Crosstalk between liver-related microRNAs and Wnt/β-catenin pathway in hepatocellular carcinoma patients

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ABSTRACT

Background and study aims: Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide with highest incidence in Asia and Africa. MicroRNAs (miRNAs), a class of non-coding single stranded RNA, which not only post transcriptionally regulate gene expression but also respond to signaling molecules to affect cell functions such as Wnt/β-catenin signaling specifically in HCC. The goal of this study is to investigate the crosstalk between Wnt/β-catenin signaling proteins and microRNAs expression in HCC patients.

Patients and methods: Fresh tissue samples of 30 primary HCC patients and 10 control subjects were included. Expression level of 13 different miRNAs (miR-10a– miR-106b– miR-99a– miR-148a– miR-125b– miR-30e– miR-183– miR-155– miR-199a– miR-199a3p– miR-24– miR-122 and miR-215) were examined using real-time PCR assay. Five proteins involved in the Wnt/β-catenin pathway (β-catenin, APC, c-myc, survivin and cyclin D1) were analysed by immunohistochemistry technique. The correlation between miRNAs expression levels with protein expressions was assessed.

Results: Up-regulation of miR-155 and miR-183 was reported in HCC patients compared to normal controls and this up-regulation was significantly correlated with liver cirrhosis in the case of miR-155 (p < 0.05) referring to their oncogenic activity. Down-regulation was observed for 11 miRNAs in HCC indicating their tumour suppressor activity. miRNA-10a, miR-30e, miR-215, miR-125b and miR-148a were significantly correlated with the expression of important players in Wnt/β-catenin pathway including β-catenin, APC, c-myc and cyclin D1. Detailed analysis revealed that miR-215 is associated with the grade of the disease and miR-125b is associated with HCV infection.

Conclusion: Collectively, our data showed potential role of miR-10a, miR-30e, miR-215, miR-125b and miR-148a as important mediators in HCC progression. Furthermore, their association with Wnt/β-catenin cascade proteins could be exploited to develop new therapeutic target strategies in HCC.

Introduction

Hepatocellular carcinoma (HCC) is the fifth most commonly diagnosed cancer worldwide but the third leading cause of cancer-related death around the world [1]. Hepatocellular carcinoma death rates have increased by almost 3% per year since 2000 (American Cancer Society’s Cancer Statistics Center; 2017). About 90% of HCC cases arise from cirrhosis, which can be attributed to a wide range of factors including chronic viral hepatitis B or C (HBV or HCV) infections, alcohol abuse, nonalcoholic steatohepatitis (NASH), autoimmune hepatitis, primary biliary cirrhosis (PBC), and carcinogens exposure [2].

The involvement of microRNAs (miRNAs) as regulators for HCC development became a focus of research in molecular biology. MicroRNAs are small, evolutionarily conserved, single stranded RNA molecules of approximately 21–24 nucleotides, which regulate gene expression by binding to specific mRNA targets and promoting their degradation and/or translational inhibition [3].
During tumour development, miRNAs function as oncogenes or tumour suppressor genes. Sun et al. [4] reported that, deregulation of different miRNAs in different types of cancer including HCCs. The Wnt pathway is a highly regulated signaling pathway that controls numerous stages of tissue homeostasis [5]. This pathway is closely regulated at both transcriptional-level regulations to post-translational modification; thus aberrant Wnt activity often results in developmental disorders and diseases including but not limited to cancer [6]. The canonical Wnt-signaling cascade refers to the transduction of series of signals mediated via the interaction of specific Wnt ligands with their target receptor resulting in the accumulation of β-catenin in the nucleus [7]. The cytoplasmic stability of β-catenin is usually maintained at a minimal level by the destruction complex composed of a scaffold combination of tumor suppressor protein adenomatous polyposis coli (APC), Axin2, casein kinase1 (CK1) and glycogen synthase kinase 3β (GSK-3β) [8]. In the absence of Wnt ligand interaction (OFF-state), the membrane receptor complex is inactivated, eventually resulting in β-catenin ubiquitination and degradation [9]. The current and widely accepted model suggests that CK1 and GSK-3β kinases phosphorylate β-catenin [10], leading to recruitment of APC that mediates the degradation of cytoplasmic β-catenin. Although it is inactive in adult livers, the Wnt pathway participates in liver pathobiology [11]. Deregulation of this pathway has previously been described in hepatoblastoma as well as in HCC [12]. In liver carcinogenesis, early deregulation of the Wnt pathway occurs, leading to increased proliferation, migration, and invasiveness of cancer cells [13]. Advanced technology in biomedical research have allowed experimental and bioinformatics approaches to identify miRNAs as regulators of components of the Wnt-signaling pathways and vice versa. Thus, both miRNAs and Wnt-signaling pathways form a network involved in the regulation of key biological processes.

