Microbial Pathogenesis 110 (2017) 688–693

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

The effect of the hepatitis C virus (HCV) NS3 protein on the expression of miR-150, miR-199a, miR-335, miR-194 and miR-27a

Sayyad Khanizadeh [a, b, *], Mehrdad Ravanshad [c], Seyed Younes Hosseini [d], Parivash Davoodian [e], Mohammad Almasian [f], Zahra Khanlari [c]

[a] Hepatitis Research Center and Department of Microbiology and Virology, Lorestan University of Medical Sciences, Khorramabad, Iran
[b] School of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran
[c] Department of Virology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
[d] Department of Bacteriology and Virology, Shiraz University of Medical Sciences, Shiraz, Iran
[e] Infectious & Tropical Diseases Research Center, Hormozgan University of Medical Sciences, Bandar Abbas, Iran
[f] Department of the English Language, School of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran

ARTICLE INFO

Article history:
Received 20 January 2017
Received in revised form
1 March 2017
Accepted 6 March 2017
Available online 9 March 2017

Keywords:
HCV
Chronic hepatitis
NS3
microRNA
LX-2
Fibrosis

ABSTRACT

Hepatitis C virus (HCV) infection is considered one of the most important causes of chronic liver diseases. Many reports have shown that the proteins of the HCV via interactions with gene expression regulatory networks such as cellular pathways and microRNAs can contribute to the development of chronic liver diseases. The present study aimed to investigate the effects of the HCV NS3 protein on the expression of miR-150 miR-199a, miR-335, miR-194, miR-27a in a cell culture model. Plasmids expressing the full length of the HCV NS3 protein were transfected into the LX-2 cell line, while at the same time a plasmid expressing empty GFP (green fluorescent protein) was used as a negative control group. Subsequently, total RNA was extracted and real-time PCR was performed to measure microRNA expression levels. Additionally, the trypan blue exclusion test was performed to examine the effect of the expressing NS3 protein plasmid on cellular viability. The analysis of microRNA gene expression in LX-2 cells indicated that the NS3 protein, which is endogenous to HCV, can significantly upregulate the expression of miR-27a and downregulate the expression of miR-335 and miR-150 in comparison with the control plasmid expressing GFP and normal cells ($p < 0.01$). These results suggest that the HCV NS3 protein may play a role in the pathogenesis of chronic hepatic diseases such as liver fibrosis via interaction with cellular microRNAs and modulation of microRNA gene expressions.

1. Introduction

Hepatitis C virus (HCV) is known as one of the most prevalent infectious agents affecting liver cells. Most cases of virus-caused infection progress as a chronic condition, which can have serious clinical outcomes, including fibrosis, cirrhosis, and hepatic cell cancer (HCC) [1]. In most cases, acute infection is not identified. Infection is mostly detected accidentally by such indirect means as detecting increased hepatic enzymes, the development of symptoms of chronic infection, or the pathology of hepatic cells [1]. Hepatic fibrosis is one of the clinical complications of chronic liver disease. Chronic HCV infection significantly contributes to the development of hepatic fibrosis. The development of hepatic fibrosis and subsequently cirrhosis is an important predictor of the prognosis of disease and the resultant mortality [2,3].

Success in the establishment of infection can be attributed to the ability of the virus to evade the immune system and the interactions of the virus proteins with cellular signaling and regulatory pathways [4,5]. HCV has several structural and nonstructural proteins, including core, E1/2, NS3/4, NS5, which can play a role in the development of infection, the pathogenesis of chronic liver diseases such as HSCs(hepatic stellate cells)-mediated fibrosis, and the suppression of the innate immune response [6,7]. Studies have shown that proteins specific to this virus can interact with various hepatic cell proteins and regulatory pathways, which can generate pathogenic molecular processes that positively affect the progression of hepatic fibrosis [5,6], for example, it is observed that HCV

* Corresponding author. Hepatitis Research Center and Department of Microbiology and Virology, Lorestan University of Medical Sciences, Khorramabad, Iran.
E-mail address: khani.virologist@gmail.com (S. Khanizadeh).

http://dx.doi.org/10.1016/j.micpath.2017.03.004
0882-4010/

proteins expressed in HSCs induce these cells [6,7].

