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Original research article

Interaction of Host miRNA and Viral RNA of Hepatitis C Virus as New Drug Targets Against Development of Hepatocellular Carcinoma

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ABSTRACT

Hepatitis C virus (HCV) infection induces significant changes in hepatocytes over time. Host-derived microRNAs (miRNAs), small non-coding RNAs (22 nucleotides), are believed to influence the persistence or suppression of chronic HCV infection by modulating viral genome replication, transcription, and translation. However, their contribution to the development of hepatocellular carcinoma (HCC), as well as the specific interactions between miRNAs and distinct HCV genotypes, remain insufficiently understood. This study explores the potential involvement of host miRNAs in HCC progression among patients infected with different HCV genotypes. Using various bioinformatics tools—such as Clustal Omega, ChimeraX: AlphaFold, EMBOSS Transeq, UNAFOLD, and Freiburg IntaRNA—we analyzed the site-specific binding of key miRNAs (miR-122, miR-21, miR-155, and miR-193a-5p) across all seven HCV genotypes. Our findings revealed that miR-122 predominantly targets the NS5B coding region in most genotypes, suggesting a role in inhibiting viral replication and translation. Conversely, certain miRNAs that bind to the Envelope (E) region may enhance viral gene expression. These results indicate that sequence homology between host miRNAs and HCV mRNA can either suppress or facilitate viral gene translation. Targeting miRNAs that promote HCV replication may offer a novel therapeutic strategy for managing HCV-related diseases.

Keywords: Genotypes; HCV; Hepatocellular carcinoma; miRNA

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1. Introduction

On a global scale, an estimated 50 million individuals are suffering from chronic Hepatitis C Virus (HCV) infection, while approximately 1 million new cases are added annually. According to the World Health Organization (WHO), HCV was responsible for 242,000 deaths during 2022, most of which were associated with liver cirrhosis and hepatocellular carcinoma (HCC), or primary liver cancer [1]. Despite this, the cause of HCC among HCV patients is unknown.

HCV is classified under the family Flaviviridae and genus Hepacivirus. Its genome (9.6 kilobases in length) is comprised of a positive-sense single stranded RNA [2, 3] with an open reading frame that, upon translation, encodes a polyprotein which is later converted into 10 viral proteins [3]. The virus includes three structural proteins viz; Core (C), Envelope 1 (E1) and Envelope 2 (E2) and seven non-structural proteins viz; p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B [4].

The HCV structural Core protein, weighing 21KDa comprised of 177 amino acids, acts as the viral capsid, enveloping and safeguarding the viral RNA within a spherical structure [3]. This protein regulates transcription of genes responsible for metabolism disturbance and cellular death, leading to oxidative stress, liver steatosis, and HCC [4]. The humoral immune response primarily targets glycosylated proteins (23 KDa, 192 amino acids) & E2 (70 KDa, 363 amino acids), which are responsible for membrane fusion and entering the host cells [5, 6].

HCV was discovered in 1989 [7] and due to its remarkable genetic diversity, the number of known subtypes has grown from 18 -67 in 2005; to 86-90 in 2013 [8], reaching 93 subtypes by March 2022. At present, 8 genotypes and 93 subtypes have been reported [9].

HCC is the most common malignant liver tumour and a leading cause of cancer-related deaths globally. HCC is the third most common cause of cancer-related mortality, with a poor prognosis due to late detection, limited therapy options, and high recurrence rates [10].

MicroRNAs (miRNAs) have emerged as potent and dynamic regulators of the progression from chronic HCV infection to HCC. miRNAs are short non-coding RNAs, approximately 22 nucleotides in length, that can regulate gene expression by binding to a coding mRNA and silencing or degrading it [11].

Previous studies have indicated that miRNAs play a crucial role in regulating liver development, regeneration, and metabolism. This perhaps is the reason that changes in intrahepatic miRNA networks have been associated with liver diseases such as hepatitis, cirrhosis, steatosis, and HCC. Among the list of miRNAs, miR-122 is the most prevalent miRNA in the adult liver and plays a key role in liver function as well as in liver disease [12].

