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Differences Between Sorafenib and Lenvatinib Treatment from Genetic and Clinical Perspectives for Patients with Hepatocellular Carcinoma

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Data Collection B
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Background: The aim of this work was to systematically compare the differences between sorafenib and lenvatinib for patients with hepatocellular carcinoma (HCC) from genetic and clinical perspectives.

Material/Methods: The mRNA and miRNA sequencing information of patients with HCC treated with either sorafenib or lenvatinib was analyzed using differential expression and a protein–protein interaction assay. The clinical manifestations and adverse events of the 2 drugs were also investigated.

Results: Compared with patients with HCC treated with sorafenib, patients treated with lenvatinib developed 8 differentially expressed genes (*DEGs*, *FGF4*, *FGF23*, *UNC13C*, *RIMBP2*, *STXBP5L*, *PHOX2B*, *NEUROD4*, and *POU4F2*) and 3 miRNAs (DEMs, *has-miR-548ah*, *has-miR-888*, and *has-miR-196a-1*), of which *hsa-miR-548* regulated 4 target genes, the largest number among the 3 miRNAs. The functions of these DEMs and DEGs were verified by external experiments in the HCC cell line Hep3B2.1-7. We further investigated the adverse events of the drugs for patients with advanced HCC in clinical treatment. The patients in the sorafenib group developed less frequent symptoms of hypertension and diarrhea. Also, the frequency of hand-foot skin reactions in patients treated with lenvatinib was lower than that of patients treated with sorafenib ($P<0.05$). There were no significant differences in nausea, fatigue, frequent urination, and dizziness ($P>0.05$).

Conclusions: In a time of increasing interest in chemotherapy drug treatments for patients with HCC, this study provided a better understanding of the clinical evaluations of sorafenib and lenvatinib.

Keywords: Carcinoma, Hepatocellular • Lenvatinib • Sorafenib

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Background

Hepatocellular carcinoma (HCC) is the most frequent primary malignancy of the liver, comprising 75% to 85% of cases of liver cancer [1], creating a critical medical problem worldwide. There were 782 000 cases diagnosed and 746 000 deaths in 2012, giving HCC the rank as the sixth most common neoplasm and the second leading cause of cancer-related deaths [2]. The worldwide incidence of HCC is heterogeneous owing to the prevalence of risk factors. The formation of HCC is closely associated with the presence of chronic liver disease [3]. The hepatitis B virus (HBV) and hepatitis C virus (HCV) are the primary causes, which subsequently lead to chronic liver disease [4]. The pathogenesis of virus-induced HCC has been suggested to be associated with various mechanisms, including the integration of HBV-DNA into the host's genetic machinery, selective immunosuppression for virus presentation, down regulation of viral protection gene expression, virus-specific T-cell suppression for recognizing HBV antigens, and DNA methylation [5]. In addition to virus-induced HCC, growing evidence from retrospective studies supports the connection between HCC and other non-viral risk factors, such as diabetes, alcoholism, and dyslipidemia, especially in developed regions [6]. For most patients, the disorder is diagnosed at an advanced stage, when surgical treatment is no longer an option. Clinically, patients with advanced HCC need chemotherapy to improve treatment. Based on decades of efforts, researchers have provided several potential systemic therapies targeting advanced HCC, namely sorafenib, lenvatinib, regorafenib, cabozantinib, atezolizumab plus bevacizumab, and ramucirumab in phase III trials [7].

Until 2008, there was still no effective therapy for patients diagnosed with advanced-stage HCC or patients who transitioned into it as other therapies failed. Sorafenib, developed by the Bayer and Onyx companies, was initially approved by the FDA for advanced HCC treatment in 2006 [8]. A year later, it proved to be a unique target drug for HCC [9,10]. Sorafenib is a small polytyrosine kinase inhibitor, which dominantly suppresses Raf kinase, vascular endothelial growth factor, and platelet-derived growth factor function [11]. Sorafenib was the first systemic therapy approved in HCC as the result of 2 positive randomized placebo-controlled trials, with 1 multicenter trial done predominantly in Europe and the United States, and the other trial done in the Asia-Pacific area. Lenvatinib is a receptor tyrosine kinase oral small-molecule inhibitor, which was recently approved for first-line treatment in patients with unresectable advanced HCC in the United States, the European Union, Japan, and China [12]. Lenvatinib was clinically initiated as a substitution for sorafenib. However, the molecular mechanism of lenvatinib is poorly understood. Meanwhile, it is still controversial whether lenvatinib could replace sorafenib since both of them cause diverse adverse events, including hand-foot skin reactions, arterial hypertension, fatigue, and diarrhea [13].