MicroRNAs are small noncoding RNA molecules with very high expression levels [8]. These molecules can regulate the expression of cellular genes by posttranscriptional regulations and by binding with the 3′ section of the untranslatable region (3′UTR) of the transcript of the cellular genes. These molecules are linked to many basic cellular processes, including apoptosis, reproduction, and differentiation [8]. Many studies have shown that the HCV virus can communicate with cellular microRNA networks. Moreover, it has been observed that cellular microRNAs play a considerable part in HCV infection and the pathogenesis of chronic liver diseases, including hepatic fibrosis [9,10]. Various reports have shown that miR-194, miR-150, and miR-335, due to their anti-fibrotic properties, and miR-27a and miR-199a, due to their pro-fibrotic properties, play important roles in the pathogenesis and progression of hepatic fibrosis [11]. Most studies on HCV proteins have been conducted on the core protein, while other proteins have been studied less extensively [12,13]. Nonstructural proteins 3 (NS3) is one of the nonstructural proteins of HCV, which also known as p-70 plays an important role in viral infection and the pathogenesis of virus-caused diseases due to its multiple enzymatic. It acts as a serine protease [14]. The objective of the present study was to investigate the effect of NS3 on the fibrogenesis of HSCs via modulation of miR-150 miR-199a, miR-335, miR-194, and miR-27a gene expressions.

2. Materials and methods

2.1. Cell culture

The LX-2 cells (an immortalized human HSC cell line) were kindly gifted by Professor Scott Friedman (Mount Sinai School of Medicine, New York, USA). They were cultured in the DMEM (Gibco USA) medium along with a 10% bovine serum (FBS, Sigma, St. Louis USA), 100 μg/mL of the penicillin-streptomycin antibiotics (Gibco), and 2 μM of L-glutamine under the atmospheric conditions of 5% CO_2 and the temperature of 37 °C. In this stage, the cells were divided into 4 groups: 1. The first group included the cells that were treated with the fibrotic leptin hormone using a previously described method [15] and were considered as the fibrosis model. 2. The second group included the cells that were transfected with the expressing HCV NS3 protein plasmid. 3. The third group was made up of natural untreated cells used as the normal control group. 4. The fourth group comprised the cells that were transfected with a blank plasmid expressing GFP and were considered the negative control group.

2.2. Plasmids and transfection

The plasmids (gWiz) expressing the full-length NS3 protein of HCV [16] was a kind gift from Dr. Gloria Gonzalez-Aseguinolaza (CIMA Research Center, Navarra, Spain). On the day prior to the transfection, about 4.10^5 LX-2 cells were cultured on 6-well flasks. When 36 h had passed since cell growth and at the cell growth density of 70%, the LX-2 cells were transfected with plasmids expressing HCV NS3 protein using Lipofectamine 2000 (Invitrogen) in accordance with the manufacturer's instructions and at the same time a blank plasmid expressing GFP was used as a negative control group. 6 h after transfection, the cell culture medium was changed, and then the cells were incubated for 18 h.

2.3. Cell viability test

In order to understand the effect of the plasmid expressing HCV NS3 protein on the viability or apoptosis rates of the transfected cells, the trypan blue exclusion cell viability test was conducted and the cell phenotypes were observed under an optic microscope. To perform this test, 100 cells were stained and examined using the trypan blue vital stain during cell counting, and the ratio of live cells to unstained cells was divided by the total number of cells (dead stained cells + unstained live cells) and the results were compared among different groups.

