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ORIGINAL ARTICLE

MicroRNA let-7b inhibits hepatitis C virus and induces apoptosis in human hepatoma cells

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Abstract

Background Small non-coding RNAs have emerged as essential modulators of viral infections such as hepatitis C virus (HCV). Cellular miRNAs directly regulate the viral infectivity and indirectly by targeting virus-host factors. The current study investigates the inhibitory effect of let-7b miRNA on HCV replication in the Hepatocarcinoma cell line (Huh7.5).

Methods and Results The algorithm-based search revealed that let-7b, a high score microRNA, has target sequences on the HCV genome. The Huh7.5 cells were stably transduced with let-7b lentiviral vectors (Huh7.5/let-7b) and mock (Huh7.5/scrambled). The expression of the let-7b level was assessed by real-time PCR assay and Red fluorescence microscope. A dual-luciferase assay was conducted to evaluate the liver-specific let-7b and HCV genome interaction. In the next step, for establishing HCVcc, Full-length HCV-RNA was transduced to naïve Huh7.5, Huh7.5/scrambled, and Huh7.5/let-7b cells. The results of in silico analysis and dual-luciferase reporter assay exhibited a specific interaction of HCV-NS5B and let-7b. Real-time PCR analysis revealed that in contrast to infected naïve Huh7.5 cells and Huh7.5/scrambled, a significant decrease in HCV-RNA load was seen in Huh7.5/let-7b cells. On the other hand, the Flow Cytometry test showed that let-7b could significantly induce the apoptosis pathway in Huh7.5/let-7b.

Conclusions The results also suggest that let-7b, as a target of the HCV genome, potentially reduces HCV replication and raises cell apoptosis rate. We suggest that let-7b directly downregulates HCV replication and may serve as a unique antiviral therapy.

Keywords Hepatitis C virus · Huh7.5 · MicroRNA let-7b

Introduction

Liver diseases, as one of the principal healthful concerns throughout the world, include more than 100 etiological agents of hepatic and biliary diseases that can finally lead to liver failure [1]. Hepatitis C virus (HCV) is a Hepatotropic viral infection that nearly affects 1%, equivalent to 220 million of the world population [2, 3], and annually, 399,000–495,000 patients die of this infection [4]. This viral infection is transmitted through risky injection practices and contaminated blood and blood products, leading to 30% acute HCV infections which immediately clear up and up to 70% chronic HCV infection, which can evolve into cirrhosis, as the main complication and hepatocellular carcinoma or HCC [5, 6].

HCV belongs to the *Flaviviridae* and *Hepacivirus* genus. Based on the new ICTV (International Committee on Taxonomy of Viruses) classification, *the Hepacivirus* genus is classified as *Hepacivirus* A-N and infects other mammalians [7].

HCV, as the well-characterized *Hepacivirus,* has 9.6 kb single strand positive RNA that flanks with 5'- and 3'-untranslated regions (UTR). These conserved regions are required for genome replication and translation. One large open reading frame (ORF) of HCV encodes 3 structural (core-E1-E2) and 7 non-structural proteins (NS2-NS3-NS4A-NS4B-NS5A-NS5B). In recent years, hepatoma-derived Huh 7.5 cell (Huh7) has been proposed as the susceptible surrogate cell model for a few adapted strains of

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HCV, including JFH1 and Con-1(cell culture-adapted HCV [HCVcc]) [8].

More recently, the high dependency of HCV replication on the host cellular factors during the life cycle has been shown. Increasing data have revealed that a large number of HCV-host cell interactions occur in virus-infected cells, and HCV exploits host factors such as cellular protein and cellular miRNAs to support its genome replication and translation [9, 10]. For example, it reported that about 40 identified human proteins interact with at least two HCV proteins [9, 11].

miRNAs are small non-coding regulative RNAs that have a significant role in diverse arrays of biological cell processes such as death, proliferation, differentiation, apoptosis, tumorigenesis, stress, and immune response [12]. miRNAs have been shown to have different lengths and biogenesis and different distributions among the tissues and organs. Recent studies have shown that knockdown or inhibition of miRNAs inhibits the progression of HCC cells. In addition, let-7b, as a role of oncogene, has been reported to contribute to the modulation of cell proliferation by regulating the cell cycle by targeting FOXO1 [13] & IGF-1R [14] or PLK1 [15] in HCC. By targeting the *NS5B* and 5′ UTR regions of the HCV genome, let-7b inhibits the activity of the HCV replicon, thus reducing the HCVcc infectivity. On the other hand, let-7b with IFNα-2a has a synergistic inhibitory effect on HCV infection [16].

Viral infection can exert profound effects on cellular miRNAs, leading to disruption in the host gene expression. On the other hand, increasing evidence confirms that host miRNAs play different roles in accelerating or suppressing viral replication by direct or indirect interaction [17].

Although direct antiviral or DAA, as new HCV antiviral drugs, have been relatively satisfactory, the following use of these drugs suggests research into other options. By 2017, thirty-one microRNAs have been identified, interacting with the HCV genome and some causing inhibiting the promotion of viral replication. It was revealed that these microRNAs had significant roles in the prognosis and diagnosis of HCV infection at different disease stages. Also, it was found that some of them were highly attractive as therapeutic targets for the development of new anti-HCV treatments [5].