Patients and methods

Patients

Fresh tissue samples from 30 HCC patients as well as 10 healthy volunteers from liver donor prior transplantation, used as normal controls, were enrolled in the current study. All samples were processed according to the national guidelines for the use of human subject materials and approved by the Institutional Review Board of the Egyptian National Cancer Institute, Cairo University. A written informed consent was obtained from all participants involved in the study during the period 2013–2014. The HCC patients were diagnosed by abdominal ultra-sonography, triphasic CT abdomen, serum AFP and confirmed pathologically. None of the HCC patients received any previous chemotherapeutic treatments. The control volunteers showed no clinical or biochemical evidence of any liver diseases or any other diseases at recruitment time. Patients with HBV infection, multiple liver lesions, or metastasis were excluded. Liver biopsy was taken from each patient using ultrasound guided trucut needle biopsy technique. Tissues were cut immediately into two parts; one was preserved in 10% neutral buffered formalin and processed for haematoxylin and eosin staining for histopathological and immunohistochemical analysis. The other part was collected in RNAlater solution (Qiagen, Germany) and stored at –80 °C for miRNA analysis. Clinicopathological characteristics of the patients and controls are summarized in Table 1.

RNA isolation/cDNA synthesis/quantitative real-time PCR analysis

Isolation of total RNA from tissue biopsies was performed using miRNeasy Mini kit (Qiagen) according to the manufacturer’s recommendations. The quality and yield were assessed using a Nano-Drop 2000 spectrophotometer (Thermo Fisher Scientific technologies, USA). A total of 1 μg RNA was reverse transcribed into cDNA in a final volume of 20 μl using miScript Reverse Transcription kit (Qiagen, Germany) according to the manufacturer’s protocol. The expression levels of miR-10a, miR-24, miR-30e, miR-99a, miR-106b, miR-122, miR-125b, miR-148a, miR-155, miR-183, miR-199a, miR-199a-3p and miR-215 were determined by miScript SYBER Green PCR kit (Qiagen, Germany) using miScript universal primer and quantiTec SYBER green PCR master mix. RNU6 was used as internal control. All primers used are obtained from Qiagen. The reactions were incubated in a 96-well optical plate at 95 °C for 15 min, followed by 40 cycles at 94 °C for 15 s, 55 °C for 30 s and 70 °C for 30 s on ABI 7500 (Applied Biosystems, USA). All of the reactions were run in triplicate. A comparative Ct method was used. The expression levels of miRNAs relative to the internal control RNU6 was calculated using the 2^-ΔΔCT equation where ΔΔCT = (CTmiRNA - CTU6)target – (CTmiRNA - CTU6)control [14].

Immunohistochemistry (IHC)

IHC analysis for β-catenin, APC, c-myc, cyclin D1 and survivin proteins was performed on paraffin embedded tissue section from the 30 HCC cases and 10 control subjects recruited for the current study. Briefly, consecutive tissue sections (4μm) were deparaffinized using xylene, rehydrated alcohol and subjected to antigen retrieval by heating in target retrieval solution (Dako, USA) for 15 min in microwave. Hydrogen peroxide (3%) was then used to quench the sections for 10–15 min. After blocking, sections were incubated with primary mouse monoclonal antibodies for 1 h. Slides were washed in Tris-Buffered Saline and Tвин 20 (TBST) solution for 10 min. and the binding sites were stained using DAB-substrate and Diaminobenzidine-chromogen solution for 10 min until suitable staining were developed. The following primary antibodies were used: APC (Abcam, dilution 1:100); β-catenin (Invitrogen, dilution 1:100); c-myc (Thermo Scientific, dilution 1:100); cyclin D1 (R&D system, dilution, 1:50); and survivin (Enzo life, dilution 1:40). Tissue sections from human colon adenocarcinoma used as positive control for β-catenin, APC and survivin whereas human breast carcinoma were employed as positive control for c-myc and cyclin D1.