2.4. RNA extraction and reverse transcription reaction

After the transfection stage, total RNA was extracted from LX-2 cells using the trizol solution (Invitrogen) in accordance with the manufacturer's instructions. Next, in order to synthesize the microRNA cDNAs, the extracted RNA was reverse-transcribed by the cDNA synthesis kit (Thermo Fisher Scientific, USA) using the stem-loop method by means of the stem-loop RT primer specific to each microRNA (Table 1), and were converted to cDNA for use in quantitative investigations. To carry out this stage, first 5 μL of the extracted RNA was added to 1.5 μL of 50 nM primer (RT) and 4 μL distilled water in a sterile 0.2 microtube devoid of nuclease. Next, the reaction mixture was incubated in the thermocycler device at a temperature of 70 °C for 15 min. Then, the reaction tubes were placed on ice. 4 μL of X 5 buffer, 2 μL of the dNTP (10 mM) mixture, 1 μL of the RiboLock RNase Inhibitor (40 U/μL) (Thermo Scientific™), and 1 μL of RT enzyme (50U/μL) (Thermo Scientific™), were added to them, respectively. Then, the reaction mixture was incubated in the thermocycler device for 1 h at a temperature of 42 °C and for 5 min at a temperature of 72 °C (in order to deactivate the polymerase enzyme). In the end, the constructed cDNA was preserved at a temperature of −20 °C.

2.5. Real-time PCR

After the generation of the microRNA cDNA, the qRT-PCR technique was carried out using the primers specific to each microRNA (Table 1). The primer sequences of each microRNA were designed using information obtained from the miRbase database (www.mirbase.org) and also by using the oligoanalyzer and RNAfold software applications. To conduct this stage on ice, and inside a sterile microtube, 2 μL of cDNA was added to 10 μL of Real-Time SYBR green PCR master mix (Roche Diagnostics GmbH Mannheim, Germany). Then, the reaction mixture volume was increased to 19 μL using water and, in the end, 0.5 μM of each primer (forward and reverse) was added to the tube. Next, the reaction tubes were subjected to the following thermal conditions: an initial 95 °C for 5 min, 95 °C for 20 s (the denaturation phase), 60 °C for 35 s (the annealing phase), 72 °C for 20 s (the extension phase). The final extension was repeated for 10 min. To prevent variations in the measurement of expression levels of microRNAs, primers specific to the U6 snRNA gene (R-ACGCTTCACGAATTTGCGTGTC and F- CTCGCTTCGGCAGCACATATACT) were used in the Real-Time PCR as the internal control gene.

2.6. Bioinformatics approach

In order to predict miRNA target sequences, the miRNA sequences were analyzed for their predicted target proteins using miRTarBase (http://mirtarbase.mbc.nctu.edu.tw/) and TargetScan (www.targetscan.org) version 6.1 algorithms. The major proteins that were found to be common with most homology to the target sequences were selected for further analysis.

2.7. Statistical analysis

This work was a case − control study. Statistical analysis was

Table 1
Primer sequences specific to each microRNA using the stem-loop method.

miRNA	Primer sequences 5′ → 3′
hsa-miR-27a	RT: GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTGCTCA
	F: AGAGGGCTTAGCTGCTTGT
hsa-miR-194	RT: GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACTCCACA
	F: GGTGTAACAGCAACTCCATGT
hsa-miR-199a	RT: GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACGAACAG
	F: CCCAGTGTTCAGACTACC TGT
hsa-miR-150	RT: GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCACTGG
	F: CACAGTCTCCCAACCCTTGT
hsa-miR-335	RT: GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACATTT
	F: GCAGGTCAAGAGCAATAACGA

RT: stem loop reverse transcription primer.
Universal reverse primer: GAGCAGGGTCCGAGGT.
miRNA complementary specific sequences are underlined.

conducted using the Graph Pad Prism software application (version, 6). To evaluate differences among the data, one-way ANOVA was used. The statistical differences between the experimental and control groups was determined using the Tukey post-test. The significance level was set at $p \leq 0.05$.

3. Results

3.1. Lack of toxicity and cytotoxicity of the gwis plasmid expressing the NS3 protein of HCV

In this study, the trypan blue exclusion test was used to assess the effect of the expression vector of the NS3 protein of the HCV on the viability of LX-2 cells. The results of this test in different groups showed that no significant difference exists in terms of cell viability between LX-2 cells transfected by the plasmid expressing the NS3 protein of the HCV and non-transfected cells ($p < 0.01$) (Fig. 1).

