### Editorials

**Hepatocellular carcinoma—the role of the underlying liver disease in clinical practice**

*de Mattos AZ, Bombassaro IZ, Vogel A, Debes JD*

**Immunotherapy for esophageal cancer: Where are we now and where can we go**

*Shoji Y, Koyanagi K, Kanamori K, Tajima K, Ogimi M, Ninomiya Y, Yamamoto M, Kazuno A, Nahushima K, Nishi T, Mori M*

**Outpatient management of obscure gastrointestinal bleeding: A new perspective in high-risk patients**

*Riccioni ME, Marmo C*

### MiniReviews

**Pathogenesis of chronic enteropathy associated with the SLCO2A1 gene: Hypotheses and conundrums**

*Xie ZX, Li Y, Yang AM, Wu D, Wang Q*

**Present and future of new systemic therapies for early and intermediate stages of hepatocellular carcinoma**

*Urquijo-Ponce JJ, Alventosa-Mateu C, Latorre-Sánchez M, Castelló-Miralles I, Diago M*

### Original Articles

**Retrospective Cohort Study**

**Clinical manifestations, diagnosis and long-term prognosis of adult autoimmune enteropathy: Experience from Peking Union Medical College Hospital**

*Li MH, Ruan GC, Zhou WX, Li XQ, Zhang SY, Chen Y, Bai XY, Yang H, Zhang YJ, Zhao PY, Li J, Li JN*

**Prospective Study**

**Non-pancreatic hyperlipasemia: A puzzling clinical entity**

*Feher KE, Tornai D, Vitalis Z, Davida L, Szepki N, Papp M*

**Basic Study**

**HepG2.2.15-derived exosomes facilitate the activation and fibrosis of hepatic stellate cells**

*Gao Y, Li L, Zhang SN, Yang Y, Zhang XB, Feng SM*

**Cell division cyclin 25C knockdown inhibits hepatocellular carcinoma development by inducing endoplasmic reticulum stress**

*Li YF, Zheng JY, Miao XY, Liu HL, Zhang YY, Chao NX, Mo FR*

**Novel lactylation-related signature to predict prognosis for pancreatic adenocarcinoma**

*Peng T, Sun F, Yang JC, Cai MH, Huai MX, Pan JX, Zhang FY, Xu LM*
<table>
<thead>
<tr>
<th>Page</th>
<th>Title</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td>2612</td>
<td>Diagnostic value associated with the combination of saliva pepsin and microorganisms in functional heartburn and gastroesophageal reflux disease</td>
<td>Huang YQ, Yang C, Luo W</td>
</tr>
<tr>
<td>2615</td>
<td>Reinforcing the management of type 1 gastric esophageal varices</td>
<td>Majid Z, Abrar G</td>
</tr>
</tbody>
</table>
About Cover
Editorial Board Member of World Journal of Gastroenterology, Matthias Ocker, MD, Professor, Medical Department, Division of Hematology, Oncology, and Cancer Immunology, CCM, Charité University Medicine Berlin, Berlin 10117, Germany. matthias.ocker@charite.de

Aims and Scope
The primary aim of World Journal of Gastroenterology (WJG, World J Gastroenterol) is to provide scholars and readers from various fields of gastroenterology and hepatology with a platform to publish high-quality basic and clinical research articles and communicate their research findings online. WJG mainly publishes articles reporting research results and findings obtained in the field of gastroenterology and hepatology and covering a wide range of topics including gastroenterology, hepatology, gastrointestinal endoscopy, gastrointestinal surgery, gastrointestinal oncology, and pediatric gastroenterology.

Indexing/Abstracting
The WJG is now abstracted and indexed in Science Citation Index Expanded (SCIE), MEDLINE, PubMed, PubMed Central, Scopus, Reference Citation Analysis, China Science and Technology Journal Database, and Superstar Journals Database. The 2023 edition of Journal Citation Reports® cites the 2022 impact factor (IF) for WJG as 4.3; Quartile category: Q2. The WJG’s CiteScore for 2021 is 8.3.

Responsible Editors for This Issue
Production Editor: Xiao-Mei Zheng; Production Department Director: Xu Guo; Cover Editor: Jia-Ru Fan.