Immunohistochemically stained sections were grouped into three groups for statistical analysis: Control group, Normal group and HCC group. β-Catenin, APC, c-myc, cyclin D1 and survivin stained sections from the three groups were examined under light microscopy and scored manually by two unbiased pathologists (M. K. and H.A). Sections were traced, captured and measured semi-quantitatively using an image analysis system (Leica Microsystems, QWin software 3000) [15]. Five to ten fields (x20) per slide were measured and included in the analysis.

Statistical analysis

All statistical analyses were performed using SPSS 20 software (SPSS, Inc., USA). Data on the relative expression of miRNAs are performed as mean ± SEM. Chi-square test or Fisher’s exact test was used to examine the relation between qualitative variables. One-Way ANOVA calculator was used to analyse the correlation between the expression of the five IHC markers in normal and HCC sections as regards the results of their image analysis. Mann-Whitney-U test was used to compare miRNAs expression with different clinicopathological factors. Spearman’s correlation coefficients were calculated between miRNAs expression with β-catenin, APC, c-myc, cyclin D1 and survivin proteins. A p value < 0.05 was considered significant.
Results

Expression profile of miRNAs in HCC patients

The expression levels of 13 different miRNAs were investigated in tissue samples freshly collected from 30 HCC patients using qRT-PCR assay. Most of miRNAs (11 out of 13) showed a downregulation with different fold change ranging from 3.35 for miR-24 up to 16.95 for miR-148a compared to normal subjects (p < 0.05). Interestingly, miR-155 and miR-183 showed significant upregulation in HCC patients with 6.9 and 26.9 fold increases, respectively compared to normal subjects (p < 0.05). The expression levels for all miRNAs and associated correlation are shown in Tables 2 and 3.

Analysis of Wnt/β-catenin signaling pathway in HCC patients

The protein expression profile of Wnt/β-catenin pathway were investigated namely, β-catenin, APC, c-myc, survivin, and cyclin D1. IHC stained sections of HCC tissues (n = 30) were analysed compared to normal liver sections (n = 10) histopathologically and semi-quantitatively using image analysis (Figs. 1 and 2) respectively. Our results revealed a strong membranous and pale cytoplasmic β-catenin staining with absence of nuclear expression in normal hepatocytes. However, results of HCC sections showed high expression of β-catenin in both nucleus and cytoplasm. This indicates relocalization of the membranous β-catenin to the cytoplasm and nucleus (p < 0.001). Nuclear staining was observed in 25 cases of the 30 (83%) cases examined (Fig. 1A). For APC protein expression, we found that all normal liver subjects exhibited cytoplasmic staining. The intensity of APC immunostaining and the number of immunoreactive cells were absent in tumourous epithelial cells of 15/30 (50%) patients (p < 0.001). Whilst, the expression of APC was similar to normal cells in 3 patients (10%), reduced APC expression was observed in (40%) of patients (Fig. 1B). Nuclear c-myc expression was detected in 29/30 (96%) patients (p = 0.0057), whilst only one patient exhibited weak expression of c-myc compared to normal liver tissues (Fig. 1C). As anticipated, the expression of survivin protein was absent in all normal tissue liver sections analysed whilst, it was expressed with different intensities in all HCC patients (p < 0.001) as illustrated in (Fig. 1D). Furthermore, we observed overexpression of cyclin D1 in 67% of tumour tissues compared to normal tissue (p < 0.001). 25% of patients showed weak cyclin D1 protein expression and only 8% of cases didn't show any expression of cyclin D1 (Fig. 1E).