3.2. The effect of the NS3 protein of the HCV on the expression profiles of microRNAs

In order to find out whether the NS3 protein of the HCV can affect the expression of microRNAs or not, the LX-2 cells were transfected with the Gwis plasmid expressing the complete NS3 protein of the HCV. Next, to analyze the expression of the miR-150, miR-335, miR-194, miR-27a, and miR-199a genes, the expression of these microRNAs was measured using the Real Time PCR technique. As previously mentioned, the LX-2 cells surveyed in this study were divided into 4 groups. In LX-2 cells that were activated by the fibrotic hormone leptin and were considered as the model of fibrosis, it was observed that the expression of miR-150, miR-335 and miR-194, known as anti-fibrotic microRNAs, was significantly downregulated in comparison with untreated cells or normal cells and the negative control group (LX-2 cells transfected with empty plasmids expressing GFP), while the expression of miR-27a and miR-199a, known as pro-fibrotic microRNAs, when compared to the control groups, was significantly upregulated (Fig. 2). It was also observed that in the cells transfected with a plasmid expressing the HCV NS3, similar to the activated LX-2 cells in the fibrosis model, the expression levels of the two microRNAs, miR-335 and miR-150, was significantly downregulated (2.5 and 2 fold, expression levels of change for miR-335 and miR-150, respectively) compared to normal cells and the expression of miR-27a was significantly upregulated in terms of fold change (to 2 folds). On the other hand, the levels of expression of the two microRNAs, miR-194 and miR-199a, did not show any significant change in terms of fold change in comparison with the experimental and control groups. These results show that the expression plasmids of the HCV NS3 were able to significantly affect the expression of microRNAs.

3.3. The target sequences of microRNAs

On the basis of bioinformatics methods using the miRTarBase and TargetScan, TNC, C-MYB, RXRA, RAC1 and ETS2 were identified as a target gene of miRNA-335, miRNA-150, miRNA-27a, miRNA-194 and miRNA-199a respectively at the highest level of homology (Fig. 3).

4. Discussion

Many studies have shown that the proteins of the HCV virus play a crucial role in the development and progression of chronic infections caused by this virus, and the proteins of the virus have been extensively identified in infected hepatic cells. A large number of studies have demonstrated that HCV proteins can affect the expression levels of the cellular genes via interactions with the gene regulatory pathways. These events play a considerable role in persistent viral infections and the clinical consequences of chronic liver diseases, including liver fibrosis [17–19]. By targeting vital host cell factors needed for the replication of the HCV virus, microRNAs can play an important role in the infectivity and pathogenesis of the chronic infection caused by this virus, Many reports have shown that alteration in the expression of cellular microRNAs occur during chronic HCV infection [9,10]. For example, in a cellular culture model using an infectious HCV colon, Rebecca et al. showed

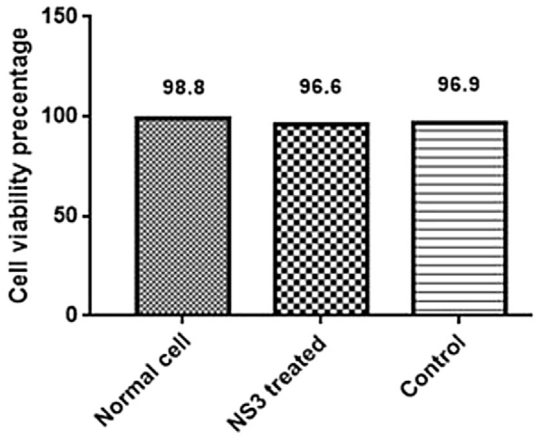

Fig. 1. The effect of the NS3 protein expression plasmids on cell viability. As can be seen in the figure, the NS3 protein expression plasmid has shown no left significant cytotoxic and stimulatory effects after trypan blue exclusion tests among different groups.