Several miRNAs, including miR-122, miR-21, miR-155, and miR-193a-5p have been linked to liver inflammation, fibrosis, and oncogenesis, particularly in HCV-infected patients [13-16].

Research into the evolution of HCV genotypes holds crucial significance for global health initiatives targeting HCV eradication [4]. Along with knowledge generated on various genotypes, the role that miRNA plays in HCC can either be as a tumor suppressor or as an oncogene, termed oncomiRs, depending on their specific targets and functions [17].

Since interaction between the HCV and liver-associated miRNAs most likely

aids in liver disease progression, the knowledge generated on the role of miRNAs in HCV-induced liver cell damage and HCC, which is unknown [18], will be the great significance.

Although the evolution of miRNA was seen long after the various genotypes were already in circulation, their comparative role in inducing HCC has not been fully uncovered. We hypothesize that during the course of intracellular replication of HCV proteins, incomplete viral progenies might have been produced (abortive infection), leaving viral RNA units inside the cell cytoplasm, which over time might have become functional miRNAs.

By studying the nucleotide sequence homology/complementarity of viral RNA and miRNA reported to be present in HCV infected cells, the present paper reports the possible causal role of host miRNA in causing HCC among HCV infected patients.

2. Materials and Methods

2.1 Data collection

The HCV genotype sequences (1–7) and host miRNAs were collected from the NCBI (National Center for Biotechnology Information) database, USA, and the miRbase database, UK (Table 1). Due to the unavailability of detailed annotation in the NCBI database and lack of details of HCV genotype 8 in FASTA sequences, we conducted in-depth analysis of 27 research publications available on Google Scholar focusing on host miRNA involved in HCV infection leading to HCC [19]. Since the published literature which we included did not have any mention of genotype 8, our study focused on HCV genotypes 1–7.

The analysis revealed that miR-122 was the most frequently reported, appearing in eight studies, predominantly exhibiting downregulation in the context of HCV infection. Whereas, miR-21 and miR-193a-5p were reported in three studies and miR-155 in two studies and were shown to be upregulated. Based on the frequency of reporting and the observed differential expression patterns, we selected miR-21, miR-193a-5p, miR-155, and miR-122 for further investigation in this study.

The complementary sequence of selective miRNAs was obtained from the Sequence Manipulation Suite (complementary) database of the miRNAs as listed in Table 2.

Following that, both the HCV genotypes and the complementary miRNA sequences were aligned via Clustal Omega database, UK. The objective of this prediction was to indicate the particular regions of interaction between the miRNA and the HCV viral genome.

2.2 Two- and Three-Dimensional structure determination

The 2D structure of miRNA was predicted using the UNAFOLD web server. FASTA sequences of miR-122, 21, 155, and 193a-5p were entered, and the ”Fold RNA” option was selected. Two possible circular structures were generated for each miRNA, along with their corresponding delta G values. The information obtained from RNAdraw, including the secondary structure in CT format, was copied and pasted into the automated 3D RNA structure modelling RNA composer tool, where it was converted into dot-bracket notation. This resulting structure was then copied and pasted into the RNA composer homepage as the FASTA sequence, leading to the generation of a PDB (Protein Data Bank) file. This PDB file was then used in conjunction with the Pymol database to design the 3D structure of the miRNA.

To determine the impact on trans-

Table 1. Different HCV genotypes and host miRNAs.

HCV Genotype	Base pairs	Accession no. (NCBI)	Host miRNAs	Base pairs	Accession no. (miRbase)
HCV-1	9646 bp	NC_004102.1	miR-122	22 bp	MIMAT0000421
HCV-2	9711 bp	NC_009823.1	miR-21	22 bp	MIMAT0000076
HCV-3	9456 bp	NC_009824.1	miR-155	24 bp	MIMAT0000646
HCV-4	9355 bp	NC_009825.1	miR-193a-5p	22 bp	MIMAT0004614
HCV-5	9343 bp	NC_009826.1			
HCV-6	9628 bp	NC_009827.1			
HCV-7	9443 bp	NC_030791.1			