Correlation between miRNA expression levels and Wnt/β-catenin protein expressions

The expression level of 13 different miRNAs analysed in the present study was correlated with the Wnt/β-catenin proteins
Table 2
Expression levels for tissue miRNAs/Wnt/β-catenin proteins that are significantly deregulated in Egyptian primary hepatocellular carcinoma patients compared to control volunteers.

<table>
<thead>
<tr>
<th>miRNA</th>
<th>Average (fold changes) ± SEM</th>
<th>Up/down expression</th>
<th>p-value</th>
<th>Differentially associated Wnt/β-catenin protein</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-10a</td>
<td>0.244 ± 0.08</td>
<td>Down</td>
<td>0.043</td>
<td>c-myc</td>
<td>0.04</td>
</tr>
<tr>
<td>miR-24</td>
<td>0.298 ± 0.17</td>
<td>Down</td>
<td>0.027</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>miR-30e</td>
<td>0.183 ± 0.03</td>
<td>Down</td>
<td>0.030</td>
<td>c-myc</td>
<td>0.004</td>
</tr>
<tr>
<td>miR-99a</td>
<td>0.147 ± 0.03</td>
<td>Down</td>
<td>0.012</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>miR-106b</td>
<td>0.268 ± 0.04</td>
<td>Down</td>
<td>0.038</td>
<td>APC</td>
<td>0.02</td>
</tr>
<tr>
<td>miR-122</td>
<td>0.101 ± 0.02</td>
<td>Down</td>
<td>0.0003</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>miR-125b</td>
<td>0.184 ± 0.03</td>
<td>Down</td>
<td>0.004</td>
<td>B-catenin</td>
<td>0.03</td>
</tr>
<tr>
<td>miR-148a</td>
<td>0.059 ± 0.01</td>
<td>Down</td>
<td>0.001</td>
<td>APC</td>
<td>0.05</td>
</tr>
<tr>
<td>miR-155</td>
<td>6.908 ± 1.8</td>
<td>Up</td>
<td>0.017</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>miR-183</td>
<td>26.983 ± 0.3</td>
<td>Up</td>
<td>0.042</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>miR-199a</td>
<td>0.363 ± 0.1</td>
<td>Down</td>
<td>0.036</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>miR-199a-3p</td>
<td>0.117 ± 0.03</td>
<td>Down</td>
<td>0.039</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>miR-215</td>
<td>0.157 ± 0.03</td>
<td>Down</td>
<td>0.013</td>
<td>c-myc</td>
<td>0.01</td>
</tr>
</tbody>
</table>

SEM: standard error of the mean, *p* is significant if <0.05.

Table 3
Parametric correlation between different miRNAs analysed in HCC patients.