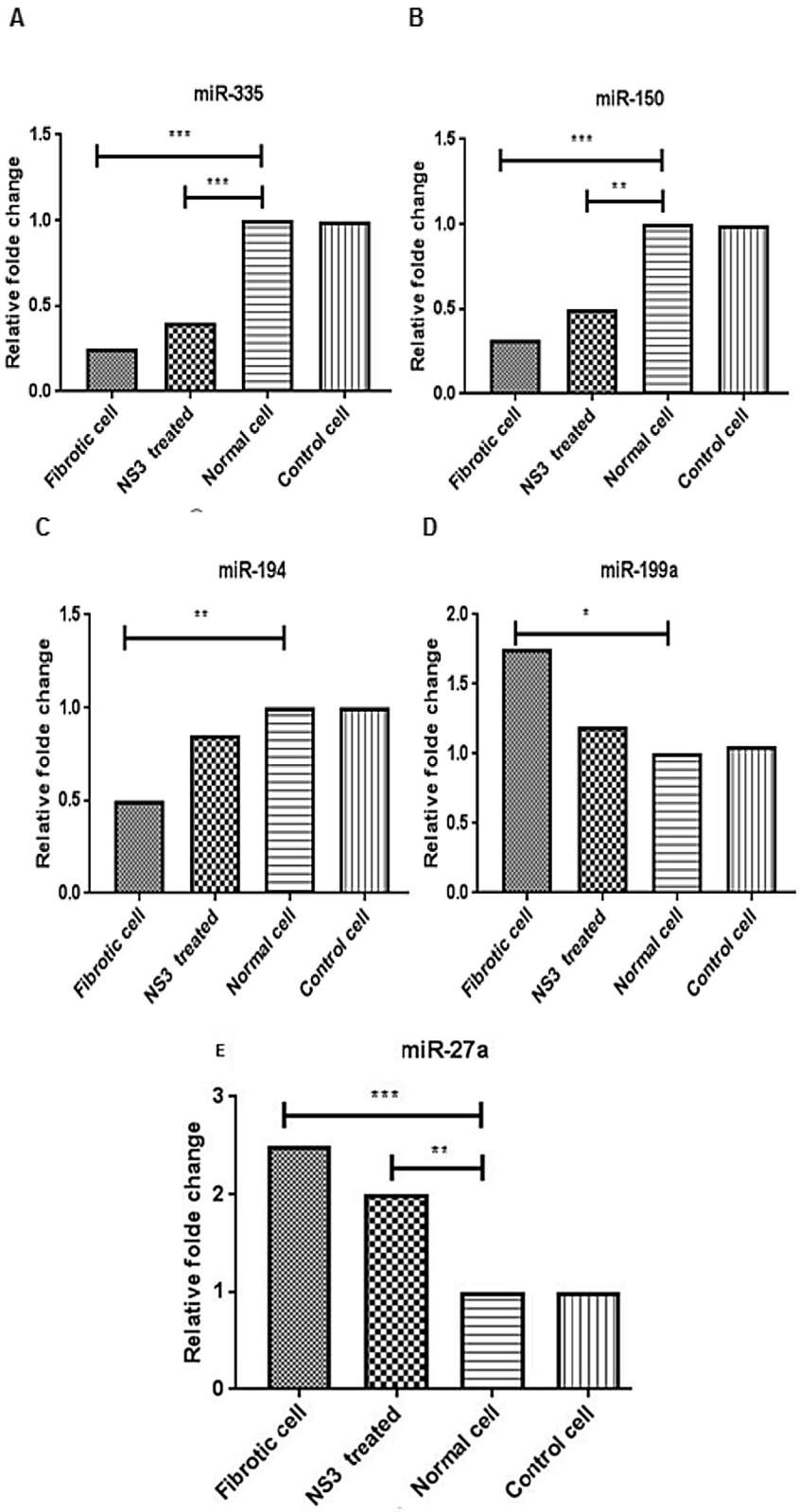

Fig. 2. The results of the analysis of gene expression microRNAs: miR-335 (2A), miR-150(2B), miR-194 (2C), miR-199a (2D), miR-27a (2E) using the Real Time-PCR technique. Each bar indicates the increases or decreases in the fold change compared to the expression of the control cells. *P < 0.05, **p < 0.01, ***p < 0.001 represents a significant change in fold change of the experimental groups of cells activated with leptin and the control groups. The data were an experiment that was set up as a triplicate.

that viral infection leads to the downregulation of the expression of miR-122 and upregulation of the expression of miR-21. Further analyses have demonstrated that miR-21 can upregulate the expression levels of TGF-β by targeting the SMAD7 gene transcripts,

and consequently increase the fibrogenesis of hepatic cells [20]. Additionally, other studies have exhibited the fibrotic effects of the HCV NS3 protein on the hepatic cells [6,21].

