their corresponding densitometric analysis, and the expression of data were performed in a manner similar to that previously described [33].

Statistical analysis
Statistical differences between groups were calculated by non-parametric Mann-Whitney U test using GraphPad Prism 6 software (GraphPad Software Inc., San Diego, CA). A p-value <0.05 was considered to be statistically significant. Correlations were observed with Pearson’s correlation coefficient and analyses were carried out using Statistical Package for Social Science (version 21.0; SPSS, Chicago, IL).

Results
HNF4A correlates with coagulation factor expression levels in human liver
To verify whether HNF4A modulates the expression of coagulation factors in human liver, we quantified mRNA levels of HNF4A and 9 genes involved in coagulation (F5, F9, F10, F11, F12, SERPINC1, PROC, PROZ, and PROS1) in 104 human liver samples. We observed a widespread interindividual variation in expression levels of the analyzed hepatic transcripts (Fig 1).

As shown, the wider expression variability was found for F9 (14–400%) and SERPINC1 (6–640%) whereas PROC and PROZ had the lower range of variability (Fig 1). The distribution by percentiles according with the expression of the analyzed factors showed up to 3-fold differences among percentile 25th and 75th (Fig 1). Similarly, a wide variability was also observed for miR-24, as shown in Fig 1. Next, we analyzed the correlation between HNF4A expression and the same 9 coagulation factors in human livers. We found that those samples with higher coagulation factor expressions had also higher HNF4A expression (all p<0.0001; Fig 2).
These results suggest that differences in HNF4A expression might explain, at least in part, interindividual variations seen in the expression of coagulation factors in human liver.
In vitro study in HepG2 cells

To study the possible indirect effect of miRNAs on coagulation factor expression, we selected two miRNAs, miR-24 and miR-34a, previously pointed as direct inhibitors of HNF4α [27,30]. Both miRNAs bind to several sites in human HNF4A, as described in Fig 3. While miR-34a interacts in three different sites located within HNF4A 3’UTR, miR-24 mostly inhibits HNF4α expression by binding to sites located within the coding region [27] (Fig 3).

MiR-24 regulates coagulation factors by targeting HNF4A. The functional role of miR-24 in regulating HNF4α downstream targets was tested in HepG2 cells by transfecting with miRNA mimics. As expected, results from western blot analysis using whole cell lysates from HepG2 confirmed a decrease of 70% of HNF4α mediated by miR-24 (p = 0.01) (Fig 4A) and a decrease of 25% in mRNA levels (Fig 4B). To investigate whether the decrease of HNF4α was...
accompanied by a decrease of coagulation factors, we determined mRNA levels by qRT–PCR analysis. HepG2 transfection with miR-24 caused a decrease in mRNA of all selected factors although such reduction was only statistically significant for \(F10, F12, PROS1\) and \(SERPINC1\) (Fig 4C).

**MiR-34a regulates coagulation factors by targeting HNF4A.** The functional role of miR-34a on HNF4α was tested in HepG2 cells. Similarly to that seen for miR-24, western blot analysis of lysates from HepG2 showed a significant decrease of HNF4α (Fig 4A) and HNF4A mRNA (Fig 4B), as previously described [27,30].

We next investigated the consequences of HNF4α inhibition by miR-34a on coagulation factors. For this, mRNA levels of selected factors were tested by qRT–PCR. As shown in Fig 4B, miR-34a induced a decrease in the expression of all tested factors.

The transcript decrease was significant for \(F10, F12, PROS1, PROC, SERPINC1\) and \(PROZ\) (Fig 4C).

Overall, these results confirmed the role of miR-24 and miR-34a in regulating HNF4α expression and showed a new trans-mechanism of regulation of several coagulation factors by miRNA through HNF4α.

**Inverse correlation between HNF4A and both miR-24 and miR-34a expression levels in human livers**

To verify the impact of miR-24 and miR-34a variations on HNF4A and because substantial variability in the expression of HNF4A and its downstream coagulation genes targets remains unexplained, we analyzed ex vivo miRNA-mRNA correlations in human liver samples. We found a slight inverse correlation between HNF4A and both miR-24 and miR-34a hepatic transcript levels (\(r = -0.170; p = 0.08\) and \(r = -0.228; p < 0.05\); respectively) (Fig 5).

Interestingly, liver samples with extreme miRNA levels (percentiles 10th and 90th) showed inverse levels of HNF4A and coagulation factors transcripts, in some cases, such correlations were statistically significant (Fig 6). As shown in Fig 6A, liver samples with lower expression of miR-24 had significantly higher expression of HNF4α, \(F9, F11\), \(PROS1\) and \(PROZ\). In turn, liver samples with lower levels of miR-34a also had the following higher coagulation factors levels: HNF4α, \(F9\), \(PROS1\) and \(PROZ\) (Fig 6B).