Since let-7b is one of the primary miRNAs with direct anti-HCV activity [10, 18], it was selected for experimental assessment in the current study. Using specific algorithms shows that let-7b targets one critical point in 5′ UTR and two in the *NS5B* gene. This study aimed to assess the increased let-7b expression effect on the downregulation of HCV replication and HCC apoptosis pathways.

Materials and methods

Prediction of miRNA targets

In silico analysis has been described previously [19, 20]. Briefly, to search for miRNAs with their complementary regions of the HCV genome, we analyzed the data based on the ViTa database. Let-7b on the genotype 1b replicon (Con-1, Gene Bank Accession No. AJ238799) had two targets, which were selected based on the highest score of the *NS5B*.

Using miRanda, TargetScan, miRBase, and ViTa databases, we determined the down-regulation effect of microRNAs on HCV replication. The ViTa algorithms revealed that the inhibitory effect of let-7b was on the NS5B gene (in 2 points) and 5′ UTR sequences.

Huh7.5 cell lines and culture

Huh7.5 cell line was a kind gift of Dr. Hosseini (Shiraz University of Medical Sciences, Iran). Stable clones of Huh7.5/let-7b and Huh7.5/scrambled pre- or post-transfected with HCV RNA transcripts as well as naïve Huh7.5 were grown in Dulbecco's modified Eagle medium (Gibco BRL), supplemented with 10% fetal bovine serum (Sigma, Germany), 0.1 M HEPES (Invitrogen), 0.5% L-glutamine, penicillin, and streptomycin sulfate (Invitrogen).

Generation of Huh7.5 /let-7b and Huh7.5/scrambled cell clones

Lentiviral vectors for overexpression of let-7b (pLV-miR-let-7b) and an unspecific control (pLV-control) were purchased from Biosttia (San Diego, CA 92,123, USA). Both of them contained the puromycin resistance gene and RFP reporter. According to the manufacturer's guideline, Huh7.5 cells were transduced with pLV-control and pLV-miR-let-7b, with 8 µg/ ml polybrene. Both stably transduced Huh7.5 clones (Huh7.5/scrambled and Huh7.5/let-7b) were isolated and expanded in the medium containing 2 µM puromycin concentration and 3 MOI/1000 cells lentiviral vectors (Sigma-Aldrich, USA).

The SYBR Green-based real-time PCR assay was used to analyze the let-7b expression. Total RNAs were isolated using Trizol reagent (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol from 3×10^5 cells/mL. According to the manufacturer's instructions for quantifying miRNAs, the SYBR® Green MicroRNA reverse transcription kit was used. BONmir High Sensitivity microRNA 1st Strand cDNA Synthesis

kit (Tehran, Iran) and BON microRNA qPCR Master mix (Tehran, Iran) was used for cDNA synthesis and quantification of let-7b, respectively. The U6 gene was used as a housekeeping gene, and relative gene expression was calculated using the $2^{-\Delta\Delta CT}$ method.

Luciferase assay

Plasmid construction

PCR amplified the open reading frame encoding the truncated *NS5B* gene from the isolated genotype 1 HCV-RNA of a high load infected patient. The 465 bp fragment encompassing let-7b respond element (MRE) was cloned and sub-cloned in pTZ57R/T cloning plasmid and psiCHECK™-2 luciferase vector. Sense and antisense primers of the *NS5B* gene designed with the CLC sequence viewer database's help were F (NS5B): 5′-CTCGAGCTT GTCGTTATCTGTGAAAG-3′ and R (NS5B):5′-CTCGAG GGCCATGGAGTCGTTG-3′ that contained *Xho*I restriction site. Ligation of the 465 bp fragment of the NS5B gene into pTZ57R/T plasmid was performed as described by the supplier (InsTAclone PCR cloning kit; Thermo Scientific™). Then, it was transformed into the *Escherichia coli* strain DH5α. The transformants were chosen on LB-Amp (50 mg/mL) agar plates supplemented with IPTG and X-Gal. Desired constructs were confirmed by PCR, restriction enzyme analysis, and DNA sequencing (Macrogene-Korea) using universal M13 PUC primers. The pTZ57R/T-NS5B construct was digested with *Xho*I and then sub-cloned into the corresponding restriction sites of the psiCHECK™-2 plasmid. The psiCHECK™-2/NS5B constructs were confirmed by PCR and restriction enzyme analysis (13, 14).

Luciferase assay

The psiCHECK ™-2/NS5B construct was used to transfect into Huh7.5/let-7b cells as well as Huh7.5/scrambled and naïve Huh7.5. psiCHECK ™-2/GAPDH construct (a gift from Dr. Atashi) as a luciferase assay control was transfected into Huh7.5/let-7b cells. Briefly, the day before transduction, 1×10^4 cells/well were grown in the 96-well dishes, and on the day of transduction, both psiCHECK ™-2/NS5B and psiCHECK ™-2/GAPDH were transfected with lipofectamine 2000. The transfected cells were harvested and lysed after 48 h to determine the luciferase activity using the Dual-luciferase Reporter Assay System kit (Promega-Germany). Assays were performed in triplicate. Luciferase activities were measured using a luminometer (POLARstar Omega-Germany).