In the present study, we investigated the effects of the NS3 protein on the expression profiles of miR-335, miR-150, miR-194, miR-27a, miR-199a in a cell culture model. These microRNAs have been explored in various studies in the context of hepatic fibrosis and it is proven that miR-150, miR-194, miR-335 have anti-fibrotic properties, and when fibrosis occurs in tissues, their expression is downregulated. On the other hand, the expression of miR-199a and miR-27a is upregulated during fibrosis [11]. Similar to previous studies conducted on the fibrotic properties of these microRNAs, in our study, it was observed that the expression levels of miR-335 and miR-150 were significantly downregulated, and the expression levels of miR-27a was upregulated in cells transfected with the NS3 protein. However, unlike other studies, our observations did not show any significant changes in the expression levels of miR-194 and miR-199a in the treated cells by the NS3 protein.

Additionally, in order to make further analyses through bioinformatics, targetscan and miRTarBase algorithms also the results of other similar studies were used and it was observed that miR-335, miR-150, miR-27a (Fig. 3) bind to their target sequence in the '3UTR region in the TNC and C-myb genes respectively and to the alpha X retinoid acid receptor at the highest hybridization level [11]. Chen et al. have shown that miR-335 binds to TNC, which is a glycoprotein on the extracellular matrix. The protein product of this gene is one of the activating factors for the HSCs and consequently the fibrogenic properties of these cells, which plays a major role in their division and migration [22]. C-myb is a proto-oncogene, which encodes a transcription factor, which is important in the replication, differentiation, and viability of hematopoietic cells [23]. Reports suggest that activated HSCs also express this protein, and its expression in these cells plays a role in the formation of hepatic fibrosis [24]. Furthermore, studies have shown that hepatic fibrosis downregulates the expression of miRNA-150, such that the upregulation of the expression of this microRNA in fibrotic liver tissues is associated with decreased hepatic fibrogenesis [25]. In addition, previous studies have demonstrated that during chronic infection of liver cells infected with HCV, the expression of the C-myb protein is upregulated, and its expression plays a part in the development and progression of the disease in these cells to more serious complications [26,27]. RXR-alpha is a nuclear receptor, which acts as an intermediary of the biological effects of the retinoid by intervening in the cellular activities resulting from retinoic acid. This protein forms a heterodimer complex with PPARA that is needed for the PPARA transcription activity in the metabolism of fatty acids [28]. Previous studies have shown that the upregulation of the expression of miR-27a in cultured HSCs can disrupt the pathways of fat metabolism by affecting the RXR-alpha gene transcription product and consequently lead to the accumulation of fat in the affected cells. As a result of this, the cellular oxidative stress is increased and HSCs are activated [29]. Furthermore, in another study, it was observed that the Core protein of the HCV can upregulate the expression of TGF-β via same mechanism and also increase the oxidative stress in HSCs and as a result intensify fibrogenic activities in these cells [30].

5. Conclusion

In conclusion, according to the results of this study and similar studies, it seems that the HCV proteins can lead to alterations in the expression levels of intracellular microRNAs and thus the interactions between the virus proteins with the cellular microRNAs networks, as gene expression regulation factors, can play an important role in the persistent progression of chronic liver diseases. However, it is necessary to study the exact hepatic fibrogenic mechanisms of these proteins as well as the factors and intracellular targets, which affect the expression levels of microRNAs via these proteins. Overall, the results of this study indicated that the NS3 protein of the HCV virus can affect the expression levels of miR-335, miR-150, miR-27a, and thus this mechanism may play a role in the pathogenesis of chronic liver diseases and the fibrotic

Fig. 3. The predicted sequences for TNC, C-MYB, RXRA, RAC1 and E2S2 for miRNA-335, miR-150, miRNA-27a, miR-194 and miR-199a. Two different bioinformatics approaches (Targetscan and miRTarBase) were used for the target prediction.

properties of the NS3 protein. Additionally, these results can provide insights into the pathogenesis of chronic liver diseases caused by HCV infection and the needed treatment strategies.