<table>
<thead>
<tr>
<th>miR-10a</th>
<th>miR-24</th>
<th>miR-30e</th>
<th>miR-99a</th>
<th>miR-106b</th>
<th>miR-122</th>
<th>miR-125b</th>
<th>miR-148a</th>
<th>miR-155</th>
<th>miR-183</th>
<th>miR-199a</th>
<th>miR-199a-3p</th>
<th>miR-215</th>
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<td>1.000</td>
<td>0.440</td>
<td>0.592</td>
<td>0.593</td>
<td>0.234</td>
<td>0.419</td>
<td>0.604</td>
<td>0.392</td>
<td>0.192</td>
<td>0.271</td>
<td>0.683</td>
<td>0.662</td>
<td>0.532</td>
</tr>
<tr>
<td>0.440</td>
<td>1.000</td>
<td>0.162</td>
<td>0.143</td>
<td>-0.349</td>
<td>0.533</td>
<td>0.110</td>
<td>0.038</td>
<td>-0.082</td>
<td>-0.642</td>
<td>0.277</td>
<td>0.394</td>
<td>0.103</td>
</tr>
<tr>
<td>0.592</td>
<td>0.162</td>
<td>1.000</td>
<td>0.816</td>
<td>0.502</td>
<td>0.347</td>
<td>0.714</td>
<td>0.607</td>
<td>0.126</td>
<td>-0.200</td>
<td>0.436</td>
<td>0.403</td>
<td>0.676</td>
</tr>
<tr>
<td>0.593</td>
<td>0.143</td>
<td>0.816</td>
<td>1.000</td>
<td>0.463</td>
<td>0.363</td>
<td>0.797</td>
<td>0.515</td>
<td>0.256</td>
<td>-0.225</td>
<td>0.430</td>
<td>0.369</td>
<td>0.630</td>
</tr>
<tr>
<td>0.234</td>
<td>-0.349</td>
<td>0.502</td>
<td>0.463</td>
<td>1.000</td>
<td>-0.293</td>
<td>0.545</td>
<td>0.512</td>
<td>0.190</td>
<td>0.465</td>
<td>0.362</td>
<td>0.125</td>
<td>0.184</td>
</tr>
<tr>
<td>0.419</td>
<td>0.533</td>
<td>0.347</td>
<td>0.363</td>
<td>-0.293</td>
<td>1.000</td>
<td>0.337</td>
<td>0.183</td>
<td>-0.054</td>
<td>-0.657</td>
<td>0.247</td>
<td>0.252</td>
<td>0.508</td>
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<tr>
<td>0.604</td>
<td>0.110</td>
<td>0.714</td>
<td>0.797</td>
<td>0.545</td>
<td>0.337</td>
<td>1.000</td>
<td>0.659</td>
<td>0.266</td>
<td>-0.153</td>
<td>0.397</td>
<td>0.300</td>
<td>0.372</td>
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<tr>
<td>0.392</td>
<td>0.038</td>
<td>0.607</td>
<td>0.515</td>
<td>0.512</td>
<td>0.183</td>
<td>0.659</td>
<td>1.000</td>
<td>0.013</td>
<td>0.111</td>
<td>0.263</td>
<td>0.128</td>
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<tr>
<td>0.192</td>
<td>-0.082</td>
<td>0.126</td>
<td>0.256</td>
<td>0.190</td>
<td>-0.054</td>
<td>0.266</td>
<td>0.013</td>
<td>1.000</td>
<td>0.167</td>
<td>0.504</td>
<td>0.518</td>
<td>0.136</td>
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<tr>
<td>0.183</td>
<td>-0.271</td>
<td>-0.642</td>
<td>-0.200</td>
<td>-0.225</td>
<td>0.465</td>
<td>-0.657</td>
<td>0.153</td>
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<td>0.167</td>
<td>1.000</td>
<td>-0.026</td>
<td>-0.167</td>
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<tr>
<td>0.683</td>
<td>0.277</td>
<td>0.436</td>
<td>0.430</td>
<td>0.362</td>
<td>0.247</td>
<td>0.397</td>
<td>0.263</td>
<td>0.504</td>
<td>-0.026</td>
<td>1.000</td>
<td>0.895</td>
<td>0.373</td>
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<tr>
<td>0.662</td>
<td>0.394</td>
<td>0.403</td>
<td>0.369</td>
<td>0.125</td>
<td>0.252</td>
<td>0.300</td>
<td>0.128</td>
<td>0.518</td>
<td>-0.108</td>
<td>0.895</td>
<td>1.000</td>
<td>0.282</td>
</tr>
<tr>
<td>0.532</td>
<td>0.103</td>
<td>0.676</td>
<td>0.630</td>
<td>0.184</td>
<td>0.508</td>
<td>0.372</td>
<td>0.394</td>
<td>0.136</td>
<td>-0.167</td>
<td>0.373</td>
<td>0.282</td>
<td>1.000</td>
</tr>
</tbody>
</table>

These values represent the correlation coefficient. Correlation is significant at the 0.05 level (2-tailed).

