Beni-Suef University Journal of Basic and Applied Sciences

RESEARCH

Open Access

Interaction between HOTTIP, H19, and HOTAIR long noncoding RNAs and miRNA-152 in cases of HCC caused by HCV infection

Rady E. El-Araby[1,2*], Fawzy Roshdy[1†], Mariam Zaghloul[3], Ahmed A. E. Saad[4], Maha H. Morsi[5], Wafaa M. Radwan[6], Rana M. Adel[7], Sara H. Elshafiey[7], Yasmine Elhusseny[8], Reham F. Othman[9], Hamed Helal[10], Doha E. Hassanein[5] and Hany A. Elghobary[11]

Abstract

Background Liver cancer (hepatocellular carcinoma "HCC") remains a significant health issue without prompt detection and appropriate prevention. By interacting with each other, long noncoding RNAs (lncRNAs) and microRNAs (miRNAs) can form gene regulatory networks. Specifically, we aim to determine whether the lncRNAs (HOTTIP, H19, and HOTAIR) and miRNA-152 interact in a significant manner in the progression of Hepatitis C virus (HCV) patients to HCC. This is followed by the question of whether these biomarkers can be used to diagnose and prognose noninvasively. We used online computational techniques to predict which miRNA group is likely to affect the lncRNAs being examined. This study involved 133 participants. 103 patients with HCV were included in the study, which was divided into two groups: Group I, with 65 cases of chronic liver disease without HCC, and Group II, with 38 cases of chronic liver disease with HCC. In addition, 30 healthy volunteers served as controls. In this study, a qRT-PCR was used to test gene expression.

Results A consistent reverse correlation has been observed between lncRNAs and miRNA-152 as the disease progresses.

Conclusion According to our findings, the studied biomarkers may be useful as noninvasive biomarkers for prognosis in patients with HCV Genotype 4 who develop liver cirrhosis and HCC. Many miRNAs, including miRNA-19a and miRNA-106a, may interact with lncRNAs that have been investigated in addition to miRNA-152.

Keywords LncRNAs, miRNA-152, HCV-genotype 4, Cirrhosis, HCC

†Rady E. El-Araby and Fawzy Roshdy contributed eqully to this work.

*Correspondence:
Rady E. El-Araby
rady.el_araby@tufts.edu; radyeid.elaraby@gmail.com
Full list of author information is available at the end of the article

1 Background

Among all cancers, hepatocellular carcinoma (HCC) causes the second-highest number of deaths globally [1]. Several factors are related to chronic cirrhosis, including Obesity, alcoholism, chronic hepatitis B and C, and metabolic disturbances [2]. The overall 5-year survival rate appears to be low at 18%, despite improvements in surgical procedures among HCC patients [3]. In most cases, surgical resection or liver transplantation are not possible because of the late diagnosis. It has been reported that HCC has a recurrence rate of up to 50% within five years of surgery. The development of novel treatments for late-stage HCC may significantly improve the outcome of these patients [4]. For this reason, it is imperative to diagnose HCC as soon as possible to obtain efficient and effective therapy and boost the probability of long-term survival. Understanding the genetic mechanisms involved in HCC development and progression is crucial to accurate detection at an early stage. It is also crucial to create effective approaches to treatments.

Several networks of genes and an imbalance of signaling pathways are hallmark features of HCC, as with many other cancer types [5, 6]. Genetic dysregulation affects proteins coding for genes, and non-coding RNAs (ncRNAs) are also affected [7]. There are approximately 98% of human genomes encoded by noncoding transcripts, the majority of which are processed into microRNAs and long noncoding RNAs (lncRNAs) [8]. It is a general property of these RNAs that they can effectively carry out their biological roles [9]. Newly discovered non-coding RNA (ncRNA) molecules have been proven to be essential players in a broad scope of biological and pathological processes [10, 11]. A number of ncRNAs have been shown to play a significant role in hepatocellular carcinoma pathogenesis [12, 13]. The conservation of lncRNAs and miRNAs, for example, has been demonstrated over a long period of time. Due to this, it can be argued that they play a functional role indirectly, such as through their influence on a variety of biological and pathological processes, including tumor rates [14].

These RNAs affect cell proliferation, senescence, differentiation, stress responses, immune system activation, and apoptosis, in addition to the aforementioned two major classes of noncoding RNAs [15]. Numerous medical conditions, including malignancies, neurological illnesses, and metabolic syndromes, are associated with noncoding RNAs. They serve a crucial function in gene expression, suggesting they may be viable therapeutic targets for diseases. In addition, they serve as biomarkers for disease diagnosis and prognosis. Consequently, research efforts have focused extensively on how noncoding RNAs interact with the regulatory network [16].

Along with the bioinformatics analysis we performed to select the lncRNAs (HOTTIP, H19, and HOTAIR), we found that this study summarizes seven well-documented lncRNAs in HCC: H19, HOTAIR, HULC, HOTTIP, MALAT1, MVIH, and MEG3. It was thought that upregulation of lncRNAs in HCC plays an oncogenic role [17]. Moreover, a previous study addressed the role of HOTTIP, H19, and HOTAIR in HCC progression in Egyptian HCV patients [18]. Prior studies have shown that H19 [19], HOTAIR [20], and HOTTIP [21] are upregulated in the HCC tissue in comparison with the normal tissue.

A significant decrease in miR-152-3p expression was observed in HCC tissues as compared to non-tumor tissues [22]. Furthermore, a previous study found that miRNA-152 was downregulated in HCC Egyptian patients with genotype 4 HCV infection [23]. Therefore, analyzing the expression levels of these ncRNAs in the early stages of HCC can provide valuable insight into the disease progression and might be useful in early diagnosis and treatment.

1.1 Aim of the work

Our hypothesis is that these lncRNAs (HOTTIP, H19, and HOTAIR), as well as miRNA-152, are useful in detecting HCC in patients with HCV and predicting disease progression. We used online computational tools to assess interactions between miRNAs and lncRNAs. We also examined the association of these lncRNAs and miRNA-152 with clinical outcomes. Our final objective was to investigate the association between our candidate's expression quantities and disease progression.

2 Patients and methods

Following the guidelines of the Theodor Bilharz Research Institute's Research Ethics Committee (TBRI-REC) (FWA00010609), all patients were asked to provide informed consent according to the Helsinki Declaration of 1975. The TBRI-REC number is PT (673), which indicates that the local ethical committee approved this project.

2.1 Inclusion criteria

The Gastroenterology and Hepatology Section at the TBRI in Giza, Egypt, provided the data for the present study. The study took place over six months from April to October 2020. During this period, 103 patients were included, and their data regarding demographics, clinical diagnosis, and treatment were collected.

Patients were devided into two groups: chronic HCV liver disease (CLD) without HCC as group I (65 patients) and group II chronic HCV CLD with HCC (38 patients). Group I was further subdivided according to the presence of liver cirrhosis into two subgroups, group 1a,

those without liver cirrhosis (30), and group 1b, those with liver cirrhosis (35) respectively. Moreover, we recruited 30 healthy individuals of matched gender and age as controls.

All patients and controls were subjected to a thorough history and comprehensive clinical examination.

All HCV patients included had persistent viremia for the last six months and did not receive HCV treatment with direct-acting antiviral agents (DAAS) or interferon.

Liver cirrhosis was diagnosed with characteristic abdominal ultrasound findings: coarse echotexture, an irregular border liver, and a shrunken liver. Those with focal lesions are confirmed by triphasic CT abdomen for HCC diagnosis. Additionally, HybProbe probes are used to genotype HCV.