**Discussion**

Previous data from our group showed a noticeable variability in transcript levels of \(F11\) and \(F9\) in human healthy livers [33] suggesting that a common genetic regulator is behind specific
clustering patterns for human hepatic coagulation factors. However, molecular basis underlying the wide coagulation factors variability in normal population probably involve a combination of factors. Transcriptional factors are ideal candidates to investigate in this framework given their crucial role in controlling mRNAs processing. Moreover, the tandem of some transcription factors and miRNA is an essential network for several biological processes, and its identification has elucidated some of its functions in inflammation [34] and drug metabolism [35] but still remains to be described in hemostasis. Therefore, we aimed to investigate the contribution of miRNAs as novel participants in the variable expression of coagulation factors as well as to investigate the role of HNF4α in this regulatory loop.

Our first interesting finding was positive correlations between HNF4A and the mRNAs of several coagulation genes. To our knowledge these are the first data describing transcriptional relationship between HNF4A and a large number of hepatic coagulation factors in human liver. The bibliographic review of studies that experimentally validated HNF4A regulation by miRNA, conducted us to select miR-24 and miR-34a as indirect regulators candidates of several coagulation factors [27,30]. Our in vitro results showed that miR-24 and miR-34a had a significant impact on the expression of coagulation factors in HepG2 (Fig 4B). Similarly, both miRNAs inhibited HNF4α expression in human hepatocytes, suggesting a TRANS-regulation of several coagulation factors under miRNA control as a novel mechanism in hemostasis.

Additionally, we investigated whether the integrated regulatory association of miR-24, miR-34a, and HNF4A observed in vitro could also take place in human healthy livers. We first tested the relationship between HNF4A mRNA and both miRNA levels confirming that liver samples with higher HNF4A transcript are those with the lower miRNAs levels, and vice versa. The idea that miR-24 and miR-34a gene regulation is mediating as a significant mechanism contributing to variation in gene expression has been previously documented. Lamba et al [35] demonstrated that miR-34a expression was significantly and negatively correlated with expression levels of multiple hepatic transcription factors (including HNF4α) and that it was involved in a significant mediation of the association observed between CYP2C19 and HNF4α [35].

Fig 6. Expression range of miR-24 and miR-34a with HNF4A mRNA and its downstream targets in healthy livers. Dot plot diagram of HNF4A mRNA, selected coagulation factors and miR-24 (A) or miR-34a (B) levels in livers. P10 and p90 represent 10th and 90th percentiles of miR-24 (A) and miR-34a (B), respectively. Each data point represents an individual liver tissue sample. The results are presented as fold change with respect to the normalization standard. The asterisk indicates a statistically significant difference (P < 0.05) and “ns” indicates a statistically non-significant difference (P > 0.05).

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our results while confirming the miR-34a/HNF4A interaction, for the first time also showed a key regulatory role of miR-34a in hepatic coagulation genes in humans.

In turn, we also confirmed here a regulatory connection between miR-24 and HNF4A, as Hatziapostolou et al did in samples from 12 healthy livers [34]. Our series of human liver samples extended to 104, and provide additional consequences for miR-24/HNF4A interaction, as it had repercussions on the levels of coagulation factors transcripts. Of note, liver samples with extreme expression of miR-24 levels (percentiles 10th and 90th) were, in the opposite, those with utmost HNF4A and coagulation factors expression. Similar findings can be inferred from data reported by Hatziapostolou et al [34]. Thus, these authors described that transient inhibition of HNF4A in an hepatocellular carcinoma model drives a feedback loop circuit through several inflammatory miRNAs, and among them miR-24 [34]. Moreover, our in vitro data supported a lower inhibitory effect for miR-24 in comparison with miR-34a, which might explain that only statistically significant values are found for liver samples from extreme percentiles (Fig 6). Alternatively, the sample size might be insufficient to reach statistical power (Fig 5).

In summary, our results suggest that miR-24 and miR-34a participate in the interindividual variability observed in expression of coagulation factors genes in humans. Therefore, in both circuits HNF4A would work as a key switchboard. It remains to be clarified to what extent genetic variations of miRNAs that change HNF4α expression and activity may affect the expression of hepatic coagulation factors, and to what extent this may affect the development of thrombotic or hemorrhagic disorders.

Supporting Information
S1 Table. TaqMan® probes used for real time quantitative PCR of human genes. (DOCX)

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Author Contributions
Conceived and designed the experiments: RGC CM. Performed the experiments: SSA ABA RTM NGB. Analyzed the data: SSA RGC CM. Wrote the paper: SSA RGC CM. Contributed to the critical revision: VR VV.