Table 2. Host miRNAs with original and reverse complementary sequences.

miRNAs	Original sequence	Reverse complementary sequence
miR-122	5'-UGGAGUGUGACAAUGGUGUUUG-3'	3'-CAAACACCATTGTCACACTCCA-5'
miR-21	5'-UAGCUUAUCAGACUGAUGUUGA-3'	3'-TCAACATCAGTCTGATAAGCTA-5'
miR-155	5'- UUAAUGCUAAUCGUGAUAGGGGUU-3'	3'-AACCCCTATCACGATTAGCATTAA-5'
miR-193a-5p	5'- UGGGUCUUUGCGGGCGAGAUGA-3'	3'-TCATCTCGCCCGCAAAGACCCA-5'

lational protein synthesis, a comparison was made between functional protein synthesis (without miRNA binding) and non-functional protein synthesis (with miRNA bound to the HCV region). Functional protein sequences were acquired from the NCBI by entering specific accession numbers and selecting the appropriate mat peptide sequences. These sequences were converted from nucleotides to amino acids using the FASTA format. For the non-functional protein synthesis, the portion where the miRNA attaches was removed from the specific binding protein FASTA sequence, and the remaining sequence was converted into amino acids using the EMBOS Transeq (European Molecular Biology Open Software Suite).

Further functional and modified protein sequence structures were obtained from the ChimeraX, UC San Francisco. The sequences were submitted to the Chimerax for structure prediction using AlphaFold. The resulting structures were enhanced for clarity and quality by importing the PDB file into Pymol and adjusting various settings such as transparency, surface display, and so on.

The Freiburg IntaRNA is a software tool developed to precisely analyze RNA-RNA interactions. Its primary purpose is to predict mRNA binding sites for specified non-coding RNA (ncRNAs), such as eukaryotic miRNAs or bacterial small RNAs (sRNAs). The host miRNA FASTA sequence was entered into the Query ncRNA box while the HCV genotype FASTA sequence was entered into the target RNA (long) box.

The homology or complementarity of four miRNAs i.e., miR-122, miR-21, miR-155, and miR-193a-5p with seven HCV genotypes were studied. The affinity of complementary binding was evaluated by the binding energy scores.

3. Results and Discussion

3.1 miRNA and HCV genotypes interaction

The detailed interaction of individual miRNA with HCV genotypes are mentioned in Table 3.

In HCV genotype 1, the mRNA-miRNA interactions were as follows. miR-122 bound NS5B with a binding energy of -6.01 Kcal/mol. The interaction involved

Table 3. Interaction of individual miRNAs with various HCV genotypes.