Name of Journal
World Journal of Gastroenterology

ISSN
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

Launch Date
October 1, 1995

Frequency
Weekly

Editors-in-Chief
Andrzej S Tarnawski

Executive Associate Editors-in-Chief
Xian-Jun Yu (Pancreatic Oncology), Jian-Gao Fan (Chronic Liver Disease), Hou-Bao Liu (Biliary Tract Disease)

Editorial Board Members
http://www.wjgnet.com/1007-9327/editorialboard.htm

Publication Date
May 21, 2024

Copyright
© 2024 Baishideng Publishing Group Inc

Publishing Partner
Shanghai Pancreatic Cancer Institute and Pancreatic Cancer Institute, Fudan University
Biliary Tract Disease Institute, Fudan University

Instructions to Authors
https://www.wjgnet.com/bpg/gerinfo/204

Guidelines for Ethics Documents
https://www.wjgnet.com/bpg/GerInfo/287

Guidelines for Non-Native Speakers of English
https://www.wjgnet.com/bpg/gerinfo/240

Publication Ethics
https://www.wjgnet.com/bpg/GerInfo/288

Publication Misconduct
https://www.wjgnet.com/bpg/GerInfo/208

Policy of Co-Authors
https://www.wjgnet.com/bpg/GerInfo/310

Article Processing Charge
https://www.wjgnet.com/bpg/gerinfo/242

Steps for Submitting Manuscripts
https://www.wjgnet.com/bpg/gerinfo/239

Online Submission
https://www.f6publishing.com

Publishing Partner’s Official Website
https://www.shca.org.cn
https://www.zs-hospital.sh.cn
Basic Study

HepG2.2.15-derived exosomes facilitate the activation and fibrosis of hepatic stellate cells

Yang Gao, Li Li, Sheng-Ning Zhang, Yuan-Yi Mang, Xi-Bing Zhang, Shi-Ming Feng

The role of exosomes derived from HepG2.2.15 cells, which express hepatitis B virus (HBV)-related proteins, in triggering the activation of LX2 liver stellate cells and promoting liver fibrosis and cell proliferation remains elusive. The focus was on comprehending the relationship and influence of differentially expressed microRNAs (DE-miRNAs) within these exosomes.

AIM

To elucidate the effect of exosomes derived from HepG2.2.15 cells on the activation of hepatic stellate cell (HSC) LX2 and the progression of liver fibrosis.

METHODS

Exosomes from HepG2.2.15 cells, which express HBV-related proteins, were isolated from parental HepG2 and WRL68 cells. Western blotting was used to confirm the presence of the exosomal marker protein CD9. The activation of HSCs was assessed using oil red staining, whereas DiI staining facilitated the observation of exosomal uptake by LX2 cells. Additionally, we evaluated LX2 cell proliferation and fibrosis marker expression using 5-ethynyl-2′-deoxyuracil staining and western blotting, respectively. DE-miRNAs were analyzed using DESeq2. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways were used to annotate the target genes of DE-miRNAs.

RESULTS

Exosomes from HepG2.2.15 cells were found to induced activation and enhanced proliferation and fibrosis in LX2 cells. A total of 27 miRNAs were differentially expressed in exosomes from HepG2.2.15 cells. GO analysis indicated that these DE-miRNA target genes were associated with cell differentiation, intracellular...
signal transduction, negative regulation of apoptosis, extracellular exosomes, and RNA binding. KEGG pathway analysis highlighted ubiquitin-mediated proteolysis, the MAPK signaling pathway, viral carcinogenesis, and the toll-like receptor signaling pathway, among others, as enriched in these targets.

**CONCLUSION**
These findings suggest that exosomes from HepG2.2.15 cells play a substantial role in the activation, proliferation, and fibrosis of LX2 cells and that DE-miRNAs within these exosomes contribute to the underlying mechanisms.

**Key Words:** Hepatic stellate cells; Liver fibrosis; Exosomes; Small RNA sequencing; HepG2.2.15

©The Author(s) 2024. Published by Baishideng Publishing Group Inc. All rights reserved.

---

**Core Tip:** This study investigated the effects of exosomes, particularly those derived from HepG2.2.15 cells, on the activation of LX2 stellate cells and the progression of liver fibrosis. Exosomes from HepG2.2.15 cells were found to enhance LX2 cell activation, proliferation, and fibrosis. These exosomes contained 27 differentially expressed microRNAs that target various cellular functions and pathways, including differentiation, signal transduction, and apoptosis regulation. This suggests a significant role for HepG2.2.15-derived exosomes in liver fibrosis, with potential therapeutic implications.

**Introduction**
Introduction of chronic hepatitis B infection (CHB) induced by the hepatitis B virus (HBV) is a considerable public health concern, as it causes substantial morbidity, contributing to nearly one-third of liver cirrhosis cases worldwide [1-3]. Hepatocyte damage and subsequent necrosis are the primary clinical manifestation of CHB that occur due to the immune response generated by the HBV-infected liver cells [4]. Moreover, there is a strong association between liver injury and the onset of liver fibrosis. Current therapeutic measures prioritize the use of antioxidants and hepatoprotective agents. However, these treatments have limited efficacy in attenuating liver damage and inhibiting the advancement of fibrosis [5]. Therefore, thorough research on HBV-induced fibrogenesis and the pursuit of superior therapeutic approaches are indispensable.