References
1. Van Hylckama Vlieg A, Callas PW, Cushman M, Bertina RM, Rosendaal FR. Inter-relation of coagulation factors and d-dimer levels in healthy individuals. J Thromb Haemost. 2003; 1: 516–22. PMID: 12871460
2. Mari D, Mannucci PM, Coppola R, Bottasso B, Bauer K A, Rosenberg RD. Hypercoagulability in centenarians: the paradox of successful aging. Blood. 1995; 85: 3144–3149. PMID: 7756646
3. Hellgren M. Hemostasis during normal pregnancy and puerperium. Semin Thromb Hemost. 2003; 29: 125–30. doi: 10.1055/s-2003-38897 PMID: 12709915
4. Rosendaal FR. Venous thrombosis: A multicausal disease. Lancet. 1999; 353: 1167–1173. doi: 10.1016/S0140-6736(98)10266-0 PMID: 10209995

5. Rosendaal FR. Venous thrombosis: the role of genes, environment, and behavior. Hematology Am Soc Hematol Educ Program. 2005; 1–12. doi: 10.1182/asheducation-2005.1.1 PMID: 16304352

6. Sladek FM, Zhong WM, Lai E, Damell JE. Liver-enriched transcription factor HNF-4 is a novel member of the steroid hormone receptor superfamily. Genes Dev. 1990; 4: 2353–2365. doi: 10.1101/gad.4.12b.2353 PMID: 2279702

7. Babeu JP, Boudreau F. Hepatocyte nuclear factor 4-alpha involvement in liver and intestinal inflammatory networks. World J Gastroenterol. 2014; 20: 22–30. doi: 10.3748/wjg.v20.i1.22 PMID: 24415854

8. Walesky C, Apte U. Review Role of Hepatocyte Nuclear Factor 4 a (HNF4 a) in Cell Proliferation and Cancer. 2015; 16: 101–108.

9. Coelie H, Spaargaren-Van Riel CC, De Jong M, Bertina RM, Vos HL. Functional characterization of transcription factor binding sites for HNF1-alpha, HNF3-beta (FOXA2), HNF4-alpha, Sp1 and Sp3 in the human prothrombin gene enhancer. J Thromb Haemost. 2003; 1: 1688–98. 393 [pii] PMID: 12911579

10. Carew J a, Pollak ES, High K a, Bauer K A. Severe factor VII deficiency due to a mutation disrupting an Sp1 binding site in the factor VII promoter. Blood. 1998; 92: 1639–1645. PMID: 9716591

11. Erdmann D, Heim J. Orphan nuclear receptor HNF-4 binds to the human coagulation factor VII promoter. J Biol Chem. 1995; 270: 22988–96. PMID: 7559437

12. Figueiredo MS, Brownlee GG. cis-acting elements and transcription factors involved in the promoter activity of the human VIII gene. J Biol Chem. 1995; 270: 11828–38. PMID: 7744832

13. Reijnen MJ, Sladek FM, Bertina RM, Reitsma PH. Disruption of a binding site for hepatocyte nuclear factor 4 results in hemophilia B Leyden. Proc Natl Acad Sci U S A. 1992; 89: 6300–3. PMID: 1631121

14. Naka H, Brownlee GG. Transcriptional regulation of the human factor IX promoter by the orphan receptor superfamily factors, HNF4, ARP1 and COUP/Ear3. Br J Haematol. 1996; 92: 231–240. PMID: 8562402

15. Miao CH, Leytus SP, Chung W, Davie W. Liver-specific Expression of the Gene Coding for Human Factor X, a Blood Coagulation Factor. J Biol Chem. 1992; 7395–7401. PMID: 1313796

16. Tarumi T. Cloning and Characterization of the Human Factor XI Gene Promoter. Transcription Factor Hepatocyte Nuclear Factor 4alpha (HNF-4alpha) is Required for Hepatocyte-Specific Expression of Factor XI. J Biol Chem. 2002; 277: 18510–18516. doi: 10.1074/jbc.M20186200 PMID: 11891231

17. Farsetti A, Moretti F, Narducci M, Misiti S, Andreoli M, et al. Orphan receptor hepatocyte nuclear factor-4 antagonizes estrogen receptor (alpha)-mediated induction of human coagulation factor XII gene. Endocrinology. 1998; 139: 4581–4589. doi: 10.1210/endo.139.11.6299 PMID: 9794469

18. Hall AJ, Peake IR, Winship PR. Regulation of the human protein S gene promoter by liver enriched transcription factors. Br J Haematol. 2006; 135: 538–46. doi: 10.1111/j.1365-2458.2006.06327.x PMID: 17061980

19. Sugawara H, Iwata H, Souri M, Ichinose A. Regulation of human protein Z gene expression by liver enriched transcription factor HNF 4alpha and ubiquitous factor Sp1. JThrombHaemost. 2007; 5: 2250–2258.