Flow Cytometry and apoptosis evaluation

The apoptosis was evaluated by V-FITC/PI apoptosis detection kit (BD-Pharminogen) 24 h post-transfection in naive, Huh7.5/scrambled and Huh7.5/let-7b, according to the manufacturer's instructions. Cells were assayed with flow cytometry on BD FACSCalibur (BD Biosciences, San Jose, CA) and analyzed with Flow Jo 7.5.5 to determine the population of early and late apoptotic cells.

HCVcc establishment and in-vitro HCV infection

HCV-RNA transfection To develop a cell-cultured hepatitis C virus (HCVcc) of gt 1b, we plated naïve Huh7.5 cells the day before transfection in 6-well plates and incubated them at 37 °C under 5% CO2 condition (Optimization of modification). After reaching 80% confluence, the growth medium was removed from the cells and washed twice with PBS, and then resuspended into 2 mL of Opti- media (Invitrogen, CA). The cells were inoculated with the serum containing 10^5 HCV-RNA copies prepared from HCV positive blood [33]. The cells were plated in 10% DMEM and then passaged every 2–4 days. Using real-time PCR assay, we assessed the titer of progenies viruses. To the generation of an HCV stock, 0.1 moi of the virus was added to 30% confluent naïve Huh7.5 cells, and after 3–4 days post-infection, the cells were trypsinized and replated before the complete confluence. The supernatants were harvested 7-days post infection, harvested, titrated, and stored at – 80 °C.

Infectivity inhibition assay In vitro HCV infection was performed, as described previously. Briefly, the supernatant of 7-days post-infection was serially diluted tenfold in complete DMEM and used to infect 10^4 Huh 7.5/let-7b, Huh7.5/mock, and naive Huh7.5 cell. Following 30–45 min incubation of the inoculum, the supernatant was discarded, and the cells were washed twice with PBS, replaced with fresh complete DMEM, and incubated for 3–4 days at 37 °C under 5% CO_2 condition. Supernatants from infected cells were then harvested in the indicated time point's post-transfection and then analyzed by Taq-man real-time PCR assay.

HCV level quantification and tracking changes HCV genome levels in naïve Huh7.5, Huh7.5/let-7b, and Huh7.5/scrambled cells were assessed using commercially available HCV quantification kits (HCV high pure quantitative viral RNA Kit, Roche, Germany), according to the manufacturer's instructions, using StepOne plus Real-Time.

Statistical analysis

All experimental data were analyzed with at least three independent t-test and Mann- Whitney test, ANOVA ± SD, and

Pearson correlation test. Statistical analyses and draw charts were performed using SPSS 16.0 statistical software package and Graph Pad prism 6. Any differences were assessed using t-test, and $0.01 < P < 0.05$ was considered as significant.

Results

NS5B-HCV is a putative gene for let-7b target sequences

Following in silico search with the ViTa software algorithm, it was revealed that let-7b, as a high score microRNA, is a two-point of NS5B and one point of 5'-NCR genotype 1b replicon complementary (pCon-1 Gene Bank Accession No: AJ238799), (Fig. 1).

Let-7b up-regulated in Huh7.5/let-7b cells, but not in Huh7.5/scrambled and naïve Huh7.5 cells

The red fluorescent protein was measured by FACS/Microscopy, red fluorescence six days after transduction in naïve Huh7.5, Huh7.5/scrambled, and Huh7.5/let-7b cells to count the transduced Huh7.5 cells with lentiviral vectors (Fig. 1.S.). The results revealed that the let-7b expression was markedly increased following pseudo viral vector transduction. As shown in Fig. 2, the expression level of let-7b in Huh7.5 /let-7b transduced cells increased nearly 2.7 folds (Fig. 2).

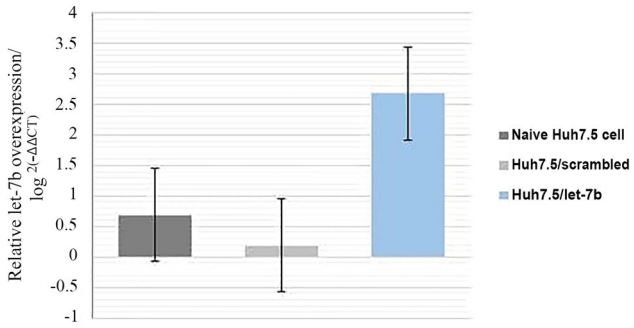


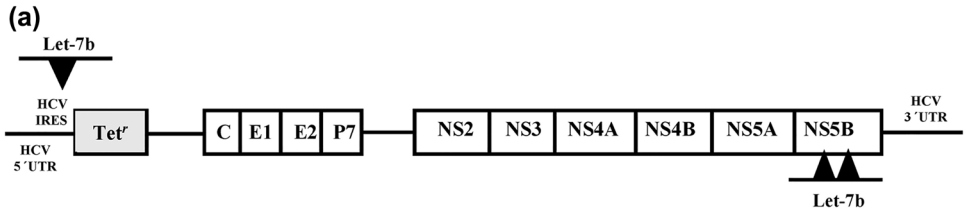
Fig. 2 let-7b miRNA expression in Huh7.5 cells. The real-time PCR analysis of relative expression level (fold change) of let-7b in Huh7.5 cells transduced with scrambled control and pseudo viral vector that overexpressed let-7b. The mean values of three independent experiments (*P- value* < 0.05)