Hepatic stellate cells (HSCs), which are widely dispersed throughout the liver as perisinusoidal cells, perform various functions ranging from vascular regulation to drug metabolism in the healthy liver [5]. Typically, quiescent cells are activated upon liver injury, proliferate, and exhibit heightened contractility, inflammation, chemotaxis, and an increase in extracellular matrix (ECM) production, which critically contribute to fibrosis [6]. Consequently, elucidating the role of HSCs in fibrogenesis may herald novel treatments for liver fibrosis.

Exosomes are small extracellular vesicles, approximately 100 nm in size, secreted by various cells and present ubiquitously in biological fluids [7]. Their composition includes diverse proteins and nucleic acids such as double-stranded DNA, mRNA, microRNAs (miRNAs), and long non-coding RNA (lncRNAs), enabling them to merge with target cells and modulate their function [8]. Exosomes actively engage in the pathophysiological mechanisms of numerous diseases [9,10]. Recent evidence suggests that exosomes from hepatitis C virus-infected hepatocytes containing specific miRNAs induce HSC activation, thereby facilitating fibrogenesis [11]. They are integral to the immune defense against HBV, carrying virus-derived nucleic acids that provoke macrophages [12], whereas cholangiocyte-originating exosomes containing H19 increase HSC differentiation and activation, promoting fibrosis [13]. However, the specific roles of exosomes in HBV-related liver fibrosis require further investigation.

In this study, we found that exosomes emanating from HBV-infected hepatocytes induced the activation and proliferation of LX2 cells, an immortalized line of human HSCs. Using small RNA sequencing, differentially expressed microRNAs (DE-miRNAs) in HepG2.2.15 cell-derived exosomes (an HBV-infected hepatocellular carcinoma cell line) and their non-infected counterparts were identified and profiled. Subsequent annotations based on Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) illuminated the miRNA target gene functions, thus presenting the pathways implicated in HBV-mediated liver pathology.

**Materials and Methods**
On February 21, 2022, the Ethics Committee of The Affiliated Calmette Hospital of Kunming Medical University and the First Hospital of Kunming approved the ethical review of this research. All procedures were conducted in accordance
with the relevant laws and institutional guidelines.

**Cell culture**

HepG2.2.15, HepG2, and WRL68 cell lines were obtained from the American Type Culture Collection (United States). WRL68 cells, which resemble hepatocytes, are suitable for in vitro liver studies because of their similar morphology, protein secretion, cytokeratin patterns, and key liver enzyme activities[14]. HepG2.2.15 cells were cultured in DMEM (Thermo Fisher Scientific, Waltham, MA, United States) supplemented with 10% fetal bovine serum (FBS; Gibco BRL, Grand Island, NY, United States) and G418 (Thermo Fisher Scientific, United States). HepG2 cells were grown in DMEM supplemented with 10% FBS. WRL68 cells were maintained in DMEM supplemented with 10% FBS and 1 mmol/L sodium pyruvate (Thermo Fisher Scientific, United States). All cell lines were cultivated at 37 °C in a humidified atmosphere containing 5% CO₂.

**Isolation and identification of exosome**

Exosomes were isolated from the culture media of HepG2.2.15, HepG2, and WRL68 cells following a previously described protocol[15]. Initially, media samples were centrifuged at 2000 g for 30 min at 4 °C, followed by a secondary centrifugation at 10000 g for 45 min at 4 °C. Afterward, the supernatant was filtered through a 0.45 μm filter and ultracentrifuged at 100000 g for 70 min at 4 °C. The pellet was suspended in 100 μL of chilled PBS. Portions of the resuspended exosomes (20 μL for electron microscopy, 10 μL for particle size analysis) were set aside, with the remainder stored at -80 °C. LX2 cells were co-cultured with exosomes using a Transwell system, following the methods outlined in a previous study[16].

**Transmission electron microscopy**

For transmission electron microscopy (TEM) analysis, 10 μL of exosome suspension and acetone dioxide were applied to a copper grid and left to settle for 1 min. After drying with a filter paper for 2 min at room temperature, the samples were examined using a Hitachi H-7650 transmission electron microscope (Hitachi, Tokyo, Japan) at an acceleration voltage of 100 kV. A NanoSight NS300 system (Malvern Panalytical, Shanghai, China) was employed to measure exosome size distribution and concentration by leveraging Brownian motion[17].

**Western blotting**

RIPA buffer (Abcam) was used to extract total protein from LX2 cells exposed to exosomes. Protein concentrations were quantified using the BCA Protein Assay Kit (Abcam, China). Proteins were resolved by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto PVDF membranes, which were blocked with 5% fat-free milk at room temperature overnight. Primary antibodies used were CD9, α-smooth muscle actin (α-SMA), COL1A1, MMP-2, TIMP-1, CTGF, and β-actin, all at 1:1000 dilutions (Abcam), followed by incubation with Goat Anti-Rabbit immunoglobulin G H&L (HRP, 1:10000, ab7090, Abcam) for 2 h at room temperature. Beta-actin served as a loading control. Relative expression levels were calculated using grayscale values of target proteins normalized to β-actin, with analysis conducted in Image J software (National Institutes of Health, United States).