Ethics approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors.

Competing interests

The authors declare that they have no competing interest.

Acknowledgments

The authors would like to express their gratitude to all colleagues at the Hepatitis Research Center and Department of Microbiology and Virology, Lorestan University of Medical Sciences, Khorramabad, Iran, and Department of Bacteriology and Virology, Shiraz University of Medical Sciences, Shiraz, Iran, and Infectious & Tropical Diseases Research Center, Hormozgan University of Medical Sciences, Bandar Abbas, Iran, who contributed to this work.

Abbreviations

HCV	hepatitis c virus
HSC	hepatic stellate cell
NS3	nonstructural protein 3
MIR	microRNA
UTR	translated region

References

[1] Y.C. Kwon, R.B. Ray, R. Ray, Hepatitis C virus infection: establishment of chronicity and liver disease progression, EXCLI J. 27 (2014) 977–996.
[2] J.M. Pawlotsky, Pathophysiology of hepatitis C virus infection and related liver disease, Trends Microbiol. 12 (2004) 96–102.
[3] S. Khanizadeh, M. Ravanshad, S.Y. Hosseini, P. Davoodian, A. Nejati Zadeh, J. Sarvari, Blocking of SMAD4 expression by shRNA effectively inhibits fibrogenesis of human hepatic stellate cells, Gastroenterol. Hepatol. Bed Bench 8 (2015) 262–269.
[4] N. Vio, M.M. Lai, J. Biosci, The hepatitis C virus persistence: how to evade the immune system? J. Biosci. 28 (2003) 287–304.
[5] C. Wu, R. Gilroy, R. Taylor, M. Olyaee, B. Abdulkarim, J. Forster, Alteration of hepatic nuclear receptor-mediated signaling pathways in HCV patients with and without a history of alcohol drinking, Hepatology 54 (2011) 1966–1974.
[6] R. Bataller, Hepatitis C virus core and nonstructural proteins induce fibrogenic effects in hepatic stellate cells, Gastroenterology 126 (2004) 529–540.
[7] C.F. Wu, Y.L. Lin, Y.T. Huang, Hepatitis C virus core protein stimulates fibrogenesis in hepatic stellate cells involving the obese receptor, J. Cell Biochem. 114 (2013) 541–550.
[8] A.M. Gurtan, P.A. Sharp, The role of miRNAs in regulating gene expression networks, J. Mol. Biol. 425 (2013) 3582–3600.
[9] P. Gupta, M.J. Cairns, N.K. Saksena, Regulation of gene expression by microRNA in HCV infection and HCV–mediated hepatocellular carcinoma, Virology J. 11 (2014).
[10] M.R. Therese, The Role of MicroRNAs during Hepatitis C Viral Infection and Liver Regeneration, PhD (Doctor of Philosophy) thesis, University of Iowa, 2009.
[11] A. Noetel, M. Kwiecinski, N. Elfimova, J. Huang, M. Odenthal, microRNA are central players in anti- and profibrotic gene regulation during liver fibrosis, Front. Physiol. 19 (2012) 3–9.
[12] G. Barba, F. Harper, T. Harada, M. Kohara, S. Goulinet, Y.G. Matsuura, Z. Eder, M.J. Schaff, T. Chapman, C. Miyamura, Bréchot, Hepatitis C virus core protein shows a cytoplasmic localization and associates to cellular lipid storage droplets, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 1200–1205.
[13] A. Banerjee, R.B. Ray, R. Ray, Oncogenic potential of hepatitis C virus proteins, Viruses 2 (2010) 2108–2133.
[14] M. Baril, M.E. Racine, F. Penin, D. Lamarre, MAVS dimer is a crucial signaling component of innate immunity and the target of hepatitis C virus NS3/4A protease, J. Virol. 83 (2009) 1299–1311.