Luciferase assay

Plasmid construction

NS5B gene PCR: PCR was performed using the designed specific primers to obtain the truncated *NS5B* gene. PCR product was evaluated through electrophoresis on 1.5% agarose gel, showing a band of about 465 bp (Fig. 3A).

pTZ57R/T-NS5B construct: The purified *NS5B* gene was cloned in pTZ57R/T cloning vector. Following the transformation and plating of the bacterial cells on LB agar containing appropriate antibiotics, some of the transformed colonies and the extracted recombinant plasmids were PCR-positive. The recombinant plasmid was confirmed by restriction digestion (Fig. 3B). Sequencing analysis (Macrogen, Korea) of the recombinant plasmid also confirmed no amplification errors in the cloned *NS5B* gene sequence.



(b)

Target start location	Score in ViTa	UTR pairing	Mfe (Minimum free energy)	Name/Infected tissue
8962-8980 (*NS5B* gene)	169	3' 5´ miR-let-7b UUGGUGUGUUGGAUGAUGGAGU AGCCACTTGAC---CTACCTCA *NS5B* gene 5´ Seed region 3´	−20.4 Kcal/ mol	Let-7b/ Liver

Fig. 1 **A** Hepatitis C virus complete genome, isolate pCon1/ pCon1/SG-Bsd (I) cell/ 9605 bp/ T7 promoter (genotype 1b HCV)/: (accession NO: AJ238799 in ViTa virus miRNA target). **B** Bioinformatic analysis of miRNAs targeting NS5B. The sequence of let-7b aligned to its predicted target NS5B sequence. The regions of complementary are indicated. Predicted let-7b interaction with NS5B-HCV, according to the web-based ViTa (http://vita.mbc.nctu.edu.tw/)

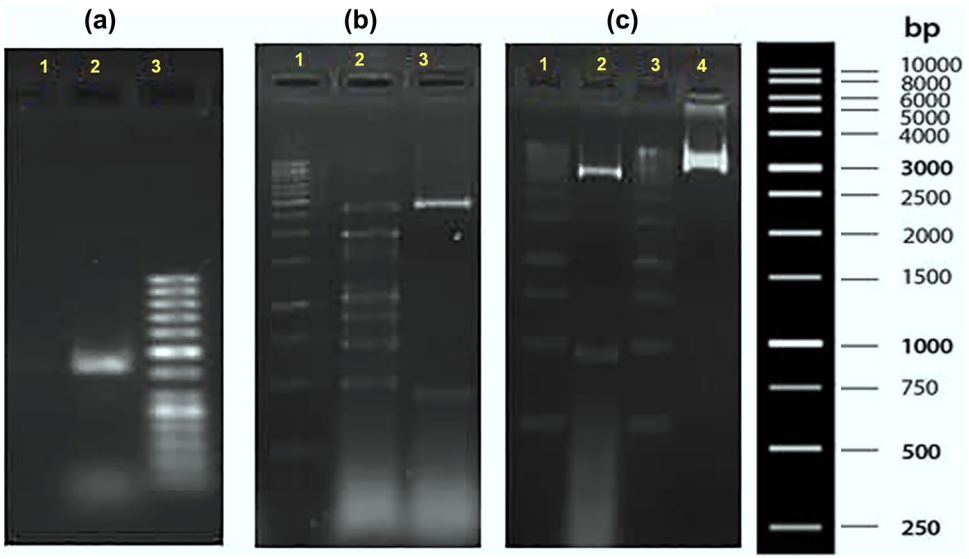


Fig. 3 The gel electrophoresis results of PCR cloning and subcloning, respectively. **A** PCR amplification of NS5B. The fragment size is 465 bp **B** Enzymatic Digestion with restriction enzyme analysis of T/A vector- NS5B. Lane 1 was the DNA size marker (ladder 1 Kb). Lane 2 digested with the EcoR-1 enzyme (Invitrogen). Lane 3 digested with *Xho*1 (Promega). T/A vector (pTZ57R/T: 2886 bp) and NS5B fragment (465 bp) bands were observed. **C** Restriction enzyme analysis of psiCHECK right-oriented ™-2-NS5B. Lane 1 and 3, DNA size marker (ladder 1 kb); Lane 2, digested with *Xho*1; Lane 4, psiCHEEK-2- NS5B digested with BamH-1

psiCHECK™-2-NS5B construct: The *Xho*1 digested construct was subcloned into the corresponding restriction site of the psiCHECK™-2. The existence of the right-oriented *NS5B* gene in the psiCHECK™-2-NS5B construct was confirmed by restriction enzyme analysis as well as gene sequencing (Fig. 3C).