2.2 Exclusion criteria

Based on the inclusion criteria, the study excluded patients with Schistosomiasis, dual Hepatitis B and C infections, nonalcoholic steatohepatitis, another chronic viral infection, biliary disorders, autoimmune hepatitis, non-HCC cancer patients, hepatotoxic drugs, alcoholism, diabetes, or patients with HCV treated with DAAS or interferon.

2.3 Sampling and storage

Under strict aseptic conditions, about 7 ml of peripheral venous blood were collected using vacuum blood collection tubes by clean venipuncture and distributed as 2.5 ml in EDTA tubes as a complete blood picture, 2.5 ml in another sterile EDTA tube (stored at −80 °C) as a viral RNA extract for HCV genotyping, miRNA, and mRNA extraction. Furthermore, 2 ml were centrifuged at 3000 rpm for 10 min after clotting at 37 °C, and the collected serum was stored at −80 °C for liver, kidney, and other specific serological tests.

2.4 Molecular analysis

2.4.1 Bioinformatics analysis

In the analysis of bioinformatics, we selected H19, HOTTIP, and HOTAIR as targets for miRNA-152 based on an integration of online databases: miRNet version 2.0, a database derived from the following meticulously documented databases: miRecords and miRTarBase v8.0, as well as a gene prediction tool for lncRNA target genes (miRnet) and miRNA-centric network visual analytics platform.

2.4.2 HCV genotyping

The HCV genotyping was performed using the HybProbe probes for PCR Cat. No. (03003248001), which can be found at (https://www.roche.com).

2.4.3 Expression of the targeted gene

Extraction of total RNA was performed using a high-purity RNA isolation kit (version 12, 2011), Cat. No: (11828665001, ROCH). For gene expression determination, RNA SYBR green I (Lightcycler EvoScript, 2017) master mix, Roche, Cat No: (07800134001), was used. Table 1 contains the primer sequences. The results of the lightCycler RNA SYBR green I master are to be analyzed using the combination of the SYBR green I filter (465–510). The CT approach was used to analyze this study. MiRNA quantity and quality between samples

Table 1 Oligo nucleotides seq. used in RT-PCR

Gene	Sequence	Tm (°C)	Reference
HOTTIP			
Forward	5-GTG GGG CCC AGA CCC GC-3	58.0	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4637691/
Reverse	5-AAT GAT AGG GAC ACA TCG GGG AAC T-3	58.0	
H19			
Forward	5-TGC TGC ACT TTA CAA CCA CTG-3	58.0	http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0000845
Reverse	5-ATG GTG TCT TTG ATG TTG GGC-3	58.0	
HOTAIR			
Forward	5-GCA GTA GAA AAA TAG ACA TAG GAGA-3	58.0	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4774541/
Reverse	5-AAT GAT AGG GAC ACA TCG GGG AAC T-3	58.0	
miRNA-152			
Forward	5-CCCAGGTTCTGTGATACACTCC-3	58.0	http://www.mirbase.org
Reverse	5-CTTCCGGGCCCAAGTTCTG-3	58.0	
U6 RNA			
Forward	5-CTCGCTTCGGCAGCACA-3	58.0	https://pubmed.ncbi.nlm.nih.gov/16728577/
Reverse	5-AACGCTTCACGAATTTGCGT-3	58.0	

are compensated by using the housekeeping gene U6 [24–26], while B-actin (used as an endogenous control to normalize the amount of total mRNA in each sample) of HOTTIP, H19, and HOTAIR between different samples. Comparisons of gene expression levels between test and control samples were performed (served as a calibration sample) according to the formula $2^{-\Delta\Delta CT}$.

2.5 Statistical analysis

For the analysis of the data, Microsoft Excel 2016 and IBM SPSS Statistics for Windows, version 26, were used (IBM Corp., Armonk, New York, USA). For non-normal variables, a 0.05 p value was determined as statistically significant when the 25th and 75th percentiles were present. Statistical analysis was conducted using the Mann–Whitney U test for non-normal variables. In order to assess the diagnostic performance of the studied genes, receiver operating characteristic curves (ROC) were calculated. ROC curves and their areas under ROC curves (AUC) are accurate metrics for assessing the prediction accuracy of selected tests. Study groups were diagnosed based on the point with the greatest combination of sensitivity and specificity. We used bivariate Pearson correlation (r) to analyze the relationship between the markers under examination. The performance of prognosis and/or risk assessment was analyzed using binary logistic regression analysis.

3 Results

3.1 Interactions and bioinformatics analysis perspectives

In Fig. 1, using the miRNet web server database, it can be seen that HOTTIP, H19, and HOTAIR, along with miRNA-152 and all functionally targeted miRNAs, influence one another in multiple ways. In this study, miRNA-106a and miRNA-119a were presented as targeted miRNAs. Particularly, these miRNAs are closely associated with HOTAIR, H19, and HOTTIP, which are associated with HCCs with different expression variants. In addition, this could reveal new relationships between them as well as potential associations with HCC development.

3.2 Gene expression

It is evident that the expression of HOTTIP, H19, and HOTAIR is consistently increased, while the expression of miRNA-152 is consistently decreased, according to the fold change principle (Table 2) (Fig. 2). The expression values of HOTTIP (p 0.001), H19 (p 0.001), and HOTAIR (p 0.01) were statistically different from controls.

Compared to noncirrhotic patients, HCC patients and patients with cirrhosis expressed significantly higher levels of HOTTIP (p 0.03), H19 (p 0.04), and HOTAIR (p 0.001). These values were also higher among patients with HCC when compared with patients without cirrhosis (p values of 0.001, 0.001, and 0.001). There was a significant increase in HOTTIP (p 0.001), H19 (p 0.013), and HOTAIR (p 0.005) expression in patients with HCC compared with patients with cirrhosis (Table 2, Fig. 2a, b, c).

The expression of miRNA-152, however, decreased significantly (p 0.04) in all patient groups in comparison to the control group. Compared to non-cirrhotic patients, the expression of miRNA-152 was lower in cirrhosis patients (p 0.007) and HCC patients (p 0.001). MiRNA-152 expression was significantly lower in patients with HCC cases (p 0.001) compared to those with cirrhosis (Table 2, Fig. 2d).

3.3 Correlation study

The correlation coefficients between miRNA152 and HOTTIP, H19, and HOTAIR are −0.206, −0.218, and −0.259, respectively (Fig. 3).

It is also noteworthy that there is a direct positive correlation between the HOTAIR and HOTTIP genes (r = 0.813, p 0.001). Additionally, there is a significant direct positive correlation between the HOTAIR gene and the H19 gene (r = 0.349, p 0.001). Moreover, H19 has a positive correlation with HOTTIP (r = 0.315, p 0.001).

3.4 Diagnostic accuracy

Utilizing ROC curves, it was evaluated how successfully the tested groups could be diagnosed by the HOTTIP, H19, HOTAIR, and miRNA-152 genes. Neither the HOTTIP nor the HOTAIR genes can be used to distinguish between cirrhotic and non-cirrhotic cases. In contrast, H19 showed an AUC of 0.77, p 0.0001, while miRNA-152 showed an AUC of 0.7, p 0.007 (Table 3, Fig. 4a).

According to the results in Table 3, Fig. 4b, HOTTIP, H19, HOTAIR, and miRNA-152 all showed AUCs of 0.67, p 0.07; 0.69, p 0.003; and 0.71, p 0.001, respectively, to distinguish the HCC from that of the cirrhosis group.

Interestingly, according to Table 3, Fig. 4c, the HOTTIP, H19, HOTAIR, and miRNA-152 showed AUCs of 0.712, p 0.0001, 0.768, p 0.0001, 0.786, p 0.0001, and 0.779, p 0.779, $p < 0.0001$, respectively, for discriminating between HCC and CLD patients.