**5-ethynyl-2′-deoxyuracil proliferation assay**

Cell proliferation was measured using 5-ethynyl-2′-deoxyuracil (EDU) kit (RiboBio, Guangzhou, China), and the ratio of EDU staining cells (with red fluorescence) to Hoechst33342-stained cells (with blue fluorescence) was used to evaluate cell proliferation rate. LX2 cells were seeded into 96-well plates at a concentration of 2 × 10⁴ per well and incubated with EDU reagent. After incubation for 2 h, the experiments were performed according to the kit instruction[18]. Finally, Hoechst staining was performed for 30 min, the stained cells were observed under a microscope, and ImageJ software (National Institutes of Health, Bethesda, MD, United States) was used for data analysis.

**Dil and Oil red staining**

For Dil staining, exosomes were incubated with 10 μL of Dil solution (Invitrogen, Carlsbad, CA, United States) at 37° for 30 min and then washed with PBS. After 10 μg/mL of Dil-labeled exosomes were co-cultured with LX2 cells for 24 h, the cells were fixed with 4% paraformaldehyde. Cytoplasm was stained with α-SMA, nucleus was stained with DAPI, and uptake of exosomes was observed by an inverted fluorescence microscope.

For Oil red staining, cells were fixed in 10% formalin, washed with 60% propylene glycerol, and then stained with 0.5% oil red (Sigma-Aldrich, St. Louis, MO, United States) with propylene glycerol for 10 min at 60 °C. The red lipid droplets were observed under a microscope and photographed.

**Small RNA sequencing**

Small-RNA sequencing and subsequent bioinformatics analyses were performed by Shanghai Oebiotech Co., LTD (China). Differential expression analysis was based on the DESeq2 package of R software[19]. DE-miRNAs (P < 0.05, log₂ FC > 1) were selected for subsequent analyses. The prediction of target genes of DE-miRNAs was performed in the miRNADA database[20] with threshold parameters of S ≥ 150 and ΔG ≤ −30 kcal/mol. GO function enrichment analysis and KEGG pathway enrichment analysis of targets were performed using ClusterProfiler package[21]. P value ≤ 0.05 were considered statistically significant.
RESULTS

Identification of exosomes from HepG2.2.15, HepG2 and WRL68 cells
Exosomes were isolated from HepG2.2.15, HepG2, and WRL68 cells and their presence was confirmed. Initially, the exosome marker protein CD9 was identified by western blot analysis, which demonstrated its expression in exosomes from all three cell types (Figure 1A). TEM and particle size analyses were performed for further verification. The exosomes displayed characteristic double-layered membranes and predominantly cup-shaped morphologies, with diameters ranging between 50 and 140 nm and an average size of approximately 82.47 nm (Figure 1B and C), thereby confirming that the particles were exosomes.

HepG2.2.15-derived exosomes regulate the activation of LX2
Resting HSCs are rich in lipid droplets. However, during HSC activation, these droplets either decrease or disappear. Exosomes derived from HepG2.2.15, HepG2, and WRL68 cells (termed HepG2.2.15-exo, HepG2-exo, and WRL68-exo, respectively) were co-cultured with LX2 cells. After 24 h of co-culture, compared to the control and other exosome-treated LX2 cells (Figure 2A-C), oil red staining indicated the lack of lipid droplets in LX2 cells treated with HepG2.2.15 exosomes (Figure 2D). This suggests that exosomes from HBV-infected hepatocytes potentiate HSC activation.

HepG2.2.15-derived exosomes promote LX2 proliferation and fibrosis
Exosomes from HepG2.2.15 and HepG2 cells were tagged with DiI and co-cultured with LX2 cells for 24 h. The fibrogenic marker α-SMA was detected in the LX2 cytoplasm, as indicated by green fluorescence, whereas the nuclei were stained blue with DAPI. Morphologically, LX2 cells exhibited a spindle shape and prominent pseudopods upon up-taking HepG2.2.15-derived exosomes (yellow), as evidenced by a higher expression of α-SMA. This was in contrast to the lower expression of α-SMA in LX2 cells treated with HepG2-derived exosomes, which retained the majority of exosomes within the cytoplasm (Figure 3A and B). These findings illustrate that LX2 cells internalize exosomes from both cell lines in vitro, but it is primarily the HepG2.2.15-derived exosomes that augment α-SMA expression in HSCs.