Transient transfection and luciferase activity assay result

To examine whether let-7b miRNA can downregulate translation of *NS5B* gene, we transfected Huh7.5/let-7b with psiCHECK™-2-NS5B construct and psiCHECK™-2-GAPDH as a negative control. Three days after

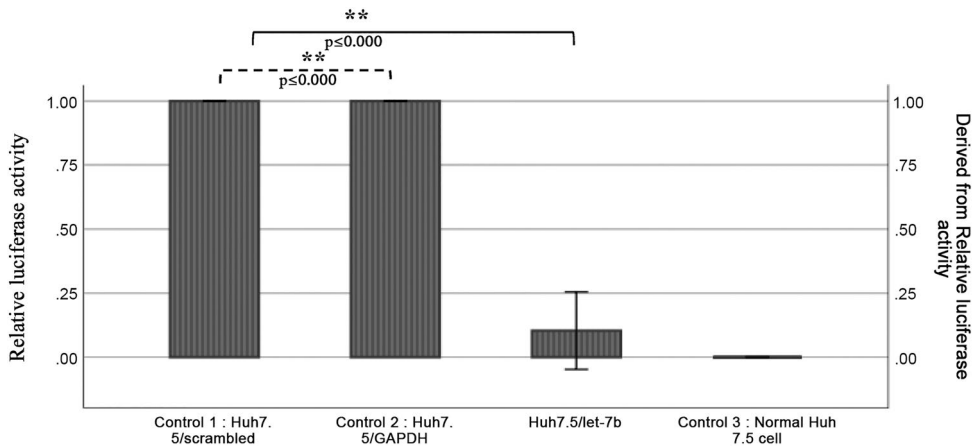


Fig. 4 Luciferase reporter assay. In-vitro dual-luciferase reporter assays of let-7b/NS5B mRNA interactions in Huh7.5. U6 normalized the luciferase reporter activity of each sample. The mean ± SEM of the relative luciferase expression ratio (Firefly luciferase/Renilla luciferase) was calculated for three biological replicates and compared with the negative control (NC), miRNA mimics treatment. All data were analyzed with ANOVA and Mann Whitney ([**] $P < 0.01$). **(Column-1)** Control1: Dual-luciferase reporter assays of NS5B and scramble lentiviral vector showing a high level of luciferase activity **(Column-2)** Control2: Dual-luciferase reporter assays of GAPDH mRNA and let-7b showing no specific miRNA-mRNA interaction and a high level of luciferase activity. **(Column-3)** Dual-luciferase reporter assays of NS5B confirmed its let-7b/NS5B mRNA interaction resulting in a significant decline in luciferase activity. **(Column-4)** Control3 (NC): naiveHuh7.5 cell without any treatment

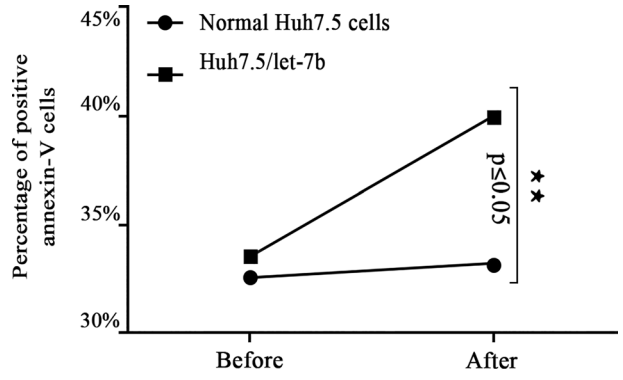


Fig. 5 Let-7b miRNA overexpression induce apoptosis in Huh 7.5. Data represent the percentage of apoptotic cells measured by Annexin V-positive Huh 7.5 cells transduced with the lentiviral vector that overexpresses let-7b. The percentage of apoptotic cells was determined by Annexin V binding using FACS. Data represent the mean percentage of apoptotic cells (±SEM). P-values were calculated by one-way ANOVA and Mann Whitney with a Tukey's post-test analysis

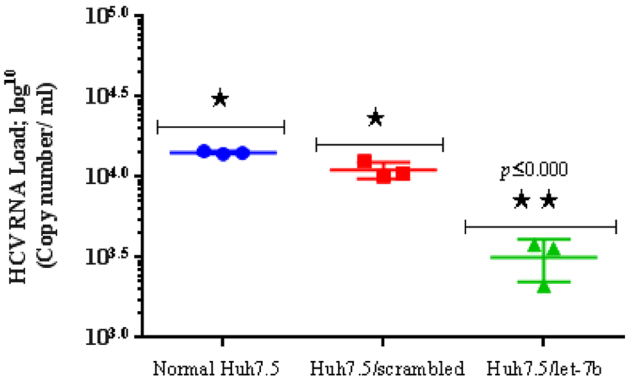


Fig. 6 HCV replication was suppressed in Huh7.5/let7b cells. Overexpression of let-7b leads to the down-regulation of the HCV RNA replication. The data shown are means ± SD of three independent t-test experiments. *, **Significant difference at $p < 0.05$ and $p < 0.01$ respectively. There is a robust and negative correlation between the expression of Huh7.5/let-7b and the suppression of HCV replication (r: −0.854 and p-Value: 0.003**)

transfection, the Huh7.5/let-7b cells showed a significant decrease in the luciferase activity compared to Huh7.5/GAPDH and Huh7.5/scrambled, as shown in Fig. 4.