These findings suggest that these four biomarkers can distinguish between HCC and cirrhotic patients with a reasonable degree of sensitivity, specificity, and AUC. In addition, their respective cut-off values can be utilized for accurate patient diagnosis.

3.5 Prognostic performance

Univariate logistic regression analysis determined that H19 and miRNA-152 were prognostic and/or predictive

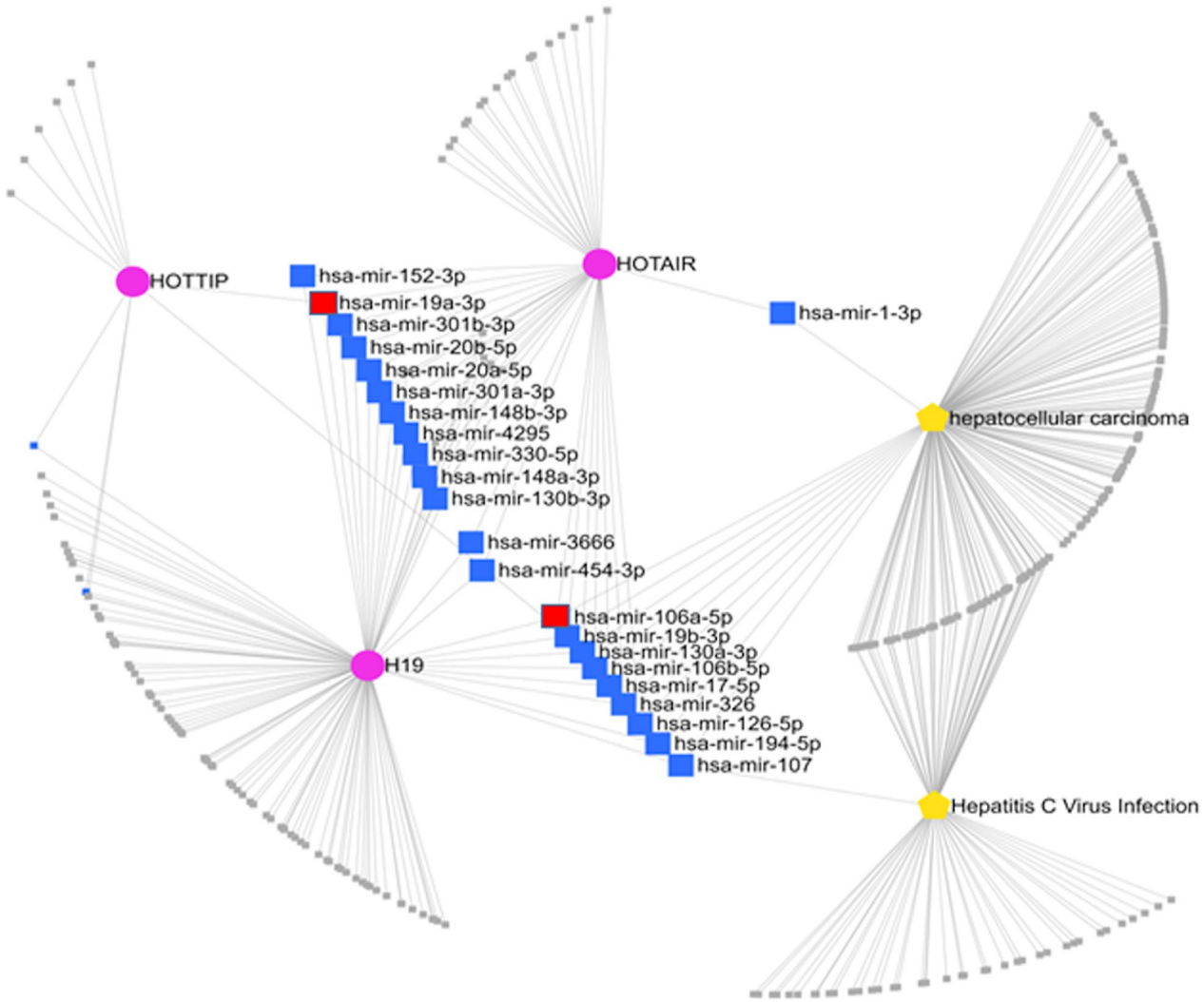

Fig. 1 Outlook of all possible functional target miRNAs from the miRNet webserver database. According to hepatocellular carcinoma and HCV infection, the network appears to consist of all interacted miRNAs plus miRNA-152. Also, the interactions with targeted lncRNAs that could interact with them should they be exposed to a specific disease

factors for cirrhosis progression. The possibility of developing cirrhosis increases by factors of 1.36 (p 0.02) and 2.32 (p 0.01) as H19 and miRNA-152 are both increased by one degree. On the basis of the HOTAIR and HOTTIP genes, patients with cirrhosis cannot be distinguished from those without cirrhosis (Table 4).

As potential prognostic indicators for HCC progression, HOTTIP, H19, HOTAIR, and miRNA-152 were evaluated in the present study. Every 1-unit increase in HOTTIP (1.08, p 0.01), H19 (1.07, p 0.03), HOTAIR (1.12, p 0.01), or miRNA-152 (2.54, p 0.001) increased the probability of developing HCC (Table 4).

HOTTIP, H19, HOTAIR, and miRNA-152 levels were raised by 1 degree in CLD overall (group I: noncirrhotic and cirrhotic). This led to an increase in the likelihood of getting HCC of 1.10, p 0.001; 1.08, p 0.02; 1.15, p 0.001; or 3.27, p 0.001, respectively (Table 4).

4 Discussion

In the absence of preemptive determination and appropriate treatment, hepatocellular carcinoma remains a significant health problem. Clinical diagnostics have successfully used a limited number of molecular biomarkers. HCC biomarkers have been extensively used as diagnostic or prognostic tools, as well as therapeutic targets. By interacting with microRNAs, messenger RNAs, and proteins, lncRNAs contribute significantly to cancer carcinogenesis and development [27]. Furthermore, lncRNAs are implicated in tumorigenesis and cancer development based on numerous clinical observations