Currently, immune-based combinations of drugs (mainly targeting the immune checkpoint PD L-1) seem to have shifted the direction of future first-line therapies [14]. For instance, the combination of lenvatinib and pembrolizumab is now being evaluated as a front-line treatment in patients with advanced HCC, and the early phase clinical trials have already reported promising results [15,16].

Although new types of drugs have been discovered gradually, sorafenib and lenvatinib are still the mainstream therapies for patients with advanced HCC. However, the overall effects of sorafenib and lenvatinib are far from satisfactory, and the clinical therapy selection is still an issue owing to the unknown molecular mechanisms. To address these issues, in this study, a differential expression analysis was performed on patients with HCC treated with either sorafenib or lenvatinib. The profile of target mRNAs and associated miRNAs was also developed. The clinical symptoms and adverse reactions in patients with HCC for the 2 different drug administrations were further explored. This study provides potential guidance for the precise administration of sorafenib and lenvatinib in HCC treatment.

Material and Methods

Participants

This study was a follow-up analysis of our previous study, which was given ethics approval, and all patients gave informed consent to participate [17]. All analyses of human data were carried out in strict compliance with relevant ethics regulations. Overall, 120 patients with advanced HCC who were admitted to our hospital from September 2019 to December 2020 were randomly selected to participant in the study. The medical records of all the recruited individuals were retrospectively reviewed. All individuals in this study were randomly selected based on the following inclusion criteria: (1) age from 40 to 80 years; (2) for laboratory measurement, the expression level of alpha-fetoprotein was greater than 400 μg/L over 1 month or 200 to 400 μg/L lasting for over 2 months; and (3) for imaging examination, the liver mass displayed more than 2 cm in diameter and typical HCC characteristics based on either computed tomography (CT) or magnetic resonance imaging (MRI) analysis. If the liver mass was 1 to 2 cm in diameter, CT and MRI were required. Participants were excluded if they had chronic liver disease or the presence of a malignancy other than HCC. The recruited individuals were untreated with chemotherapy for the previous 3 months.

The treatment rationale was as follows: 70 patients were treated with sorafenib, 40 patients were treated with lenvatinib, and 10 patients had no chemotherapy drug treatment, starting at the same time point. There was no significant difference

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in basic clinical data between these groups. For sorafenib administration, hospitalized patients with advanced HCC were treated with sorafenib tosylate tablets (Bayer, Germany) at a dosage of 0.4 g twice daily on an empty stomach or with a low-fat or medium-fat diet for 6 months. At the same time, for lenvatinib administration, hospitalized patients with advanced HCC were treated with lenvatinib mesylate capsules (Merck & Co, Inc, USA) at a dosage as 12 mg once daily on an empty stomach or with food for 6 months.

Differentially Expressed mRNA and miRNA Analysis

According to a previous report [18], the cancerous tissues of all patients with HCC were dissected and snap-frozen during surgery after the chemotherapy treatment and stored in liquid nitrogen immediately. The total RNA was extracted using an RNAiso Plus kit (Takara, Beijing, China). The total RNA samples were collected and the mRNA and miRNA expression were analyzed by Geneseed Co (Guangdong, China). The expression analysis was conducted using the Affymetrix GeneChip Pico kit and hybridized to Affymetrix Clariom S arrays as described by the manufacturer (Affymetrix, USA).

The robust multi-array average method was first utilized to normalize the original data measured by the chip. After that, the normalized value was calculated using the log2 logarithm to provide the data after normalization, following differential expression analysis. The edgeR package in R language was developed to analyze differentially expressed genes (DEGs) and differentially expressed miRNAs (DEMs) between different treatments. The absolute values of logarithmic transformed differential expression multiples (Log2FC) (used for DEGs and DEMs screening) were the indications of absolute values for different selected targets. The values of Log2FC >1 and $P<0.05$ were used as criteria for screening [19]. The P value in the results represented the adjusted P values (false discovery rate) with considering multiple comparison criterion, which was between all the genes and miRNA together in expression analysis.

Protein–Protein Interaction Networks and Analysis of miRNA Target Genes

The STRING (https://string-db.org/, version 11.0) was generated to analyze the functional connections and interactions of candidate proteins [20]. Cytoscape (https://cytoscape.org/, version 3.7.2) analysis was performed to visualize the protein–protein interaction (PPI) network [21].

The target genes of miRNAs were predicted through the miRDB (http://mirdb.org/index.html, version 6.0) database [22]. Meanwhile, Cytoscape was used to visualize the miRNA-mRNA regulatory network.