Let-7b sensitizes Huh7.5 to the apoptosis pathway

The flow cytometry result demonstrated that let-7b overexpression sensitizes Huh7.5 /let-7b cells to apoptosis. Compared with the control cells, let-7b displayed higher apoptotic rates 24 h (Fig. 2.S and Fig. 5).

HCV replication was inhibited in Huh7.5/let-7b

The HCVcc system was utilized to realize the role of let-7b miRNA in HCV infectivity. Huh7.5/let-7b and Huh7.5/scrambled and naïve Huh7.5 were infected by HCV particles derived from Con-1 plasmid (Fig. 3S). HCV particle production was then monitored by Taq-man real-time PCR assay. Our data revealed that new HCV particle production declined significantly 0.6 log10 ($p < 0.001$), (Fig. 6).

Discussion

After Lecellier et al. demonstrated the inhibitory role of miRNAs in the foamy virus replication, limited but valuable studies were conducted on the pivotal role of miRNAs in the proliferation of other viruses such as HCV replication [21]. For example, Jopling et al. conducted the first research in the HCV field. They showed that miR-122 had a proviral activity and can act as a facilitator of viral RNA replication. They suggest that miR-122 may serve as a target for HCV antiviral intervention [22]; based on the importance of miR-122 in HCV infection and HCC progression, several clinical trials of miR-122 are ongoing [23]. Researchers showed that miRNAs were important parts of initial immunity and demonstrated that miR-199a* had antiviral activity and directly targeted the HCV genome, thereby inhibiting the viral replication [19]. Qisheng Li et al. demonstrated that HCV combated the host antiviral barrier through confining hepatocellular miRNAs expression. They reveal that, unlike miR-122, miR-151-5p and miR-17-5p that have proviral activity, let-7a, let-7b, miR-130a, miR-148a, miR-181a, miR-196a, miR-30a-5p, miR-99b and miR-25 have antiviral activity [10].

In another study by our team, Shafaati et al. examined the effects of mir-196a on reducing HCV proliferation through microRNAs and tracing apoptosis [20]. However, we believe that a preliminary study of the apoptosis pathway of Huh7.5/let-7b will be an introduction to emphasize the association of the effect of let-7b in HCVcc. In the upcoming study, we will evaluate the effect of the apoptotic pathway on HCVcc-let-7b as an impact of the tumor suppressor gene.

Let-7 (lethal-7) is the prototype of human miRNAs and one of the first identified microRNAs [24] whose expression changes in different kinds of liver diseases. In the present study, in silico analysis and virologic tests were conducted to determine the role of let-7b, as a novel cellular miRNA, in controlling HCV genome replication. Our data revealed that let-7b targeted the HCV RNA, leading to a decrease in HCV RNA accumulation and viral production. Sherivesta et al. also revealed that let-7b could target the HCV genome at one point at 5'-NCR and two points at the NS5B region of the HCV genome [18, 25–28].

Emerging data demonstrated the let-7/Lin28 molecular switch, as the main regulator of multi-organ harms and cancer progression. Lin28 is a small RNA-binding protein that contains three RNA-binding domains. Lin28B inhibited the let-7 through sequestering of pri-miRNA in the nucleus from the microprocessing complex; in this way, the let-7 maturation and performance are interrupted [29, 30].

In many cases, liver injury is not enough to activate the lin28/let-7 pathway, and "hepatitis" can occur. At this time, the let-7 expression level reportedly is downregulated, and, conversely, the expression of lin28 is up-regulated for injury compensation [30]. If the upregulation of lin28 goes above a milestone, a checkpoint does not occur, and immature hepatocyte as less differentiated cells develops in the liver.

Some studies have shown lin28 overexpression and let-7 suppression in hepatocellular carcinoma patients [31, 32]. Other research found that reduction of let-7 expression could lead to more severe liver injuries in alcoholic and chronically affected patients [28, 33].

Our experimental data not only revealed that let-7b could directly target the HCV RNA, but also it could indirectly increase cell apoptosis. Ras, Myc, HMGA2, CDC25A, and CDK6, as some popular types of oncoproteins, have been identified to be targeted by let7 miRNAs [24]. Since these oncoproteins are mainly involved in cell proliferation, it is believed that let-7 can serve as a tumor suppressant [34]. Shimizu et al. demonstrated that the let-7 miRNA family directly regulated the apoptosis pathway through Bcl-xl direct targeting [24]. Bcl-xl, as well as Bcl-2, Bcl-w, Mcl-1, and Bfl-1, are anti-apoptotic members of the Bcl-2 family and are mainly involved in the mitochondrial apoptosis pathway. Previous studies showed the association of apoptosis pathway and miR-196a/ miR-196b / let-7b expression. In the study conducted by Ren et al. (2019), knockdown of miR-196a or miR-196b inhibited the progression of HCC cells. Also, Yang et al. (2017) and Xu et al. (2016) showed that miR-196a knockdown repressed cell proliferation, colony formation, migration, and invasion but affected cell apoptosis/ cell death by targeting forkhead transcription factor O1 (FOXO1) in HCC. Moreover, miR-196a contributed to cell proliferation by regulating the cell cycle via targeting FOXO1 in HCC [35].