In assessing the effect of HepG2.2.15-derived exosomes on the fibrotic processes of LX2 cells, the expression of fibrosis-associated proteins such as α-SMA, COL1A1, MMP2, TIMP-1, and CTGF was found to be elevated in the HepG2.2.15-exo group compared to the HepG2-exo group (Figure 3C and D), suggesting that exosomes from HepG2.2.15 hasten LX2 fibrosis.[22]

The proliferation of LX2 cells was examined after co-incubation with HepG2.2.15-exo and HepG2-exo for 24 h. EDU staining was conducted to discriminate between proliferating and non-proliferating cells; the nuclei of proliferating cells were stained red, while all nuclei were counterstained blue. The proliferation rate of LX2 cells in the HepG2.2.15-exo group was substantially higher than that in the HepG2-exo group (Figure 3E and F), indicating that HepG2.2.15-derived exosomes facilitated both fibrosis and proliferation in LX2 cells.

DE-miRNAs in HepG2.2.15-derived exosomes
The miRNA expression profiles of exosomes derived from HepG2.2.15 and HepG2 cells were studied using small RNA sequencing. The subsequent volcano plot revealed 27 DE-miRNAs between the two exosome populations, with 14
Figure 2 The impact of HepG2.2.15-derived exosomes on the activation of LX2 cells. A: Display of LX2 cells in a normal culture environment, showing red lipid droplets; B: Illustration of LX2 cells co-cultured with HepG2-exo. Similar to the normal culture, red lipid droplets are present; C: Depiction of LX2 cells co-cultured with WRL68-exo. Also maintains presence of red lipid droplets; D: Representation of LX2 cells when co-cultured with HepG2.2.15-exo. Notably, after 24 h of oil red staining, the red lipid droplets disappear, indicating a significant change caused by the HepG2.2.15-exo. Magnification Times: 20 ×.

Figure 3 The role of HepG2.2.15-derived exosomes in LX2 proliferation and fibrosis. A: HepG2.2.15-derived exosomes (colored in yellow), highly expressing α-smooth muscle actin (SMA) (shown in green), are illustrated as being internalized by LX2 cells. The cells showcase a spindled shape and notable pseudopodia with some exosomes penetrating the cell nucleus (depicted in blue). The scale bar signifies 100 μm for reference; B: The HepG2-derived exosomes
miRNAs upregulated and 13 downregulated in exosomes derived from HepG2.2.15 (Figure 4A). Detailed information regarding the DE-miRNAs is provided in Table 1. The heatmap in Figure 4B illustrates the variation in DE-miRNAs between the HepG2.2.15-and HepG2 exosome-treated groups.

Function annotation of target genes of DE-miRNAs
Predictive analysis using the miRanda database allowed the identification of potential target genes modulated by the DE-miRNAs (Table 2). Further enrichment analyses using GO and KEGG elucidated the biological functions of these targets (Figure 4C).

GO analysis identified the biological processes most enriched in targets, which were notably linked to cellular differentiation, intracellular signal transduction, and negative regulation of apoptosis. The cellular components that were enriched included exosomes, plasma membrane components, and cell junctions. The predominant molecular functions were RNA binding, DNA-binding transcription factor activity, and homologous protein binding. The detailed GO enrichment findings are presented in Supplementary Tables 1-3. KEGG analysis highlighted the top 20 enriched pathways, with the most significant being the tuberculosis, ubiquitin-mediated proteolysis, MAPK signaling, and toll-like receptor signaling pathways (Figure 4D).

DISCUSSION
Liver fibrosis, a common outcome of chronic liver disease, is associated with significant morbidity and mortality; however, there remains a dearth of effective treatments for this condition[23]. While traditionally considered irreversible, recent research has suggested that liver fibrosis can be reversed, as evidenced by certain experimental models and human cirrhosis studies[24]. Currently, the principal approaches to managing liver disease include the interruption of harmful stimuli and, ultimately, liver transplantation[25]. HBV core proteins has been shown to induce the production of cytokines and trigger an immune response. In our investigation, we discovered that exosomes derived from the HepG2.2.15 cell line activated LX2 cells, thereby exacerbating their fibrogenic potential[26].

HSCs exist in two states, quiescent HSC (qHSCs) and activated HSC (aHSCs). qHSCs are responsible for vitamin A storage in the liver. aHSCs are the primary source of myofibroblasts, the primary source of ECM in the damaged liver. Notably, HSC is activated after liver injury, and recent single-cell RNA sequencing studies have confirmed that activation of stellate cells involves different phenotypic changes[27]. Our results demonstrated that the expression of the fibrosis marker proteins α-SMA, COL1A1, MMP2, TIMP-1, and CTGF in the HepG2.2.15 group was higher than that in the HepG2 group, HepG2.2.15-derived exosomes accelerated LX2 HSC fibrosis and promoted LX2 proliferation in vitro.