Host miRNAs	Hepatitis C Virus genotype	Binding region	Nucleotide sequence	Binding energy Kcal/mol	No. of complementary Nucleotides	Possible Role
miR-122 (22bp)	HCV-1 (9646 bp)	NS5B	7602-9374	-6.01	8	Inhibition of HCV translation
	HCV-2 (9711 bp)	**E2**	1490-2590	-15.03	13	Promotes HCV translation
	HCV-3 (9456 bp)	NS5B	7630-9402	-14.63	16	Inhibition of HCV translation
	HCV-4 (9355 bp)	NS5B	7531-9303	-14.07	14	Inhibition of HCV translation
	HCV-5 (9343 bp)	NS5B	7549-9321	-13.2	13	Inhibition of HCV translation
	HCV-6 (9628 bp)	NS5B (two different locations)	7627-9399	n/a	n/a	Inhibition of HCV translation
	HCV-7 (9443 bp)	NS5A & NS5B	(6268-7605) (7606-9443)	n/a	n/a	Inhibition of HCV translation
miR-21 (22 bp)	HCV-1 (9646 bp)	E2 & NS4B	(1491-2579) (5475-6257)	n/a	n/a	Indeterminant
	HCV-2 (9711 bp)	NS2 & NS3	(2780-3430) (3431-5323)	n/a	n/a	Inhibition of HCV translation
	HCV-3 (9456 bp)	NS4B	5491-6273	-7.17	13	Inhibition of HCV translation
	HCV-4 (9355 bp)	E1	853-1428	-5.94	13	Promotes HCV translation
	HCV-5 (9343 bp)	NS5A & NS5B	(6199-7548) (7549-9321)	n/a	n/a	Inhibition of HCV translation
	HCV-6 (9628 bp)	NS5A	(6274-7626)	-7.51	13	Inhibition of HCV translation
	HCV-7 (9443 bp)	E2 & NS3	(1489-2589) (3430-5322)	n/a	n/a	Indeterminant
miR-155 (24 bp)	HCV-1 (9646 bp)	E2 & 3'UTR	(1491-2579) (9378-9646)	n/a	n/a	Promotes HCV translation
	HCV-2 (9711 bp)	NS5B	7667-9439	-11.05	13	Inhibition of HCV translation
	HCV-3 (9456 bp)	E2	1489-2595	-9.05	13	Promotes HCV translation
	HCV-4 (9355 bp)	NS3	3358-5250	-9.74	16	Inhibition of HCV translation
	HCV-5 (9343 bp)	E2	1429-2520	-13.28	19	Promotes HCV translation
	HCV-6 (9628 bp)	NS5B & 3'UTR	(7627-9399) (9400-9628)	n/a	n/a	Indeterminant
	HCV-7 (9443 bp)	NS5B	7606-9378)	-9.12	9	Inhibition of HCV translation
miR-193a-5p (22 bp)	HCV-1 (9646 bp)	NS3	3420-5312	-10.48	12	Inhibition of HCV translation
	HCV-2 (9711 bp)	p7 & NS5B	(2591-2779) (7667-9439)	n/a	n/a	Inhibition of HCV translation
	HCV-3 (9456 bp)	NS3 & NS5B (Two locations)	(3436-5328) (7630-9402)	n/a	n/a	Inhibition of HCV translation
	HCV-4 (9355 bp)	NS5B	6196-7530	-9.04	13	Inhibition of HCV translation
	HCV-5 (9343 bp)	E2	1429-2520	-12.13	10	Promotes HCV translation
	HCV-6 (9628 bp)	NS3 & NS5B	(3436-5328) (7627-9399)	n/a	n/a	Inhibition of HCV translation
	HCV-7 (9443 bp)	NS2	2779-3429	-15	10	Inhibition of HCV translation

*n/a: not applicable

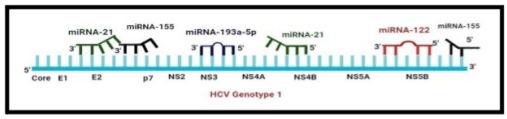

Fig. 1. Schematic representation of Hepatitis C Virus genotype 1 along with host miRNAs (miR-122,21,155, and 193a-5p) complementary regions.

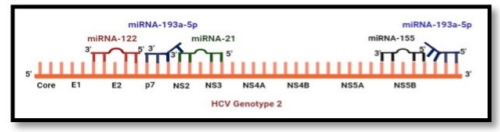

Fig. 2. Schematic representation of Hepatitis C Virus genotype 2 along with host miRNAs (miR-122,21,155 and 193a-5p) complementary regions.

14 nucleotides and 8 nucleotides were complementary. miR-21 bound E2 and NS4B. The interaction involved 15 nucleotides. miR-155 bound E2 and the 3'UTR. miR-193a-5p bound the protein coding region of NS3 with a binding energy of -10.48 Kcal/mol. The interaction involved 12 nucleotides, and 12 nucleotides were complementary (Fig 1).

In HCV genotype 2, the mRNA-miRNA interactions were as follows. miR-122 bound the protein coding region of E2 with a binding energy of -15.03 Kcal/mol. The interaction involved 15 nucleotides and 13 nucleotides were complementary. miR-21 bound NS2 and the protein coding region of NS3. The interaction involved 15 nucleotides. miR-155 bound the protein coding region of NS5B with a binding energy of -11.05 Kcal/mol. The interaction involved 15 nucleotides, and 13 nucleotides were complementary. miR-193a-5p bound the protein coding region of P7 and NS5B. The interaction involved 17 nucleotides (Fig 2).