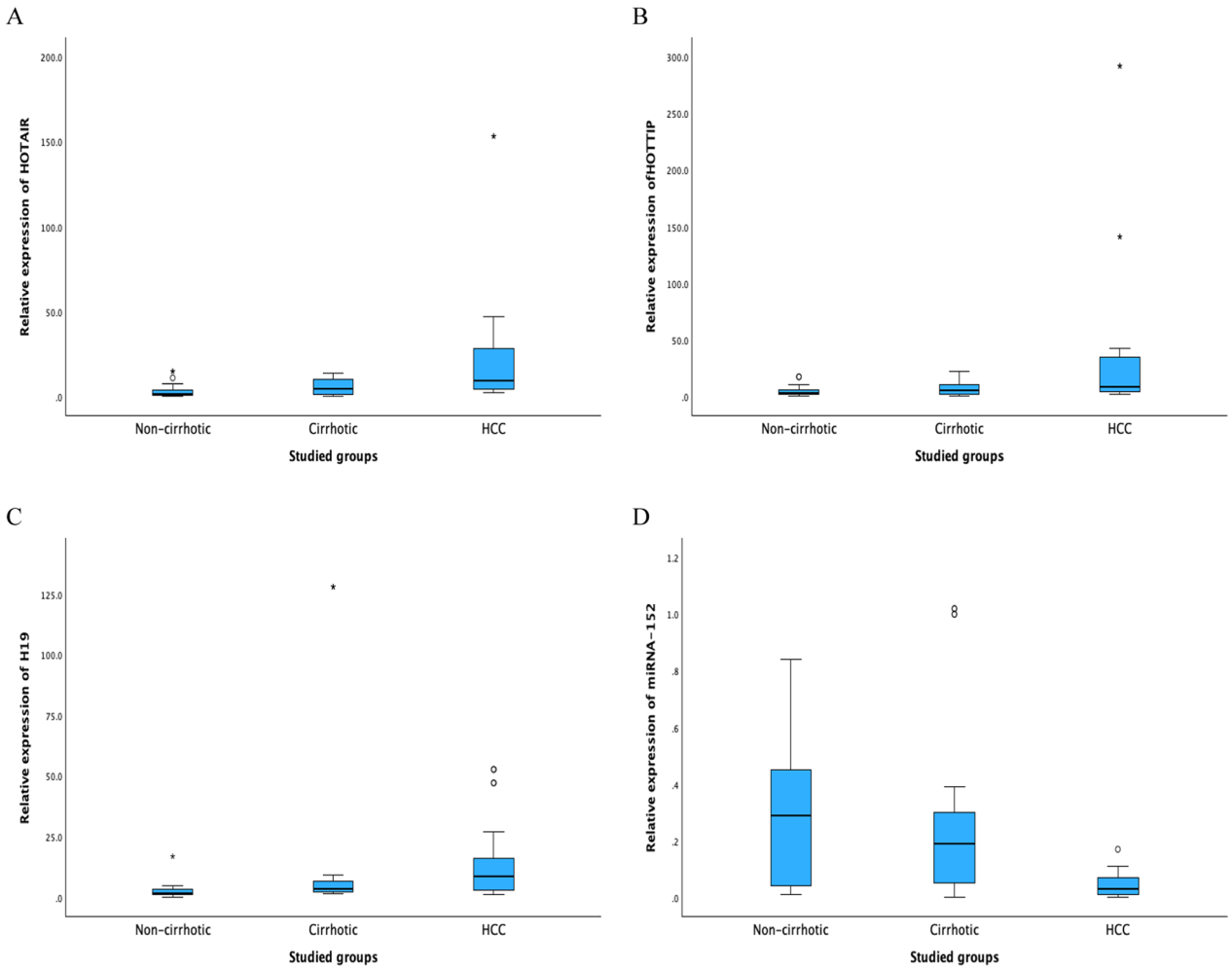

Fig. 2 Relative gene expression of the studied genes in the studied groups: **A** HOTAIR gene expression in the studied groups. **B** HOTTIP gene expression in the studied groups. **C** H19 gene expression in the studied groups. **D** miRNA-152 gene expression in the studied groups. U6 was used as an endogenous control for miRNA, while B-actin were used for HOTTIP, H19, and HOTAIR. Gene expression was calculated using three replicates for the fold change, while the fold change was calculated using the Law of Fold Change, which is $2^{-\Delta\Delta CT}$

and experimental studies. In order to control gene expression, long noncoding RNAs and microRNAs work together. However, just a handful of lncRNA-miRNA interactions have been uncovered so far, and there is a lack of widespread access to computational approaches for predicting novel connections. Due to the intrinsic patterns in their interactions that lncRNAs and miRNAs share, it is possible to predict the underlying lncRNA-miRNA interactions from known ones [8]. The study examined the relationship between H19, HOTTIP, and HOTAIR as noninvasive biomarkers for identifying HCC accompanying genotype 4 HCV in patients with HCC on top of HCV genotype 4.

In our study, online computational methods were used to show that HOTTIP, H19, and HOTAIR, as well as miRNA-152 and all functionally targeted miRNAs, influence each other in multiple ways. The new miRNAs (miRNA-106a and miRNA-119a) also interacted with the lncRNAs we were interested in. A number of these miRNAs are closely associated with HOTAIR, H19, and HOTTIP, which are associated with HCCs with different expression variants. Additionally, this may reveal new relationships between them as well as potential associations with the development of HCC (Fig. 1).

There has been much research examining the relationship between miRNAs and lncRNAs, and more information is now becoming available regarding how miRNAs function in lncRNAs [8]. Due to the role that miRNAs and long noncoding RNAs play in regulating gene expression and getting together to network, their interactions are strictly controlled. There is considerable evidence that lncRNAs and miRNAs are involved in many diseases, but further clarification is needed [28]. Because of this, the new lncRNA-miRNA regulatory circuit HOTTIP, H19,

Table 2 Gene expression of the studied genes in the studied groups

	Control N = 30	Group I (CLD without HCC) N = 65		Group II (CLD with HCC) N = 38
		Group Ia Non-cirrhotic N = 30	Group Ib Cirrhotic N = 35	
HOTTIP	1	3.10(1.43–5.62)[aa]	5.31(1.83–10.34)[aa,b]	8.07(3.81–35.44)[aa,bb,c,*]
H19	1	1.84(1.00–3.25)[a]	3.68(2.14–6.50)[aa,bb]	8.69(3.01–16.11)[aa,bb,cc,*]
HOTAIR	1	1.59(0.68–4.07)[a]	4.53(1.21–9.99)[aa,b]	9.11(3.97–29.09)[aa,bb,cc,*]
miRNA-152	1	0.32(0.06–0.65)[a]	0.16(0.02–0.30)[aa,bb]	0.03(0.01–0.07)[aa,bb,cc,*]

The fold change results depend on the fold change low: Fold-Change ($2^{-\Delta\Delta CT}$) is the normalized gene expression (ΔCT) in the test sample divided the normalized gene expression (ΔCT) in the control sample. (Fold-change values less than one indicate a negative or down-regulation)

All parameters are represented as Median with Interquartile range (25–75%) of the fold change of the studied groups, the data were analyzed by Mann–Whitney *U* test

*p value is significantly different comparing with CLD group (I)

[a] p value is significantly different comparing with control group

[b] p value is significantly different comparing with non-cirrhotic group

[c] p value is significantly different comparing with Cirrhotic group

[1 Initial] p value < 0.05 is significant, [2 Initial] p value < 0.01 is highly significant

and HOTAIR-miRNA-152 could give patients with HCC on top of HCV a new epigenetic therapeutic target.

Gene expression results showed upregulation in H19, HOTTIP, and HOTAIR expression gradually with the progression of disease. At the same time, miRNA-152 levels are reduced in the studied patients when HOTTIP, H19, and HOTAIR are upregulated, which is directly associated with disease progression (Table 2, Fig. 2). In addition, the correlation study showed an inversal correlation between the expression of miRNA-152 and H19, HOTTIP, and HOTAIR expression (Fig. 3). Based on the results of the mechanistic study, the expression of miRNA-152 is inversely related to the expression of HOTTIP, H19, and HOTAIR. The preceding relationship comes mostly in harmony with similar studies, which found that an lncRNA-miRNA-mRNA network is associated with recurrence in HCC and that lncRNA 'SNHG3' could promote the recurrence of HCC by regulating ASF1B expression via sponging of miR-214-3p [29].

A ROC curve was constructed to analyse the diagnostic accuracy of gene expression markers in cirrhotic individuals compared to non-cirrhotic individuals with varying cut-off points. Regarding H19 and miRNA-152, there was a significant difference in this study. The HOTTIP and HOTAIR lncRNA levels were not significantly different between cirrhotics and non-cirrhotics. That means HOTTIP and HOTAIR were unable to discriminate between cirrhotics and non-cirrhotics, which is contrary to H19 and miRNA-152.

HOTTIP, H19, HOTAIR, and miRNA-152 showed significant differences between patients with cirrhosis and HCC. All of them appear to be able to distinguish between patients with HCC and those with cirrhosis, according to the evidence.