Fig. 1. Light microscopic examination of IHC stained sections. Positive control (+ve CTR) tissue sections (x20): (A-I) Colonic adenocarcinoma showing strong β-Catenin expression. (B-I) Colonic adenocarcinoma showing focal APC expression. (C-I) Mammary IDC showing strong C-myc expression. (D-I) Colonic adenocarcinoma showing strong Survivin expression. (E-I) Mammary IDC showing strong Cyclin D1 expression. Normal tissue sections (x40): (A-II) Normal liver tissue showing faint positive cytoplasmic and strong membranous staining for β-Catenin. (B-II) Normal liver tissue showing faint positive cytoplasmic and membranous staining for APC. (C-II) Normal liver tissue showing positive cytoplasmic and membranous staining for C-myc. (D-II) Normal liver tissue showing negative Survivin expression. (E-II) Normal liver tissue showing positive cytoplasmic and membranous staining for Cyclin D1. HCC tissue sections (x20): (A-III) Tumour tissue showing minima, focal membranous staining with diffuse cytoplasmic and nuclear β-Catenin staining (relocalization). (B-III) Tumour tissue showing faint and focal cytoplasmic and membranous APC staining. (C-III) Tumour tissue showing strong nuclear staining together with faint and diffuse cytoplasmic and nuclear C-myc staining. (D-III) Tumour tissue showing strong and diffuse nuclear, cytoplasmic and membranous Survivin staining. (E-III) Tumour tissue showing strong and diffuse cytoplasmic and membranous Cyclin D1 staining. HCC, hepatocellular carcinoma. IDC, invasive duct carcinoma.

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including β-catenin, APC, c-myc, cyclin D1 and survivin. Our data revealed significant positive correlations between 6 out of the 13 miRNAs studied and the level of APC, β-catenin and c-myc protein expression level in which APC was positively correlated with each of miR-106b (p = 0.02), miR-125b (p = 0.05), and miR-148a (p = 0.04). β-Catenin was positively correlated with the level of miR-30e (p = 0.03) and miR-125b (p = 0.03). C-myc showed also significant positive correlation with miR-10a (p = 0.04), miR-30e (p = 0.004) and miR-215 (p = 0.01) as shown in Table 2.

Correlation between miRNA expression levels/protein expression levels with several clinico-pathological parameters

Our data indicated a significant correlation between miR-125b with presence of HCV infection, being higher expressed in HCC/HCV+ patients (n = 18) compared to HCC/HCV free; (n = 12) (fold changes were 0.17 vs 0.03, respectively; p = 0.049). Furthermore, protein expression of survivin detected in HCC/HCV+ patients was higher compared to HCC/HCV free patients (p = 0.05). High expression of miR-155 (12.8 fold change) in HCC associated with cirrhosis (n = 11) was observed compared to non-cirrhotic patients (n = 19) (3.43 fold change, p = 0.027). Finally, highly significant correlation was observed in miR-215 level with tumour grades where the mean fold changes recorded in early grade (grade I) (n = 7) was 0.01 compared to its level in grade II (n = 23) (0.017 fold change; p = 0.006). No significant correlation was recorded with other clinico-pathological factors analysed. Fig. 3 shows representative miRNAs expression level with some clinico-pathological features.

**Discussion**

Hepatic carcinogenesis is a complex multiple steps process, which requires both genetic and epigenetic alterations in hepatocytes. Deregulation of the Wnt pathway is an early event in hepatic carcinogenesis and microRNAs have critical roles in HCC progression by targeting many critical proteins coding genes [16].