In HCV genotype 3, the mRNA-miRNA interactions were as follows. miR-

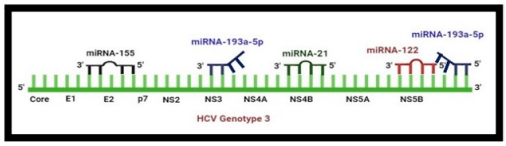

Fig. 3. Schematic representation of Hepatitis C Virus genotype 3 along with host miRNAs (miR-122,21,155 and 193a-5p) complementary regions.

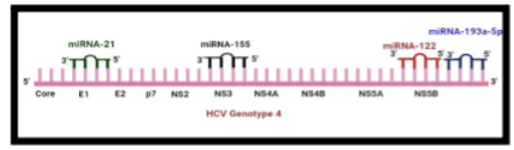

Fig. 4. Schematic representation of Hepatitis C Virus genotype 4 along with host miRNAs (miR-122,21,155 and 193a-5p) complementary regions.

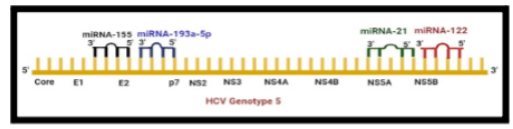

Fig. 5. Schematic representation of Hepatitis C Virus genotype 5 along with host miRNAs (miR-122,21,155 and 193a-5p) complementary regions.

122 bound the protein coding region of NS5B with a binding energy of -14.63 Kcal/mol. The interaction involved 15 nucleotides, and 16 nucleotides were complementary. miR-21 bound NS4B with a binding energy of -7.17 Kcal/mol. The interaction involved 14 nucleotides, and 13 nucleotides were complementary. miR-155 bound the protein coding region of E2 with a binding energy of -9.05 Kcal/mol. The interaction involved 15 nucleotides, and 13 nucleotides were complementary. miR-193a-5p bound the protein coding region of NS3 and NS5B. The interaction involved 15 nucleotides (Fig 3).

In HCV genotype 4, the mRNA-miRNA interactions were as follows. miR-122 bound the protein coding region of NS5B with a binding energy of -14.07 Kcal/mol. The interaction involved 14 nucleotides, and 14 nucleotides were complementary. miR-21 bound the protein coding region of E1 with a binding energy of -5.94 Kcal/mol. The interaction involved 13 nucleotides, and 13 nucleotides were complementary. miR-155 bound the protein coding region of NS3 with a binding energy of -9.74 Kcal/mol. The interaction involved 16 nucleotides, and 16 nucleotides were complementary. miR-193a-5p bound the protein coding region of NS5B with a binding energy of -9.04 Kcal/mol. The interaction involved 14 nucleotides, and 13 nucleotides were complementary (Fig 4).

In HCV genotype 5, the mRNA-miRNA interactions were as follows. miR-122 bound the protein coding region of NS5B with a binding energy of -13.2 Kcal/mol. The interaction involved 14 nucleotides, and 13 nucleotides were complementary. miR-21 bound NS5A and the protein coding region of NS5B. The interaction involved 16 nucleotides. miR-155 bound the protein coding region of E2 with a binding energy of -13.28 Kcal/mol. The interaction involved 15 nucleotides, and 19 nucleotides were complementary. miR-193a-5p bound E2 with a binding energy of -12.13 Kcal/mol. The interaction involved 15 nucleotides, and 10 nucleotides were complementary (Fig 5).

In HCV genotype 6, the mRNA-miRNA interactions were as follows. miR-122 bound NS5B in two different locations. The interaction involved 15 nucleotides. miR-21 bound the protein coding region of NS5A with a binding energy of -7.51 Kcal/mol. The interaction involved 16 nucleotides, and 13 nucleotides were complementary. miR-155 bound the protein cod-

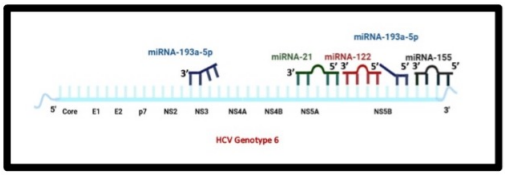

Fig. 6. Schematic representation of Hepatitis C Virus genotype 6 along with host miRNAs (miR-122,21,155 and 193a-5p) complementary regions.