[15] N.K. Saxena, M.A. Titus, X. Ding, J. Floyd, S. Srinivasan, S.V. Sitaraman, F.A. Anania, Leptin as a novel profibrogenic cytokine in hepatic stellate cells: mitogenesis and inhibition of apoptosis mediated by extracellular regulated kinase (Erk) and Akt phosphorylation, FASEB J. 18 (2004) 1612–1614.
[16] Z. Khanlari, F. Sabahi, S.Y. Hosseini, M. Ghaderi, HCV NS3 blocking effect on IFN induced ISGs like viperin and IL28 with and without NS4A, Hepat. Mon. 14 (2014) e17822.
[17] M. Dimitrova, L. Imbert, M.P. Kieny, C. Schuster, Protein-protein interactions between hepatitis C virus nonstructural proteins, J. Viol. 77 (2003) 5401–5414.
[18] N. Hagen, K. Bayer, K. Rösch, M. Schindler, The intraviral protein interaction network of hepatitis C virus, Mol. Cell Proteomics 13 (2014) 1676–1689.
[19] T.L. Tellinghuisen, C.M. Rice, Interaction between hepatitis C virus proteins and host cell factors, Curr. Opin. Microbiol. 5 (2002) 419–427.
[20] R.T. Marquez, S. Bandyopadhyay, E.B. Wendlandt, K. Keck, B.A. Hoffer, M.S. Icardi, R.N. Christensen, W.N. Schmidt, A.P. McCaffrey, Correlation between microRNA expression levels and clinical parameters associated with chronic hepatitis C viral infection in humans, Lab. Invest. 90 (2010) 1727–1736.
[21] K. Sakata, M. Hara, T. Terada, N. Watanabe, D. Takaya, S.I. Yaguchi, HCV NS3 protease enhances liver fibrosis via binding to and activating TGF-β type I receptor, Sci. Rep. 3 (2013) 3243.
[22] C. Chen, C.Q. Wu, Z.Q. Zhang, D.K. Yao, L. Zhu, Loss of expression of miR-335 is implicated in hepatic stellate cell migration and activation, Exp. Cell Res. 317 (2011) 1714–1725.
[23] R.G. Ramsay, T.J. Gonda, MYB function in normal and cancer cells, Nat. Rev. Cancer 8 (2008) 523–534.
[24] H.H. Ma, J.L. Yao, G. Li, C.L. Yao, X.J. Chen, S.J. Yang, Effects of c-myb antisense RNA on TGF-β1 and α1-I collagen expression in cultured hepatic stellate cells, World J. Gastroenterol. 10 (2004) 3662–3665.
[25] S.K. Venugopal, J. Jiang, T.H. Kim, Y. Li, S.S. Wang, N.J. Torok, J. Wu, M.A. Zern, Liver fibrosis causes downregulation of miR-150 and miR-194 in hepatic stellate cells, and their overexpression causes decreased stellate cell activation, Am. J. Physiol. Gastrointest. Liver Physiol. 298 (2010) 101–106.
[26] T. Kitada, S. Seki, K. Nakatani, N. Kawada, T. Kuroki, T. Monna, Hepatic expression of c-Myb in chronic human liver disease, Hepatology 26 (1997) 1506–1512.
[27] G. Tappero, M. Farina, F. Negro, G. Anfossi, L. Mattiello, P.D. Giuli, P. Colombatto, M.R. Brunetto, A. Angeli, F. Bonino, Intrahepatic expression of c-fos, c-myb and c-myc oncogenes: correlation with virus-induced chronic liver disease and response to interferon, Ital. J. Gastroenterol. Hepatol. 29 (1997) 148–154.
[28] H.H. Steineger, B.M. Arntsen, O. Spydevold, H.N. Sørensen, Retinoid X receptor (RXR alpha) gene expression is regulated by fatty acids and dexamethasone in hepatic cells, Biochimie 79 (1997) 107–110.
[29] J. Ji, J. Zhang, G. Huang, J. Qian, X. Wang, S. Mei, Over-expressed microRNA-27a and 27b influence fat accumulation and cell proliferation during rat hepatic stellate cell activation, FEBS Lett. 18 (2009) 759–766.
[30] A.V. Ivanov, B. Bartosch, O.A. Smirnova, M.G. Isaguliants, S.N. Kochetkov, HCV and oxidative stress in the liver, Viruses 5 (2013) 439–469.