Interestingly, HOTTIP, H19, HOTAIR, and miRNA-152 levels were significantly different between the HCC and CLD groups. In order to differentiate HCC patients from those with CLD generally, several factors have been demonstrated to be useful. A number of these factors can be identified, including HOTTIP, H19, HOTAIR, and miRNA-152. Our results are consistent with theirs, despite the fact that we used a different case study and strategy [30]. Their findings indicate that lncRNAs, miRNAs, and mRNAs are differentially expressed in normal and cancerous tissues. Additionally, altered lncRNAs and miRNAs may serve as potential diagnostic biomarkers for papillary thyroid carcinoma (PTC).

According to regression analysis, the expression levels of H19 and miRNA-152 were reliable prognosticators in patients with cirrhosis. The expression levels of HOTAIR and HOTTIP did not reach statistical significance, despite this fact. When comparing patients with cirrhosis or chronic liver disease to those with HCC, the levels of HOTTIP, H19, HOTAIR, and miRNA-152 were found to be strong predictors of an HCC diagnosis. According to [31], lncRNA-CASC7 was significantly upregulated in the serum of patients with HCC and closely associated with tumor number, IM, tumor size, and TNM stage, which could be used as a promising diagnostic biomarker. In a recent study [18], the serum levels of HOTTIP, H19, and HOTAIR were upregulated in Egyptian HCC patients with HCV genotype 4. Moreover, they can be used as noninvasive early-diagnosis biomarkers for HCC. Several potential biomarkers for HCC have recently been

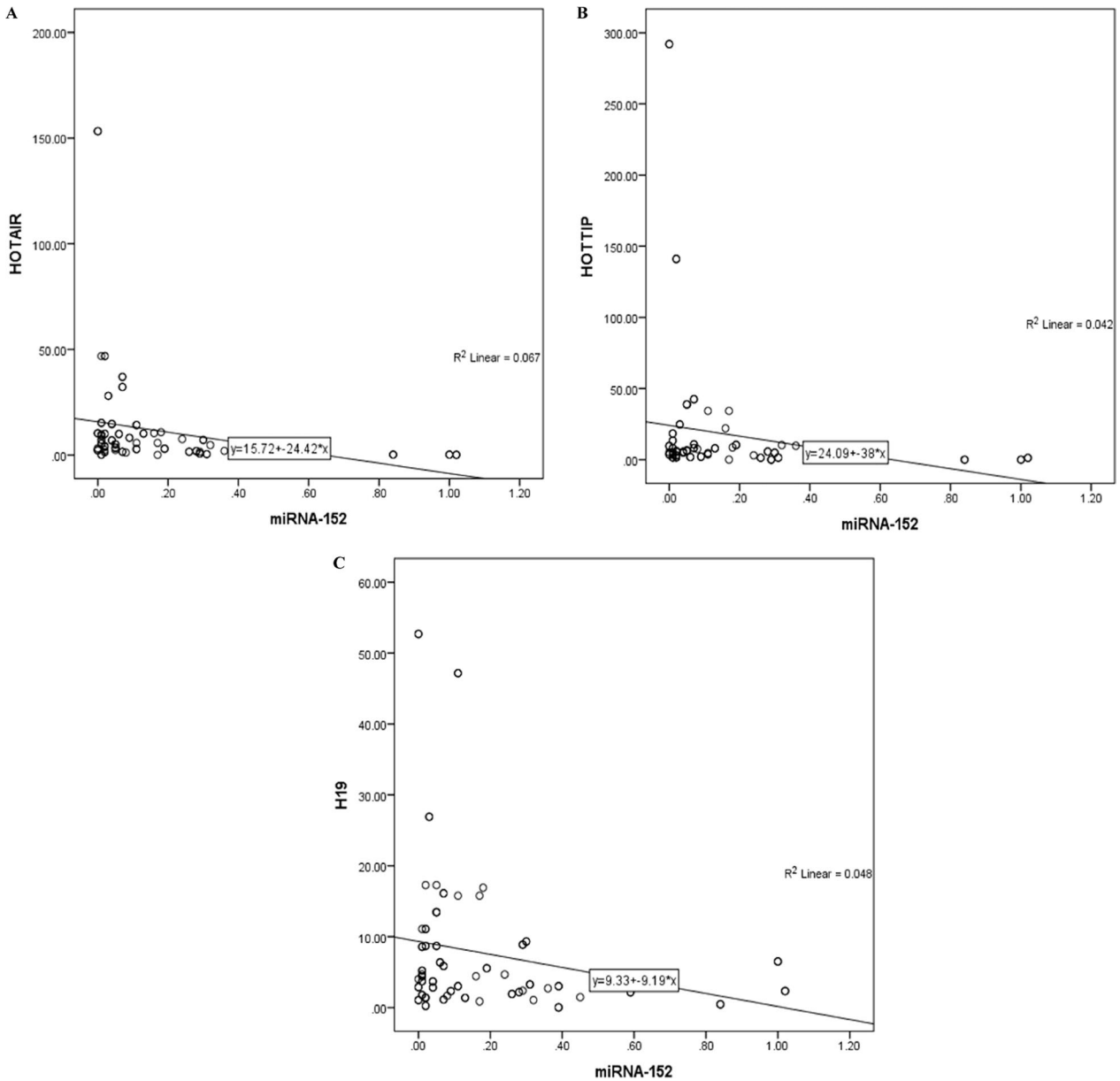

Fig. 3 Correlation study between miRNA-152 and the studied lncRNAs. **A** Correlation between miRNA-152 and HOTAIR. **B** Correlation between miRNA-152 and HOTTIP. **C** Correlation between miRNA-152 and H19. Bivariate Pearson correlation (r) to analyze the relationship between the markers under examination

identified through recent research. These results show that lncRNAs that are high may be useful molecular targets for treating HCC because they are turned down by miRNAs [17].

Biomarkers for cardiac, neurological, and cancer disorders are commonly derived from both non-coding RNA groups [32–37], and tissue and liquid biopsies have been used to examine their potential as biomarkers for these conditions. It has been found that established protein biomarkers can be used in conjunction with miRNA and lncRNA-based tests to improve the specificity and sensitivity of the diagnosis [38].

In a later study, the visual analytics platform server on miRNet was used to look at the regulatory interactions between HOTTIP, H19, and HOTAIR as lncRNAs targeting miRNA-152. Other miRNAs may target all of these targets as well. This analysis identified all miRNAs that may target the lncRNAs studied in HCC development. There is a surprising finding from this study: the targeted miRNAs (miRNA-106a and miRNA-19a) are non-coding