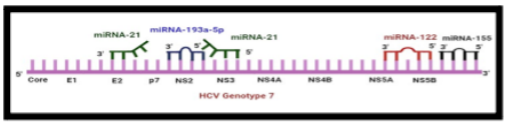

Fig. 7. Schematic representation of Hepatitis C Virus genotype 7 along with host miRNAs (miR-122,21,155 and 193a-5p) complementary regions.

ing region of NS5B and the 3'UTR in the NS3 region. The interaction involved 17 nucleotides. miR-193a-5p bound the protein coding region of NS3 and NS5B. The interaction involved 16 nucleotides (Fig 6).

In HCV genotype 7, the mRNA-miRNA interactions were as follows. miR-122 bound NS5A and the protein coding region of NS5B. The interaction involved 16 nucleotides. miR-21 bound E2 NS3. The interaction involved 16 nucleotides. miR-155 bound the protein coding region of NS5B with a binding energy of -9.12 Kcal/mol. The interaction involved 15 nucleotides, and 9 nucleotides were complementary. miR-193a-5p bound the protein coding region of NS2 with a binding energy of -15 Kcal/mol. The interaction involved 15 nucleotides, and 10 nucleotides were complementary (Fig 7).

We found that the core protein coding region at the 5' end is not involved in complementary recognition with miRNAs (miR-122, 21, 155, and 193a–5p). The

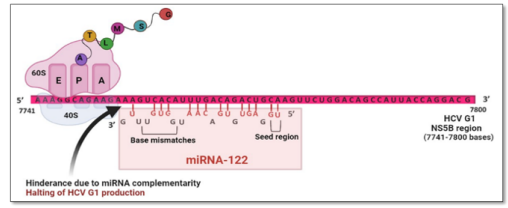

Fig. 8. Schematic diagram showing complementary nucleotide sequences matches with Hepatitis C virus 1 genotype with miR-122 leads to inhibition of translation (created with https://www.biorender.com/).

HCV mRNA may encode abundant viral core proteins, which contribute to the disturbing of the host cellular metabolism and progression of HCC. According to Moriya et al., 1998, work with transgenic mice demonstrated that core protein of HCV contributes important role in the development of HCC. A similar observation was seen in other study by Nguyen et al., 2006.

Another observation that can be made from these interactions is that the possibility of binding of these miRNAs on the HCV genotypes at one particular time, if they are synthesized by the liver at one particular time, leaving the C and E1 region unattended. This further strengthens the idea that C and E1 may participate further for progression to HCC. A possible translation/inhibition model of miR-122 HCV interaction can be seen in Fig. 8.

3.2 Three dimensional structural prediction of proteins

Structural alterations were observed in the non-functional viral protein compared to the functional viral protein when they interact with host miRNAs within the genomes of the various genotypes of HCV (Fig. 9).

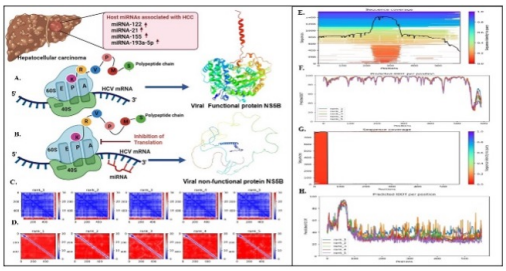

Fig. 9. Host miRNAs associated with HCC showing increased expression reported by Hussein et al., 2022, Ratnasari et al., 2022 (miRNA-21), Ullah et al., 2022(miR-122), Niu et al., 2021(miRNA-155), Loosen et al., 2020 (miRNA-193a-5p). (A)Viral functional protein synthesis (NS5B) (B.) Interaction of HCV NS5B complementary region with miR-122 resulted into synthesis of viral non-functional protein. (C.) and (D.) Prediction aligned error (PAE) score for models ranked 1 to 5 of viral Functional NS5B protein and viral non-Functional NS5B protein. (E.) and (G.) Multiple sequence alignment of viral Functional NS5B protein and viral non-Functional NS5B protein. (F.) and (H.) Predicted local distance difference test (LDDT) score per position for the five models generated by AlphaFold 2 of Functional and non-functional viral protein.