In this study, we aimed to evaluate the crosstalk between Wnt signaling proteins and microRNAs in hepatocellular carcinoma patients. Expression level of 13 liver related microRNAs were examined using RT-PCR. miR-155 and miR-183 showed upregulation compared to normal healthy tissue as reported in liver and other types of cancers [17–20]. Furthermore, miR-155 upregulation was found to be significantly more frequent in HCC associated with cirrhosis, indicating its oncogenic activity that may be associated with the progression of liver disease. A direct role of miR-155 in liver tumourigenesis was demonstrated through targeting APC which activates Wnt/β-catenin pathway [17]. However, our data did not exhibit such significant correlation which may be attributed to aetiological, environmental and genetic variations. Eleven miRNAs were found to be significantly downregulated in HCC compared to normal liver tissues indicating a tumour suppression activity. MiR-10a, miR-30e and miR-215 positively correlated with c-myc expression. MiR-30e and miR-125b were positively correlating with β-catenin. MiR-125b, miR-106b and miR-148a were associated with APC. These data indicate a possible mechanism...
for the tumour suppression mediated by the indicated miRNAs, being transcription regulators of different proteins of Wnt pathway. MiR-10a interacts with the 5′ untranslated region of mRNAs encoding ribosomal proteins (RPs) to enhance its translation and hereby affect the ability of cells to undergo transformation [21]. Furthermore, prominent oncogenes, MYC, have been shown to positively affect the production of RP genes, and other translational regulators [22–24]. Our data revealed the expected role for miR-10a in regulating the translational machinery via modulating the translation of RP through c-myc protein. MiR-30e has been involved in diverse activities in different organs. It has been engaged as a sub-specific prognostic marker in breast cancer [25,26], it was found to directly target Bmi1 (which is involved in cell self-renewal) which was up-regulated via Tumour-Associated Macrophages “TAMs”, through knocking down miR-30e in gastrointestinal cancer [27]. However, the role of miR-30e played in HCC initiation or progression is not yet fully understood [28]. Interestingly, we reported a significant correlation between miR-30e and survivin in HCC patients. In-silico analyses revealed the involvement of miR-30e in regulating autophagy pathway, explaining the correlation between miR-30e and survivin which in turn was proven to be tightly related with autophagy [29]. MiR-125b was found to be positively correlating with APC, supporting its role in blocking hepatocarcinogenesis [30] through its suppression of cell growth, induction cell cycle arrest at G1 phase, and inhibition of migration and invasion of HCC cells via targeting Lin28B oncogene [31]. Furthermore, it was shown that miR-125b suppresses EMT and EMT-associated traits of HCC by targeting SMAD 2 & 4 [32]. The dual correlation of miR125b with both β-catenin and APC may be due to that APC is not the sole factor to determine the activity of β-catenin. Moreover, β-catenin encoding gene mutation exists in around 25% of all HCC cases that might enable β-catenin to evade the interaction with the destruction complex [33] and that percentage is elevated in HCV-associated HCC to be 40% of all HCC cases [34]. Our data reported a significant correlation between miR-125b with presence of HCV infection, being higher expressed in HCC/HCV+ patients (60%) compared to HCC/HCV free. APC could be also mutated; not able to function properly and unable to provide efficient assembly and disassembly capability of β-catenin into and from the destruction complex [35]. In the current study, miR-148a expression was reported to be significantly lower in HCC patients, reassuring what previously stated: down expression of miR-148a associated with hepatic carcinogenesis and deterioration of HCC [36]. Ubiquitin specific protease 4 (USP4), was identified as a direct target of miR-148a. Downregulation of miR-148a in HCC, yielding overexpression of USP4 in mesenchymal type liver-tumour cells facilitating migration and proliferation [37]. Likewise, miR-148a was found to directly target DNA methyltransferase 1 (DNMT1), a major enzyme responsible for epigenetic silencing of tumour suppressor genes [38]. The significant correlation between miR-148a and APC protein in the present study might be explained through PTEN. The phosphatase activity of PTEN is dispensable for the regulation of APC and PTEN showed to be targeted by miR-148a and thereby contributed to hepatocarcinogenesis [39]. MiR-106b was also found to directly target APC which in turn targets β-catenin down-regulating Wnt pathway in HCC [40], which may explain the positive association between APC and miR-106b observed in our study. Finally, downregulation of miR-215 reported in HCC of the present and a highly significant correlation was observed between miR-215 level and tumour grades was consistent with several studies [41–43] indicating its role in tumour progression and its association with c-myc may be due to activating Wnt/β-catenin through increasing β-catenin phosphorylation [44]. In conclusion, our data suggest a crosstalk between the above indicated miRNAs and Wnt/β-catenin signaling proteins as well as their role in regulation of HCC progression. Better understanding of this crosstalk could be exploited for development of new therapeutic agents through targeting the canonical Wnt signaling pathway in HCC.

Conflicts of interest
The authors declared that there are no conflicts of interest.

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