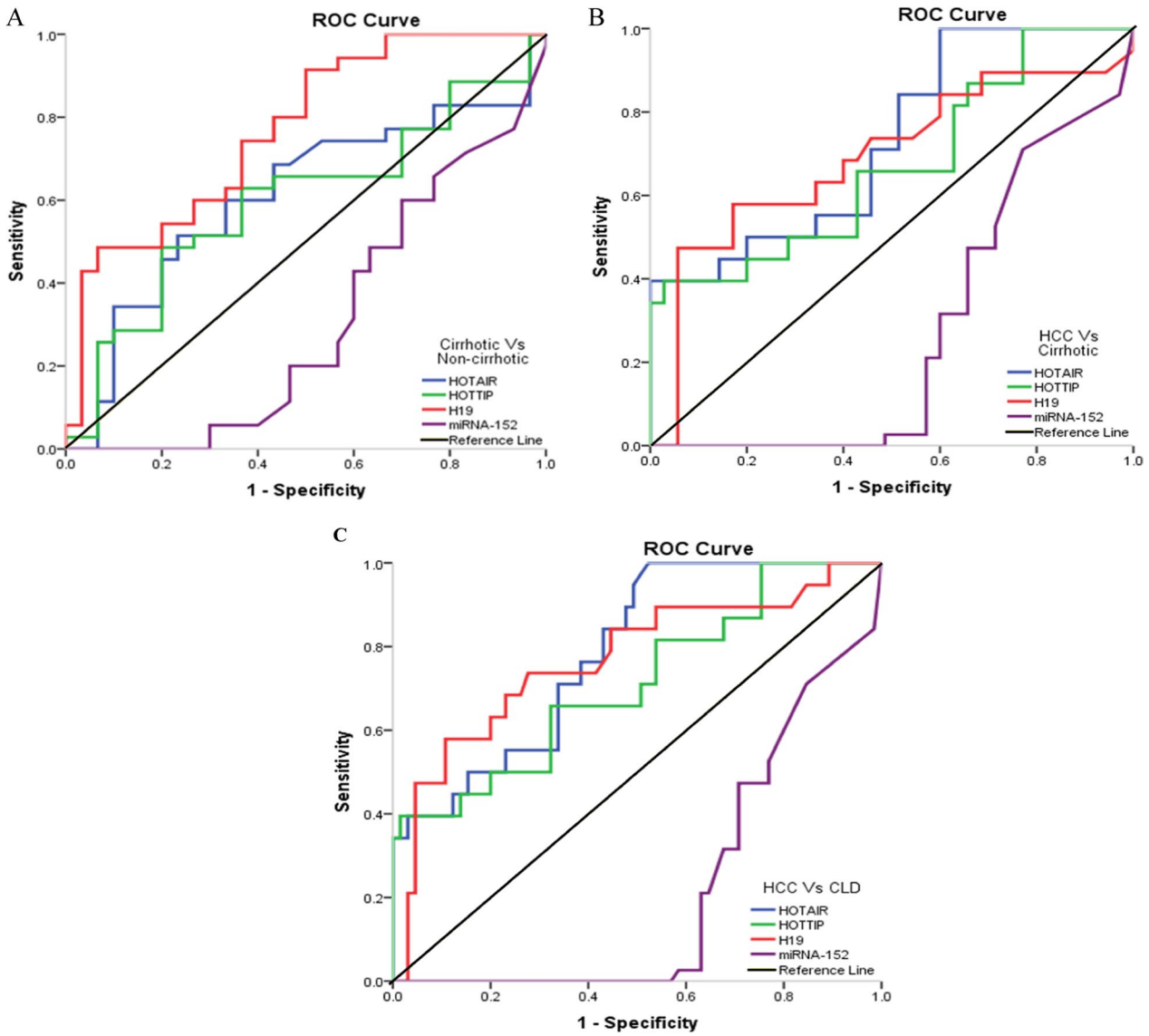

Fig. 4 ROC curve of the studied genes in the studied groups. **A** ROC curve to discriminate between the cirrhotic versus non-cirrhotic groups. **B** ROC curve to discriminate between HCC versus the cirrhotic group. **C** ROC curve to discriminate between the HCC versus CLD groups. Receiver operating characteristic curves (ROC) were done to evaluate the diagnostic performance of the studied genes. ROC curves and their areas under ROC curves (AUC) are accurate metrics for assessing the prediction accuracy of selected tests. Study groups were diagnosed based on the point with the greatest combination of sensitivity and specificity

RNAs with a high profile of association with prostate cancer. Additionally, in the previous study [39], they discovered that miRNA-106a was associated with ovarian cancer-related EMT, while miRNA-19a was associated with metastases.

There has been growing evidence that miRNAs and lncRNAs function together to establish a regulatory network for genes. As a result, lncRNA and miRNA interactions provide valuable information regarding the molecular mechanisms underlying a number of complex diseases. It is the fact that only a few lncRNA-miRNA interactions are known that poses the biggest challenge (i.e., lncRNA-miRNA interaction networks are quite sparse), as well as the insufficient knowledge regarding the mechanisms underlying these interactions [8]. The findings validate the theory we presented throughout this research and raise additional questions regarding how the remaining target miRNAs are concerned with tumor development. For a full

Table 3 Diagnostic performances of the studied genes

	Studied genes	Cutoff	Sn.	Sp.	Accuracy	AUC	95% CI	*p* value
Cirrhotic versus Non-cirrhotic	HOTTIP	> 5.618	48.6	80.0	63.1	0.598	0.458–0.738	0.171
	H19	> 3.758	48.6	93.3	69.2	0.770	0.656–0.883	< 0.0001**
	HOTAIR	> 3.837	51.4	76.7	63.1	0.610	0.470–0.751	0.124
	miRNA-152	< 0.165	51.4	70.0	67.8	0.704	0.563–0.825	0.007**
HCC versus Cirrhotic	HOTTIP	> 14.929	39.5	97.1	67.1	0.670	0.546–0.793	0.007**
	H19	> 9.318	47.4	94.3	69.9	0.691	0.566–0.816	0.003**
	HOTAIR	> 2.000	100.0	40.0	71.2	0.729	0.615–0.844	< 0.0001**
	miRNA-152	< 0.055	65.7	68.4	72.1	0.73	0.607–0.852	0.001**
HCC versus CLD	HOTTIP	> 17.268	39.5	98.5	76.7	0.712	0.607–0.817	< 0.0001**
	H19	> 8.574	57.9	89.2	77.7	0.768	0.668–0.868	< 0.0001**
	HOTAIR	> 2.000	100.0	47.7	67.0	0.786	0.700–0.872	< 0.0001**
	miRNA-152	< 0.055	68.4	70.8	78.1	0.779	0.692–0.866	< 0.0001**

Sn sensitivity, *Sp* specificity, *AUC* area under curve and CI: 95% confidence interval

***p* value < 0.01 is highly significant

understanding of their role in the development of HCC, more extensive research is required.

5 Conclusion

Accordingly, we can conclude from the above findings that lncRNAs (HOTTIP, H19, and HOTAIR) alter the expression of genes in patients with cirrhosis and HCC through their interaction with miRNA-152. Cancer disruption and development are caused by disruptions of the miRNA-152 pattern in HCC. Also, we may be able to provide reliable HCC-specific biomarkers based on the gene expression results for our candidates. Based on the results of these analyses, new therapeutic targets may be identified for treating HCV-induced cirrhosis and liver cancer. More interaction partners became apparent when computational tools were examined in more detail. There are other genes that may be present in the targeted domain of lncRNAs (HOTTIP, H19, and HOTAIR), in addition to miRNA-152. Consequently, patients with liver cirrhosis brought on by HCV and HCC would benefit from a larger, more in-depth pilot investigation that makes use of genome-wide transcriptional profiling and analytics based on bioinformatics. This study has some limitations, including a larger sample size and studying the association between different stages of cancer and these markers to assess whether these markers can be used for prognosis. Future studies should address these limitations by using a large sample size and a cross-sectional nature that would suggest correlation and need further confirmation by prospective studies.

Table 4 Prognostic performances of the studied genes

	Studied genes	OR	95% CI	*p* value
Cirrhotic versus Non-cirrhotic	HOTTIP	1.09	0.98–1.22	0.1
	H19	1.36	1.06–1.74	0.02*
	HOTAIR	1.12	0.99–1.27	0.07
	miRNA-152	2.32	1.23–3.87	0.01*
HCC versus Cirrhotic	HOTTIP	1.08	1.02–1.14	0.01*
	H19	1.07	1.01–1.23	0.03*
	HOTAIR	1.12	1.03–1.21	0.01*
	miRNA-152	2.54	1.35–4.54	0.001**
HCC versus CLD	HOTTIP	1.10	1.04–1.16	0.001**
	H19	1.08	1.03–1.21	0.02*
	HOTAIR	1.15	1.06–1.24	0.001**
	miRNA-152	3.27	1.28–6.87	0.001**

OR odd ratio, *CI* confidence interval and *p* value of Prognostic viability were calculated depending on logistic regression analysis

**p* value < 0.05 is significant, ***p* value < 0.01 is highly significant

Abbreviations

95% CI	95% Confidence interval
AUC	Area under the ROC
CLD	Chronic liver disease
r	Correlation
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HCC	Hepatocellular carcinoma
lncRNAs	Long non-coding RNAs
miRNAs	Micro RNAs
MREs	MiRNA response elements
ncRNA	Non-coding RNA
NASH	Nonalcoholic steatohepatitis
PTC	Papillary thyroid carcinoma
ROC	Receiver operating characteristic
Sn	Sensitivity
Sp	Specificity

Acknowledgements

We are very grateful to all anonymous donors of the blood samples used in this study.