One of the most critical challenges in this study was the limitations of the methodology, i.e. optimization of luciferase assay and preparation of Huh 7.5 cell line and HCVcc establishment in the cell. Due to the loss of access to some techniques and instruments (due to time constraints, insufficient financial support, and sanctions), some methods were abandoned despite their importance, such as Western blot.

In the current study, thanks to the lentiviral vector to introduce let-7b in hepatocytes, there was a permanent expression of defected miRNA to balance its impaired expression. In conclusion, a combination of bioinformatics, dual-luciferase reporter assay, and real-time PCR analysis revealed that let-7b could control the HCV infection; therefore, it can have an anti-HCV application in the future. Furthermore, it was shown that this miRNA sensitized the Huh7.5 cell line to apoptosis and could serve as an anti-tumor agent.

The data analysis of this study can be used as a step further in other related research because it provides new insight into the role of non-coding RNAs, including miRNAs, in the pathogenesis of HCV inhibition and the development of anti-HCV therapies. Let-7b overexpression in the human hepatoma cell line can regulate HCV replication. Nonetheless, there are still many issues about how miRNAs associate with HCV infection pathways, especially apoptosis and immune response pathways, and studying this is one of our future purposes.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11033-021-06955-0.

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Author contributions MJ & MS designed and performed the experiments and drafted and revised the manuscript. MJ & MS performed the experiments and analyzed data. MZ & MK & MZ developed the concept of the study and corrected the manuscript, supported the experiments technically, and revised. The manuscript all authors read and approved the final manuscript.

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Availability of data and materials The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest Marzieh Jamalidoust, Maryam Shafaati, Mehdi Kalani, Maryam Zare, Mazyar Ziyeayan declare that they have no financial or non-financial conflict of interest.

Consent to participate Not applicable.

Ethical approval Not applicable.

In the Statement of Ethics The paper is exempted from ethical committee approval. This study was done fundamentally, and clinical and animal studies and their ethical considerations are not included in this study.