Exosomes mediate intercellular communication and can package and transport lncRNAs, mRNA, proteins, and other bioactive substances. Exosome also plays a role in the occurrence and development of many diseases such as tumor metastasis and neurodegenerative diseases[28]. Exosomes containing miRNAs in the liver regulate HSC activation to control the pathogenesis of liver fibrosis. For example, the research has shown that miR-214 is transferred from HSC donor cells to HSC recipient cells via exosomes, which regulates the development of CCN2-dependent fibrosis[29]. Seo et al[30] found that TLR3 may be a new target for fibrosis, and the possible mechanism is that γδ T cells promote interleukin-17A production in liver injury, further activating exosome-mediated TLR3. Our study showed that HepG2.2.15 cells could secrete exosomes and LX2 in HepG2.2.15-derived exosomes in vitro and further promote the fibrosis of LX2.

miRNAs are the most abundant small RNA that can be detected in almost all animal models and have potential therapeutic effects and prognosis in many diseases. miRNAs recruit Argonaute protein complexes to complementary target mRNA, resulting in translation inhibition or mRNA degradation[31]. Many miRNAs are considered fibrous and are involved in cardiac fibrosis, such as miR-29, which targets a series of mRNA that encode fibrosis-related proteins[32]. The treatment of liver fibrosis is mainly based on miRNAs (miR-29b and miR-150, etc.), small drug molecules (HH inhibitors and TGF-β inhibitors, etc.), antibody therapy (TIMP-1 and Sirtuzumab, etc.), antigen therapy (ODNs and TFO, etc.)[33]. Common miRNA analysis methods include microarrays, reverse transcription quantitative polymerase chain reaction, and RNA sequencing (RNA-seq)[34]. Small RNA sequencing (sRNA-seq) can detect more than one billion RNAs in a single run and unearth new mRNA genes because of its untargeted character. sRNA-seq analysis of HepG2-derived exosomes and HepG2.2.15-derived exosomes was performed to further investigate miRNA expression and function in exosomes. We found 27 DE-miRNAs (14 upregulated miRNAs and 13 downregulated miRNAs) and 27 miRNAs were used to predict their targets, followed by target gene GO enrichment analysis and KEGG enrichment analysis.

We revealed that these targets are mainly involved in the regulation of functions such as cell differentiation, intracellular signaling, negative regulation of apoptotic processes, cellular exosome and RNA binding, mediation of protein hydrolysis, MAPK signaling pathways, viral oncogenesis, and toll-like receptor signaling pathways.
Figure 4 Differential miRNA expression in HepG2.2.15-derived exosomes. A: The volcano plot demonstrates the differentially expressed miRNAs between HepG2.2.15-derived exosomes and HepG2-derived exosomes. Using a threshold of a P value less than 0.05 and absolute value of log2FC exceeding one, the up-regulated miRNAs are illustrated in red, while down-regulated miRNAs are in green; B: A heat map represents alterations in the expression of the differential miRNAs between HepG2.2.15-derived and HepG2-derived exosomes; with the colour red symbolising up-regulated miRNAs and blue indicating down-regulated miRNAs; C: The Gene Ontology enrichment analysis provides an insightful look into the respective target genes of the differentially expressed miRNAs; D: Enriched Kyoto Encyclopedia of Genes and Genomes pathway statistics are shown in scatter plots, highlighting the top 20 enriched pathways for an easy comparison and further analysis. GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes; HBV: Hepatitis B virus; FC: Fold change.
Table 1 Differentially expressed miRNA