Author contributions
The first and second authors established the key hypotheses of this study. In the molecular biology practical section, the following authors contributed: 1st , 2nd, and 9th. Clinical data interpretation results were prepared by the third and tenth authors. 4th, 5th, 6th, 12th, and 13th authors prepared the clinical laboratory investigations and interpretations. The major manuscript texts were written by the 1st, 2nd, and 5th. A review of the manuscript was conducted by all of the authors who contributed to the project.

Funding
As a representative of my colleagues, I acknowledge that our own resources fully funded this work. All researchers contributed equally to this research. Our country's resources were not sufficient to support us, so we decided to collaborate to conduct research like that. Therefore, we decided to pool our collective resources and work together to achieve our research goals.

Availability of data and materials
Our data will be made available on reasonable request.

Declarations

Ethics approval and consent to participate
The guidelines of the Theodor Bilharz Research Institute's Research Ethics Committee (TBRI-REC) (FWA00010609), all patients were asked to provide informed consent according to the Helsinki Declaration of 1975. The TBRI-REC number is PT (673), which indicates that the local ethical committee approved this project.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Division of Oral Biology, Department of Periodontology, Tufts University School of Medicine, Boston, MA, USA. [2]Central Lab, Theodor Bilharz Research Institute (TBRI), Ministry of Scientific Research, Cairo, Egypt. [3]Hepatology, Gastroenterology, and Infectious Diseases Department, Faculty of Medicine, Kafrelsheikh University, Kafr El Sheikh, Egypt. [4]Department of Clinical Pathology, Faculty of Medicine, Al-Alazhar University, Gaza, Egypt. [5]Clinical and Chemical Pathology Department, Faculty of Medicine, Misr University for Sciences and Technology, 6th of October, Egypt. [6]Clinical Pathology Department, Faculty of Medicine, Menoufia University, Shibīn Al-Kawm, Egypt. [7]Zoology Department, Faculty of Women for Arts, Science, and Education, Ain Shams University, Cairo, Egypt. [8]Biochemistry and Molecular Biology Department, School of Medicine, Newgiza University, 6th of October, Egypt. [9]Department of Internal Medicine, School of Medicine, Newgiza University, 6th of October, Egypt. [10]Zoology Department, Faculty of Science, Al-Azhar University, Gaza, Egypt. [11]Department of Clinical and Chemical Pathology, Faculty of Medicine, Cairo University, Cairo, Egypt.

Received: 2 November 2023 Accepted: 27 April 2024
Published online: 07 May 2024

References

1. Bruix J, Gores GJ, Mazzaferro V (2014) Hepatocellular carcinoma: clinical frontiers and perspectives. Gut 63(5):844–855. https://doi.org/10.1136/gutjnl-2013-306627. (**Epub 2014 Feb 14**)
2. Lanzafame M, Bianco G, Terracciano LM, Ng CKY, Piscuoglio S (2018) The role of long non-coding RNAs in hepatocarcinogenesis. Int J Mol Sci 19(3):682. https://doi.org/10.3390/ijms19030682
3. Kulik LM, Chokechanachaisakul A (2015) Evaluation and management of hepatocellular carcinoma. Clin Liver Dis 19(1):23–43. https://doi.org/10.1016/j.cld.2014.09.002. (**Epub 2014 Oct 30**)
4. Ahn SM, Jang SJ, Shim JH, Kim D, Hong SM, Sung CO, Baek D, Haq F, Ansari AA, Lee SY, Chun SM, Choi S, Choi HJ, Kim J, Kim S, Hwang S, Lee YJ, Lee JE, Jung WR, Jang HY, Yang E, Sung WK, Lee NP, Mao M, Lee C, Zucman-Rossi J, Yu E, Lee HC, Kong G (2014) Genomic portrait of resectable hepatocellular carcinomas: implications of RB1 and FGF19 aberrations for patient stratification. Hepatology 60(6):1972–1982. https://doi.org/10.1002/hep.27198. (**Epub 2014 Sep 22**)
5. Zhang Y, Huang JC, Cai KT, Yu XB, Chen YR, Pan WY, He ZL, Lv J, Feng ZB, Chen G (2017) Long non-coding RNA HOTTIP promotes hepatocellular carcinoma tumorigenesis and development: a comprehensive investigation based on bioinformatics, qRT-PCR and meta-analysis of 393 cases. Int J Oncol 51(6):1705–1721. https://doi.org/10.3892/ijo.2017.4164. (**Epub 2017 Oct 16**)
6. Zhang X, Tang W, Chen G, Ren F, Liang H, Dang Y, Rong M (2016) An encapsulation of gene signatures for hepatocellular carcinoma, microRNA-132 predicted target genes and the corresponding overlaps. PLoS ONE 11(7):e0159498. https://doi.org/10.1371/journal.pone.0159498
7. Hashimoto K, Suzuki AM, Dos Santos A, Desterke C, Collino A, Ghisletti S, Braun E, Bonetti A, Fort A, Qin XY, Radaelli E, Kaczkowski B, Forrest AR, Kojima S, Samuel D, Natoli G, Buendia MA, Faivre J, Carninci P (2015) CAGE profiling of ncRNAs in hepatocellular carcinoma reveals widespread activation of retroviral LTR promoters in virus-induced tumors. Genome Res 25(12):1812–1824. https://doi.org/10.1101/gr.191031.115. (**Epub 2015 Oct 28**)
8. Huang YA, Huang ZA, You ZH, Zhu Z, Huang WZ, Guo JX, Yu CQ (2019) Predicting lncRNA-miRNA interaction *via* graph convolution auto-encoder. Front Genet 10:758. https://doi.org/10.3389/fgene.2019.00758
9. Zhang SW, Fan XN (2017) Computational methods for predicting ncRNA-protein interactions. Med Chem 13(6):515–525. https://doi.org/10.2174/1573406413666170510102405
10. Mishra A, Bohra A (2018) Non-coding RNAs and plant male sterility: current knowledge and future prospects. Plant Cell Rep 37(2):177–191. https://doi.org/10.1007/s00299-018-2248-y. (**Epub 2018 Jan 13**)
11. Jakobi T, Dieterich C (2018) Deep computational circular RNA analytics from RNA-seq data. Methods Mol Biol 1724:9–25. https://doi.org/10.1007/978-1-4939-7562-4_2
12. Wilson CL, Mann DA, Borthwick LA (2017) Epigenetic reprogramming in liver fibrosis and cancer. Adv Drug Deliv Rev 121:124–132. https://doi.org/10.1016/j.addr.2017.10.011. (**Epub 2017 Oct 25**)
13. Tian F, Xu J, Xue F, Guan E, Xu X (2017) TINCR expression is associated with unfavorable prognosis in patients with hepatocellular carcinoma. Biosci Rep 37(4):BSR20170301. https://doi.org/10.1042/BSR20170301
14. Oloomi M, Yardehnavi N, Bouzari S, Moazzezy N (2013) Non-coding CK19 RNA in peripheral blood and tissue of breast cancer patients. Acta Med Iran 51(2):75–86
15. Shi X, Sun M, Liu H, Yao Y, Song Y (2013) Long non-coding RNAs: a new frontier in the study of human diseases. Cancer Lett 339(2):159–166. https://doi.org/10.1016/j.canlet.2013.06.013. (**Epub 2013 Jun 18**)
16. Huang YA, You ZH, Chen X, Yan GY (2016) Improved protein-protein interactions prediction via weighted sparse representation model combining continuous wavelet descriptor and PseAA composition. BMC Syst Biol 10(Suppl 4):120. https://doi.org/10.1186/s12918-016-0360-6
17. Unfried JP, Sangro P, Prats-Mari L, Sangro B, Fortes P (2021) The landscape of lncRNAs in hepatocellular carcinoma: a translational perspective. Cancers 13(11):2651. https://doi.org/10.3390/cancers13112651
18. Roshdy F, Farag MMS, El-Ahwany E et al (2020) Long non-coding RNA HOTAIR and HOTTIP as potential biomarkers for hepatitis C virus genotype 4-induced hepatocellular carcinoma. Egypt J Med Hum Genet 21:7. https://doi.org/10.1186/s43042-020-0048-8
19. Matouk IJ, DeGroot N, Mezan S, Ayesh S, Abu-lail R, Hochberg A, Galun E (2007) The H19 non-coding RNA is essential for human tumor growth. PLoS ONE 2(9):e845. https://doi.org/10.1371/journal.pone.0000845
20. Geng YJ, Xie SL, Li Q, Ma J, Wang GY (2011) Large intervening non-coding RNA HOTAIR is associated with hepatocellular carcinoma progression. J Int Med Res 39(6):2119–2128. https://doi.org/10.1177/147323001103900608
21. Quagliata L, Matter MS, Piscuoglio S, Arabi L, Ruiz C, Procino A, Kovac M, Moretti F, Makowska Z, Boldanova T, Andersen JB, Hämmerle M, Tornillo L, Heim MH, Diederichs S, Cillo C, Terracciano LM (2014) Long noncoding RNA HOTTIP/HOXA13 expression is associated with disease progression and predicts outcome in hepatocellular carcinoma patients. Hepatology 59(3):911–923. https://doi.org/10.1002/hep.26740. (**Epub 2014 Jan 28**)