References

1. Talwani R, Gilliam BL, Howell C (2011) Infectious diseases and the liver. Clin Liver Dis 15(1):111–130
2. Jamalidoust M, Namayandeh M, Asaei S, Aliabadi N, Ziyaeyan M (2014) Determining hepatitis C virus genotype distribution among high-risk groups in Iran using real-time PCR. World J Gastroenterol: WJG 20(19):5897
3. Hajarizadeh B, Razavi-Shearer D, Merat S, Alavian SM, Malekzadeh R, Razavi H (2016) Liver disease burden of hepatitis C virus infection in Iran and the potential impact of various treatment strategies on the disease burden. Hepatitis Monthly. https://doi.org/10.5812/hepatmon.37234
4. Jamalidoust M, Namayandeh M, Moghadami M, Ziyaeyan M (2017) Comparison of HCV viral load and its genotype distributions in HCV mono-and HIV/HCV co-infected illicit drug users. Virology Journal 14(1):127
5. Cullen BR (2013) How do viruses avoid inhibition by endogenous cellular microRNAs? PLoS Pathog. 9(11):e1003694
6. Sanna A, Le Strat Y, Roudot-Thoraval F, Burban SD, Carrieri P, Delarocque-Astagneau E et al (2017) Severe liver disease related to chronic hepatitis C virus infection in treatment-naive patients: epidemiological characteristics and associated factors at first expert centre visit, France, 2000 to 2007 and 2010 to 2014. Eurosurveillance 22(30):30582
7. Prestes NGdO. Estudo metagenômico de arbovírus e outros vírus de RNA de vertebrados em roedores e marsupiais procedentes do município de Viseu no Estado do Pará-Brasil. 2019.
8. Shiokawa M, Fukuhara T, Ono C, Yamamoto S, Okamoto T, Watanabe N et al (2014) Novel permissive cell lines for complete propagation of hepatitis C virus. J Virol 88(10):5578–5594
9. Han Y, Niu J, Wang D, Li Y (2016) Hepatitis C virus protein interaction network analysis based on hepatocellular carcinoma. PLoS ONE 11(4):e.0153882
10. Li Q, Lowey B, Sodroski C, Krishnamurthy S, Alao H, Cha H et al (2017) Cellular microRNA networks regulate host dependency of hepatitis C virus infection. Nat Commun 8(1):1–16
11. Dehghani B, Dehghani A, Sarvari J (2020) Knowledge and awareness regarding hepatitis B, hepatitis C, and human immunodeficiency viruses among college students: a report from Iran. Int Q Commun Health Educ 41(1):15–23
12. He Y, Yang K, Zhang X (2014) Viral microRNAs targeting virus genes promote virus infection in shrimp in vivo. J Virol 88(2):1104–1112
13. Xu H, Liu C, Zhang Y, Guo X, Liu Z, Luo Z et al (2014) Let-7b-5p regulates proliferation and apoptosis in multiple myeloma by targeting IGF1R. Acta Biochim Biophys Sin 46(11):965–972
14. Liu Q, Shi H, Yang J, Jiang N (2019) Long non-coding RNA NEAT1 promoted Hepatocellular Carcinoma cell proliferation and reduced apoptosis through the regulation of Let-7b-IGF-1R Axis. Onco Targets Ther 12:10401
15. He Z, Deng W, Jiang B, Liu S, Tang M, Liu Y et al (2018) Hsa-let-7b inhibits cell proliferation by targeting PLK1 in HCC. Gene 673:46–55
16. Cheng J-C, Yeh Y-J, Tseng C-P, Hsu S-D, Chang Y-L, Sakamoto N et al (2012) Let-7b is a novel regulator of hepatitis C virus replication. Cell Mol Life Sci 69(15):2621–2633
17. Bruscella P, Bottini S, Baudesson C, Pawlotsky J-M, Feray C, Trabucchi M (2017) Viruses and miRNAs: more friends than foes. Front Microbiol 8:824
18. Shrivastava S, Steele R, Ray R, Ray RB (2015) MicroRNAs: role in hepatitis C virus pathogenesis. Genes & diseases 2(1):35–45
19. Murakami Y, Aly HH, Tajima A, Inoue I, Shimotohno K (2009) Regulation of the hepatitis C virus genome replication by miR-199a. J Hepatol 50(3):453–460
20. Shafaati M, Jamalidoust M, Kargar M, Arefian E, Kafilzadeh F (2021) Downregulation of hepatitis C virus replication by miR-196a using lentiviral vectors. Microbiol Immunol 65(4):161–170
21. Lecellier C-H, Dunoyer P, Arar K, Lehmann-Che J, Eyquem S, Himber C et al (2005) A cellular microRNA mediates antiviral defense in human cells. Science 308(5721):557–560
22. Jopling CL, Yi M, Lancaster AM, Lemon SM, Sarnow P (2005) Modulation of hepatitis C virus RNA abundance by a liver-specific MicroRNA. Science 309(5740):1577–1581
23. Fu X, Calin GA (2018) miR-122 and hepatocellular carcinoma: from molecular biology to therapeutics. EBioMedicine 37:17–18
24. Shimizu S, Takehara T, Hikita H, Kodama T, Miyagi T, Hosui A et al (2010) The let-7 family of microRNAs inhibits Bcl-xL expression and potentiates sorafenib-induced apoptosis in human hepatocellular carcinoma. J Hepatol 52(5):698–704
25. Tsialikas J, Romer-Seibert J (2015) LIN28: roles and regulation in development and beyond. Development 142(14):2397–2404
26. McDaniel K, Hall C, Sato K, Lairmore T, Marzioni M, Glaser S et al (2016) Lin28 and let-7: roles and regulation in liver diseases. Am J Physiol-Gastrointestinal Liver Physiol 310(10):G757–G765
27. McDaniel K, Huang L, Sato K, Wu N, Annable T, Zhou T et al (2017) The let-7/Lin28 axis regulates activation of hepatic stellate cells in alcoholic liver injury. J Biol Chem 292(27):11336–11347
28. Torres J-L, Novo-Veleiro I, Manzanedo L, Alvela-Suárez L, Macías R, Laso F-J et al (2018) Role of microRNAs in alcohol-induced liver disorders and non-alcoholic fatty liver disease. World J Gastroenterol 24(36):4104
29. Triboulet R, Pirouz M, Gregory RI (2015) A single let-7 microRNA bypasses LIN28-mediated repression. Cell Rep 13(2):260–266
30. Zhang H, Zong Y, Qiu G, Jia R, Xu X, Wang F et al (2018) Silencing Lin28 promotes apoptosis in colorectal cancer cells by upregulating let-7c targeting of antiapoptotic BCL2L1. Mol Med Rep 17(4):5143–5149
31. Cheng S-W, Tsai H-W, Lin Y-J, Cheng P-N, Chang Y-C, Yen C-J et al (2013) Lin28B is an oncofetal circulating cancer stem cell-like marker associated with recurrence of hepatocellular carcinoma. PLoS ONE 8(11):e80053
32. Zhang J, Xu A, Miao C, Yang J, Gu M, Song N (2019) Prognostic value of Lin28A and Lin28B in various human malignancies: a systematic review and meta-analysis. Cancer Cell Int 19(1):79
33. McDaniel K, Herrera L, Zhou T, Francis H, Han Y, Levine P et al (2014) The functional role of micro RNA s in alcoholic liver injury. J Cell Mol Med 18(2):197–207
34. Büssing I, Slack FJ, Großhans H (2008) let-7 microRNAs in development, stem cells and cancer. Trends Mol Med 14(9):400–409
35. Shafaati M, Jamalidoust M, Kargar M, Arefian E, Kafilzadeh F (2021) Downregulation of hepatitis C virus replication by miR-196a using lentiviral vectors. Microbiol Immunol 65:161–170