<table>
<thead>
<tr>
<th>miRNA_ID</th>
<th>log₂FC</th>
<th>Q value</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsa-let-7a-5p</td>
<td>-2.73154</td>
<td>0.00204</td>
<td>TGAGTGAGTGTTGATAGTT</td>
</tr>
<tr>
<td>hsa-let-7b-5p</td>
<td>-4.69951</td>
<td>2.33E-07</td>
<td>TGAGTGAGTGTTGATAGTT</td>
</tr>
<tr>
<td>hsa-let-7i-5p</td>
<td>-2.38322</td>
<td>0.00143</td>
<td>TGAGTGAGTGTTGATAGTT</td>
</tr>
<tr>
<td>hsa-miR-1-3p</td>
<td>4.035866</td>
<td>0.047597</td>
<td>TGGAATGTAAAGAAGTATGAT</td>
</tr>
<tr>
<td>hsa-miR-100-5p</td>
<td>-3.94205</td>
<td>1.30E-07</td>
<td>AACCCGTAGATCGAACCAGT</td>
</tr>
<tr>
<td>hsa-miR-10a-5p</td>
<td>-3.59889</td>
<td>2.26E-05</td>
<td>TACCCCTGATAGCAGAAATTG</td>
</tr>
<tr>
<td>hsa-miR-10b-5p</td>
<td>2.666194</td>
<td>0.000232</td>
<td>TACCCGTAGAAGCAGGATTG</td>
</tr>
<tr>
<td>hsa-miR-1269a</td>
<td>6.487066</td>
<td>0.002002</td>
<td>CTGAGACTGACGCCGTGCTACTGG</td>
</tr>
<tr>
<td>hsa-miR-146a-5p</td>
<td>5.734318</td>
<td>6.49E-10</td>
<td>TGGAACCTAATCCATAGGCT</td>
</tr>
<tr>
<td>hsa-miR-146b-5p</td>
<td>8.31E-05</td>
<td>9.21E-05</td>
<td>TACCATGAGTACCTGACTG</td>
</tr>
<tr>
<td>hsa-miR-148a-3p</td>
<td>-2.35416</td>
<td>0.011076</td>
<td>CCGCGTTGGAGGGCGAGATGA</td>
</tr>
<tr>
<td>hsa-miR-148a-5p</td>
<td>-7.69272</td>
<td>3.18E-07</td>
<td>TGTAAACATCCTCGACTGAGA</td>
</tr>
<tr>
<td>hsa-miR-148b-5p</td>
<td>-5.73663</td>
<td>0.011076</td>
<td>CCGCGTTGGAGGGCGAGATGA</td>
</tr>
<tr>
<td>hsa-miR-152-5p</td>
<td>2.986783</td>
<td>0.03238</td>
<td>TGGAGGAAAGCAGGTTTCCTGA</td>
</tr>
<tr>
<td>hsa-miR-185-5p</td>
<td>2.596432</td>
<td>0.041346</td>
<td>CTGAGCTTATGACAGGACG</td>
</tr>
<tr>
<td>hsa-miR-185-5p</td>
<td>3.25416</td>
<td>0.01536</td>
<td>ATACATGAGACCGAGGTTTCCT</td>
</tr>
<tr>
<td>hsa-miR-223-3p</td>
<td>-7.69272</td>
<td>3.18E-07</td>
<td>TGTAAACATCCTCGACTGAGA</td>
</tr>
<tr>
<td>hsa-miR-23a-3p</td>
<td>-3.25416</td>
<td>0.01536</td>
<td>ATACATGAGACCGAGGTTTCCT</td>
</tr>
<tr>
<td>hsa-miR-30a-3p</td>
<td>-5.80039</td>
<td>0.028767</td>
<td>CTGAGCAGAAGCAGGTTTCCTGA</td>
</tr>
<tr>
<td>hsa-miR-3158-3p</td>
<td>2.179298</td>
<td>0.003177</td>
<td>AAGGCGTTCCCTCTGCGAGAC</td>
</tr>
<tr>
<td>hsa-miR-372-3p</td>
<td>7.10648</td>
<td>4.32E-06</td>
<td>AAAGTGCTGCGACTTTGAGCC</td>
</tr>
<tr>
<td>hsa-miR-378a-3p</td>
<td>2.695553</td>
<td>0.000885</td>
<td>ACTGGACTTGGAGTCAGAAGGC</td>
</tr>
<tr>
<td>hsa-miR-483-3p</td>
<td>-4.29295</td>
<td>1.30E-07</td>
<td>CTCTTGATCCTTGTGAGCCAC</td>
</tr>
<tr>
<td>hsa-miR-483-5p</td>
<td>-6.152472</td>
<td>0.000659</td>
<td>AAAGTCTGAGACACCTCGACCT</td>
</tr>
<tr>
<td>hsa-miR-584-5p</td>
<td>-5.99766</td>
<td>0.03238</td>
<td>TTATGGTTTGCCTGGGACTGAG</td>
</tr>
<tr>
<td>hsa-miR-584-5p</td>
<td>-4.26701</td>
<td>0.00015</td>
<td>TTATGGTTTGCCTGGGACTGAG</td>
</tr>
</tbody>
</table>