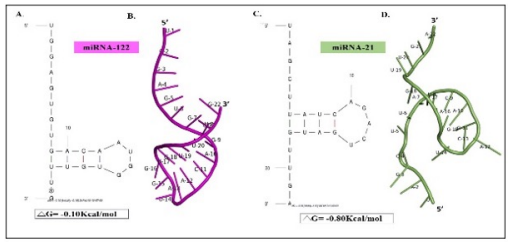

Fig. 10. 2D and 3D structures of miR-122 and miR-21 with their respective Gibbs free energies.

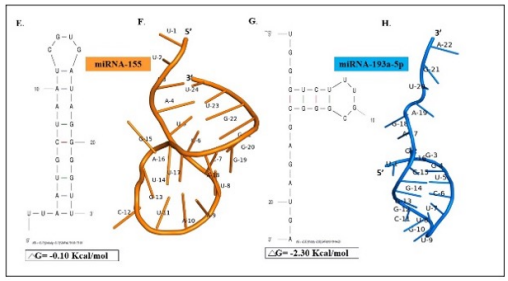

Fig. 11. 2D and 3D structures of miR-155 and miR-193a-5p with their respective Gibbs free energies.

3.3 Two- and Three-Dimensional structure prediction of miRNA

Free Gibbs energy required for the 2D structure formation in miR-122, miRNA-21, miR-155, and miRNA-193a-5p was -0.10 kcal/mol, -0.80 kcal/mol, -0.10 kcal/mol, and -2.30 kcal/mol, respectively. miR-193a-5p showed the highest binding energy among the host miRNAs (Figs. 10-11).

3.4 miRNA-HCV Genome (1-7 Genotypes) interaction

The interaction between host miR-122 and 1-7 Genotypes of HCV were studied. The binding energies were found to be -6.01kcal/mol, -15.03kcal/mol, -14.63kcal/mol, -14.07kcal/mol, and -13.2kcal/mol for HCV G1, G2, G3, G4, and G5, respectively. Maximum binding affinity was found in HCV G2 with miR-122. Higher binding affinity was seen in above 13 nucleotide base pairing in case of miR-122. The analysis was undertaken using Freiburg RNA Tools (Inta RNA-RNA interaction), considering only complementary sequences that attached to miRNAs. In some instances, complete FASTA sequences of specific binding regions were used due to the absence of interaction in sequences like HCV5 with miR-122 and G2, G5, G7 with miR-155, and G5 with miR-193a-5p. However, alignments involving two or three attached regions were not performed with this tool.

Another investigation involved the interaction of host miR-21 with HCV G3, G4, and G6, resulting in energies of -7.17kcal/mol, -5.94kcal/mol, and

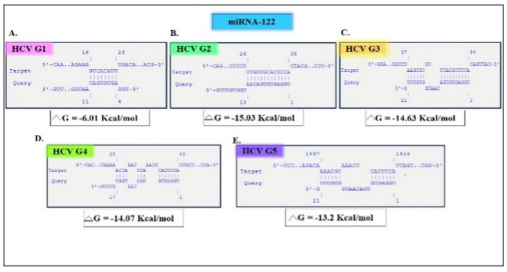

Fig. 12. RNA- RNA interaction showing energies of various HCV genotypes 1,2,3,4, and 5 with miR-122 -6.01kcal/mol, -15.03kcal/mol, -14.63kcal/mol, -14.07kcal/mol, and -13.2kcal/mol. Created with https://rna.informatik.uni-freiburg.de/.