20. Fernandez-rachubinski A, Weiner JH, Biaichman MA, Dele- H. Regions Flanking Exon 1 Regulate Constitutive Expression of the Human Antithrombin Gene. J Biol Chem. 1996; 271: 29502–29512. doi: 10.1074/jbc.271.46.29502 PMID: 8910619

21. Tremp GL, Duchange N, Branellec D, Cereghini S, Tailleux A, Berthou L, et al. A 700-bp fragment of the human antithrombin III promoter is sufficient to confer high, tissue-specific expression on human apolipoprotein A-ll in transgenic mice. Gene. 1995; 156: 199–205. Available: PMID: 7758957

22. Inoue Y, Peters LL, Yim SH, Inoue J, Gonzalez FJ. Role of hepatocyte nuclear factor 4alpha in control of blood coagulation factor gene expression. J Mol Med. 2005; 1–11. doi: 10.1007/s00109-005-0013-5

23. Safdar H, Cheung KL, Vos HL, Gonzalez FJ, Reitsma PH, Inoue Y, et al. Modulation of mouse coagulation gene transcription following acute in vivo delivery of synthetic small interfering RNAs targeting HNF-4a and C/EBPalpha. PLoS One. 2012; 7: e38104. doi: 10.1371/journal.pone.0038104 PMID: 22675511

24. Safdar H, Cleuren AC, Cheung KL, Gonzalez FJ, Vos HL, Inoue Y, et al. Regulation of the F11, Klkb1, Cyp4v3 gene cluster in livers of metabolically challenged mice. PLoS One. 2013; 8: e74637. doi: 10.1371/journal.pone.0074637 PMID: 24066149

25. Hatzis P, Talianidis I. Regulatory mechanisms controlling human hepatocyte nuclear factor 4alpha gene expression. Mol Cell Biol. 2001; 21: 7320–7330. doi: 10.1128/MCB.21.21.7320–7330.2001 PMID: 11585914
26. Hatzis P, Talianidis I. Dynamics of enhancer-promoter communication during differentiation-induced gene activation. Mol Cell. 2002; 10: 1467–1477. doi: 10.1016/S1097-2765(02)00786-4 PMID: 12504020

27. Wang Z, Burke P a. The role of microRNAs in hepatocyte nuclear factor-4alpha expression and trans-activation. Biochim Biophys Acta. 2013; 1829: 436–42. doi: 10.1016/j.bbagrm.2012.12.009 PMID: 23298640

28. Wirning A, Senkel S, Klein-Hitpass L, Ryffel GU. A systematic analysis of the 3'UTR of HNF4A mRNA reveals an interplay of regulatory elements including miRNA target sites. PLoS One. 2011; 6: e27438. doi: 10.1371/journal.pone.0027438 PMID: 22140441

29. He L, Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation. Nat Rev Genet. 2004; 5: 522–531. doi: 10.1038/nrg1415 PMID: 15211354

30. Takagi S, Nakajima M, Kida K, Yamaura Y, Fukami T, Yokoi T. MicroRNAs regulate human hepatocyte nuclear factor 4alpha, modulating the expression of metabolic enzymes and cell cycle. J Biol Chem. 2010; 285: 4415–22. doi: 10.1074/jbc.M109.085431 PMID: 20018894

31. Teruel-Montoya R, Rosendaal FR, Martinez C. MicroRNAs in hemostasis. J Thromb Haemost. 2014; 1–12.

32. Lamba V, Panetta JC, Strom S, Schuetz EG. Genetic predictors of interindividual variability in hepatic CYP3A4 expression. J Pharmacol Exp Ther. 2010; 332: 1088–1099. doi: 10.1124/jpet.109.160804 PMID: 19934400

33. Salloum-Asfar S, Teruel-Montoya R, Arroyo AB, García-Barberá N, Chaudhry A, Schuetz E, et al. Regulation of Coagulation Factor XI Expression by MicroRNAs in the Human Liver. PLoS One. 2014; 9: e111713. doi: 10.1371/journal.pone.0111713 PMID: 25379760

34. Hatziapostolou M, Polyarchou C, Aggelidou E, Drakaki A, Poultsides G a., Jaeger S a., et al. An HNF4α-miRNA inflammatory feedback circuit regulates hepatocellular oncogenesis. Cell. 2011; 147: 1233–1247. doi: 10.1016/j.cell.2011.10.043 PMID: 22153071

35. Lamba V, Ghodke Y, Guan W, Tracy TS. microRNA-34a is associated with expression of key hepatic transcription factors and cytochromes P450. Biochem Biophys Res Commun. 2014; 445: 404–11. doi: 10.1016/j.bbrc.2014.02.024 PMID: 24530915