Table 2 Differential miRNA targets genes

<table>
<thead>
<tr>
<th>miRNA_ID</th>
<th>Targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>hsa-miR-1269a</td>
<td>ADORA1, CPLX2, MECR, ADAR, EXTL2, NCKAP5L, ZDCD, HSF4, ENGA, CEMPI, TMEM231, CREBBP, PLIN4, WIPF3, TRMT9B, FOXRED2, PTGER3, LTP3, KCNK16, INAV, RBM24, OTOA, CEF5, ATP8B3, IL6ST, CPD1, TGF2, POC1B-GALNT4, RIMS4, ROHFTB1, TRRAP, PASK, CDC20, STON2, TP53I11, CHRD12, BTD, H6PD, ANLN, CI2orf43, MYCN, BRD1, CRYCN, Kncf3, ZNF618, NFAM1, MAST2, KY, DLA7, TPB, GALNT4, WASH, CDC25B, VPS9D1, ZFHX3, TMEM15, PATZ1, CTHB1, PCLO, SOX8, ATP11A, CLUH, ELAC2, MIRBP, SMG9, ROR3, VWA1, WDR92, ZNF696, CIQTNF6, ALPK2, GIPC3, SDK1, N4BP1, SLC39A3, KLIF15, LYL1, LH3X3, METTL25, LOC101927262, MLPH, DNA1H, GSTO2, ACSM1, GPBAR1, PDK2, NR2F3, CHST8, EMD1, LOC112268219, ZNF331</td>
</tr>
<tr>
<td>hsa-miR-584-5p</td>
<td>DHDDS5, IL11i</td>
</tr>
<tr>
<td>hsa-miR-30c-2-3p</td>
<td>AK1, JGF8P3, PARP3, CT45A5, CT16D, FAM227A, CT45A1, CT45A3, CT45A6, GAB4, ELMSA1, FAM160A2, ZNF30, EPHA10, ECE1, ZNF84, RA1H, CCDC66, CHAMP1, C6orf132, FREM3, THRA, TFR2, SYT7, CELP1, NOAL4, CPR161, KIAA0212, ATG4D, KCNK9, SIAH1L1, ADAP1, CT45A8, CT45A9, CT45A7, LOC7999, LOC388436, MAPK4, CCR1, PDHB, ARM7C, YXYT1, SLCC2A14, ZSCAN22, KCN51, LOC64302, SETDB1, ELAVLY13, CPS4, CHST3, CRK, KCNA10, MPP6P610, SPIG, SF3B3, ATG4B, KCNV1, KFLH20, SUSL8D, ZDHHC17, DNA1H, PDHB5, YTHDF1, AJAP1, PDCDH10, PDCDH11, PDCDH12, PDCDH13, PDCDH14, PDCDH12, PDCDH13, PDCDH15, PDCDH16, PDCDH17, PDCDH19, PDCDH18, WREN1P1, PDCDH16, NSD1, NOL2, TREML2, LRR3C, HUWE1, PRF38A, TPS3SRK, SAMD10, CT45A2, MTIE, MTIM, KNCN3, P4HA2, ZNF467, SLC28A3, LOC101274624, IL17RE1, DOCK2, ZDHHC11, CI2orf88, LOC101927462, SUOX</td>
</tr>
</tbody>
</table>

*WJG* | https://www.wjgnet.com | 2560 | May 21, 2024 | Volume 30 | Issue 19
We investigated the effects of HBV-infected hepatocytes on HSC activation, proliferation, and fibrosis. However, this study has several limitations. For example, there is no elucidation of the pathway through which HepG2.2.15-derived exosome-transported DE-miRNAs mediate phenotypic changes in HSCs. In addition, experiments in vivo are lacking to validate the effects of HepG2.2.15-derived exosomes and the DE-miRNAs carried by them on the phenotype of HSCs and liver fibrosis are lacking.

CONCLUSION

This study highlights the pronounced effect of HBV-infected hepatocytes on HSC activation, proliferation, and fibrosis. This also highlights the magnifying role of HepG2.2.15-derived exosomes in fibrogenic potential. Despite the significance of these findings, the study falls short of including in vivo experiments and leaves the pathway through which exosomes mediate HSCs' phenotypic changes of HSCs unresolved. Further research is required to elucidate these aspects fully.

FOOTNOTES

Author contributions: Gao Y was responsible for the experimental design, execution of experiments, writing, cell culture, and staining; Li L contributed to the experimental design and reviewed the data; Zhang SN participated in writing and reviewing the manuscript; Mang YY provided reagents/materials/analysis tools, conducted nucleic acid extraction, and performed PCR; Zhang XB conducted experiments; Feng SM was involved in conducting experiments, as well as in data collection, analysis, Western blotting (WB), and exosome extraction. All authors critically reviewed and revised the text and approved the final version.

Supported by The Spring City Plan: The High-level Talent Promotion and Training Project of Kunming, No. 2022SCP002; and The Research of Key Techniques and Application of Liver-Kidney Organ Transplantation, No. 202302AA310018.

Institutional review board statement: The study was reviewed and approved by the Institutional Review Board at Affiliated Calmette Hospital of Kunming Medical University & The First Hospital of Kunming.

Institutional animal care and use committee statement: This study does not involve animal research.

Conflict-of-interest statement: All authors declare that they have no conflict of interest.

Data sharing statement: Data is applicable after the approval of the authors.

Open-Access: This article is an open-access article that was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution NonCommercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: https://creativecommons.org/Licenses/by-nc/4.0/

Country/Territory of origin: China

ORCID number: Li Li 0009-0005-4372-4011.

S-Editor: Fan JR
REFERENCES


Gao Y et al. HepG2-Exos promote HSC activation and fibrosis