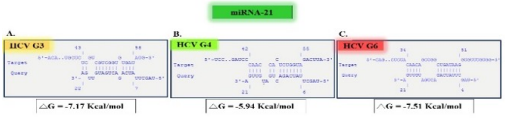

Fig. 13. RNA- RNA interaction showing energies of various HCV genotypes 3,4, and 6 with miR-21 -7.17kcal/mol, -5.94kcal/mol, and-7.51kcal/mol.

-7.51kcal/mol, respectively. Additionally, host miR-155 showed interactions with HCV G2, G3, G4, G5, and G7, yielding energies of -11.05kcal/mol, -9.05kcal/mol, 9.74kcal/mol, -13.28kcal/mol, and -9.12kcal/mol, respectively. Host miR-193a-5p exhibited interactions with HCV G1, G4, G5, and G7, resulting in energies of -10.48kcal/mol, -9.04kcal/mol, -12.13kcal/mol, and -15kcal/mol, respectively (Figs. 12-15).

The result of this study highlights molecular interactions among host miRNAs and various Hepatitis C virus genotypes. The analysis of miR-122 alignment with various HCV genotypes revealed specific binding sites unique to each genotype within the viral genome. Notably, binding sites were prominently identified within the NS5B region in many multiple genotypes,

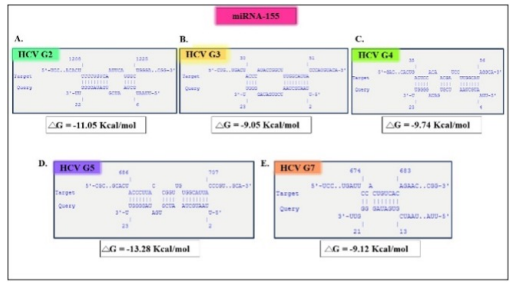

Fig. 14. RNA- RNA interaction showing energies of various HCV genotypes 2,3,4,5, and 7 with miR-155 are -11.05kcal/mol, -9.05kcal/mol, 9.74kcal/mol, -13.28kcal/mol, and -9.12kcal/mol.

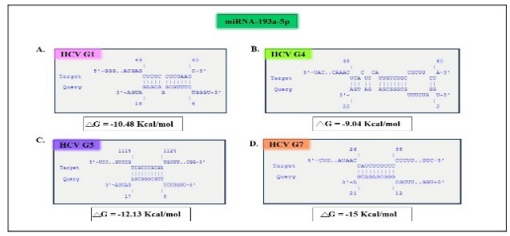

Fig. 15. RNA- RNA interaction showing energies of various HCV genotypes1, 4, 5, and 7 with miR-193a-5p are -10.48kcal/mol, -9.04kcal/mol, -12.13kcal/mol, and -15kcal/mol.

indicating possibility of role of miR-122 in inhibiting HCV replication and translation. Furthermore, the detection of genotype-specific binding in areas such as E2, NS5A, and NS5B highlights the diverse regulatory functions of miR-122 across different genotypes. Similarly, distinct binding patterns across HCV genotypes were observed for miR-21, miR-155 and miR-193a-5p, indicating genotype-specific affinities of viral genome and miR-21.

The expected alteration in viral protein structure due to interactions with host miRNAs highlight the potential influence on viral protein functionality. Furthermore, prediction of miRNA 2D and 3D structures revealed differences in binding energies among host miRNAs, with miR-193a-

5p demonstrating the highest binding affinity. This suggests a potential strong interaction with HCV genotypes.

4. Conclusion

This study indicates that host miRNAs may be responsible for the interaction with HCV in patients with single or multiple genotypes of HCV. In our previous study, the characterization of circulating HCV genotypes showed the presence of multiple genotypes such as HCV genotypes 2,3,4, and 5 in single HCV-infected individuals [26]. miRNAs may get confused about recognizing the binding site due to the presence of multiple genotypes. The present study may help develop or improve genotype-specific viral inhibition miRNA therapy. miR-122 suppresses virus in majority of cases and miR-122 will promote viral replication where HCV is mutated. Analyzing the binding energies between host miRNAs and HCV genotypes provides valuable insights into the strength of miRNA-virus interactions, with differing energies observed across various HCV genotypes. The reported observations could explore unique drug targets to inhibit HCV transformation into HCC.

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