22. Xu JH, Chang WH, Fu HW, Yuan T, Chen P (2018) The mRNA, miRNA and lncRNA networks in hepatocellular carcinoma: an integrative transcriptomic analysis from Gene Expression Omnibus. Mol Med Rep 17(5):6472–6482. https://doi.org/10.3892/mmr.2018.8694. (**Epub 2018 Mar 7**)
23. El-Araby RE, Khalifa MA, Zoheiry MM, Zahran MY, Rady MI, Ibrahim RA, El-Talkawy MD, Essawy FM (2020) The interaction between microRNA-152 and DNA methyltransferase-1 as an epigenetic prognostic biomarker in HCV-induced liver cirrhosis and HCC patients. Cancer Gene Ther 27(6):486–497. https://doi.org/10.1038/s41417-019-0123-9
24. Yin T, Zhao H (2022) miR-152-3p impedes the malignant phenotypes of hepatocellular carcinoma by repressing roundabout guidance receptor 1. Cell Mol Biol Lett 27(1):22. https://doi.org/10.1186/s11658-022-00322-y
25. Pallante P, Visone R, Ferracin M, Troncone G (2006) MicroRNA deregulation in human thyroid papillary carcinomas. EndocrineRelated Cancer 13:497
26. Duan ZY, Cai GY, Li JJ, Bu R, Wang N, Yin P, Chen XM (2018) U6 can be used as a housekeeping gene for urinary sediment miRNA studies of IgA nephropathy. Sci Rep 8(1):10875. https://doi.org/10.1038/s41598-018-29297-7
27. Yousuf T, Dar SB, Bangri SA, Choh NA, Rasool Z, Shah A, Rather RA, Rah B, Bhat GR, Ali S, Afroze D (2022) Diagnostic implication of a circulating serum-based three-microRNA signature in hepatocellular carcinoma. Front Genet 13:929787. https://doi.org/10.3389/fgene.2022.929787
28. Karreth FA, Pandolfi PP (2013) ceRNA cross-talk in cancer: when ce-bling rivalries go awry. Cancer Discov 3(10):1113–1121. https://doi.org/10.1158/2159-8290.CD-13-0202. (**Epub 2013 Sep 26**)
29. Zhan T, Gao X, Wang G, Li F, Shen J, Lu C, Xu L, Li Y, Zhang J (2021) Construction of Novel lncRNA–miRNA–mRNA Network Associated With Recurrence and Identification of Immune-Related Potential Regulatory Axis in Hepatocellular Carcinoma. Front Oncol 11:626663. https://doi.org/10.3389/fonc.2021.626663
30. Yang F, Zhang J, Li B, Zhao Z, Liu Y, Zhao Z, Jing S, Wang G (2021) Identification of Potential lncRNAs and miRNAs as Diagnostic Biomarkers for Papillary Thyroid Carcinoma Based on Machine Learning. Int J Endocrinol 2021:3984463. https://doi.org/10.1155/2021/3984463
31. Liao L, Chen X, Huang H et al (2023) Long non-coding RNA CASC7 is a promising serum biomarker for hepatocellular carcinoma. BMC Gastroenterol 23:324. https://doi.org/10.1186/s12876-023-02961-7
32. Hahne JC, Valeri N (2018) Non-Coding RNAs and Resistance to Anticancer Drugs in Gastrointestinal Tumors. Front Oncol 8:226. https://doi.org/10.3389/fonc.2018.00226
33. Hahne JC, Mirchev M, Kotzev I, Lampis A, Valeri N (2017) Biomarkers for monitoring response to therapies and detection of acquired resistance in advanced gastrointestinal cancers. Front Clin Drug Res 4:1–73. https://doi.org/10.2174/9781681084817117040003
34. Hobuß L, Bär C, Thum T (2019) Long non-coding RNAs: at the heart of cardiac dysfunction? Front Physiol 10:30. https://doi.org/10.3389/fphys.2019.00030
35. Nana-Sinkam SP, Croce CM (2014) MicroRNA regulation of tumorigenesis, cancer progression and interpatient heterogeneity: towards clinical use. Genome Biol 15(9):445. https://doi.org/10.1186/s13059-014-0445-8
36. Sánchez Y, Huarte M (2013) Long non-coding RNAs: challenges for diagnosis and therapies. Nucleic Acid Ther 23(1):15–20. https://doi.org/10.1089/nat.2012.0414
37. Stępień E, Costa MC, Kurc S, Drożdż A, Cortez-Dias N, Enguita FJ (2018) The circulating non-coding RNA landscape for biomarker research: lessons and prospects from cardiovascular diseases. Acta Pharmacol Sin 39(7):1085–1099. https://doi.org/10.1038/aps.2018.35. (**Epub 2018 Jun 7**)
38. Ratti M, Lampis A, Ghidini M, Salati M, Mirchev MB, Valeri N, Hahne JC (2020) MicroRNAs (miRNAs) and long non-coding RNAs (lncRNAs) as new tools for cancer therapy: first steps from bench to bedside. Target Oncol 15(3):261–278. https://doi.org/10.1007/s11523-020-00717-x
39. Gambari R, Brognara E, Spandidos DA, Fabbri E (2016) Targeting oncomiRNAs and mimicking tumor suppressor miRNAs: vew trends in the development of miRNA therapeutic strategies in oncology (Review). Int J Oncol 49(1):5–32. https://doi.org/10.3892/ijo.2016.3503. (**Epub 2016 May 4**)

Publisher's Note