Cell Culture

The human HCC cell line Hep3B2.1-7 was purchased from American Type Culture Collection Co (China). The cells were cultured at 37 °C in 5% CO_2 in Eagle's minimum essential medium supplemented with 10% fetal bovine serum, 100 units/mL penicillin, and 100 µg/mL streptomycin, based on the manufacturer's instruction. As previously reported [23], the cells were treated with either sorafenib or lenvatinib at 3 µM for 4 days.

For functional experiments, a specific has-miR-548ah mimic/inhibitor (Ambion) (50 nmol/L) and a corresponding negative control (Ambion) were transfected into the Hep3B2.1-7 cells. The transfection was achieved using Lipofectamine 2000 (Sigma-Aldrich, Beijing, China).

Quantitative Real-Time Polymerase Chain Reaction

The total RNA from the cancerous tissues of patients with HCC were extracted using an RNAiso Plus kit (Takara, Beijing, China). The TRIzol reagent was used to extract total RNA from the HCC cell line Hep3B2.1-7. The diverse expression activities of potential target genes were compared using the quantitative real-time polymerase chain reaction (qPCR) method. The high-capacity RNA to cDNA kit (Applied Biosytems) was used to reversely transcribe RNA (1000 ng) to cDNA, and qPCR amplification was performed with SYBR Green (Qiagen). The resulting value was normalized according to the internal reference gene GAPDH of each parallel sample. For miRNA expression analysis, the TaqMan advanced MicroRNA assay kit (Applied Biosystems) and miRNA-specific primers reverse transcription RNA (100 ng) were used in this study. PCR conditions were set up according to the instructions: 1 cycle at 95°C 5 min; 40 cycles at 95°C for 15 s; followed by 60°C for 40 s. The relative expression was measured based on the 2-ΔΔCt method. All experiments were performed in triplicate. The DEGs primers were designed based on the PrimerBank site (https://pga.mgh.harvard.edu/primerbank/) as follows:
FGF4 Forward (5'-3'): CTCGCCCTTCTTCACCGATG;
Reverse (5'-3'): GTAGGACTCGTAGGCGTTGTA;
FGF23 Forward (5'-3'): CAGAGCCTATCCCAATGCCTC;
Reverse (5'-3'): GGCACTGTAGATGGTCTGATGG;
UNC13C Forward (5'-3'): GAGTATCGTCAGCAGAAAAAGGA;
Reverse (5'-3'): CTCAGTGGATAAGTTGTGAGTGG;
RIMBP2 Forward (5'-3'): CAGAAGTCCAAGGTTCGAGAGC;
Reverse (5'-3'): GAGGTGGCTAGGCCATTCAT;
STXBP5L Forward (5'-3'): GCTGGAAGTGGTTCCGTACAT;
Reverse (5'-3'): GAACTGGATCAAAGGCTAATGCT;
PHOX2B Forward (5'-3'): AACCCGATAAGGACCACTTTTG;
Reverse (5'-3'): AGAGTTTGTAAGGAACTGCGG;
NEUROD4 Forward (5'-3'): GAGAGCTAGTCAACACACCATC;
Reverse (5'-3'): GCATCCCATAAGTACCTGGTCTG;
POU4F2 Forward (5'-3'): CAAGCAGCGACGCATCAAG;

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Reverse (5'-3'): GGGTTTGAGCGCGATCATATT;
has-miR-548ah Forward (5'-3'): ATTGGAACGATACAGAGAAGATT;
Reverse (5'-3'): GGAACGCTTCACGAATTTG;
has-miR-888 Forward (5'-3'): GAAGTTCCATCGAAAAGTGATTG;
Reverse (5'-3'): TATGCTTGTTCTCGTCTCTGTGTC;
has-miR-196a-1 Forward (5'-3'): GCCCTGCTTGCCTTTGCCCTG;
Reverse (5'-3'): TGCAGGGTCGTAGGT.
The final PCR conditions were set up as follows: 1 cycle at 95°C 5 min; 40 cycles at 95°C for 15 s; followed by 60°C for 40 s. The RT-PCR relative expression analysis was conducted using the 2-ΔΔCt method with GAPDH as an internal control gene. All experiments were performed 3 times independently.

Statistical Analysis

SAS 9.4 and SPSS 19.0 statistical software were used for data analysis. The continuous variables were tested for normal distribution, and the variables conforming to the normal distribution were expressed as mean±standard deviation. The *t* test was used for data comparison. The comparison of safety and adverse reactions of the 2 groups was done using the chi-squared and Fisher's accurate probability test. The $P<0.05$ was considered as a significant difference.

Results

Comparisons of DEGs and DEMs for the 2 Drugs

Following the previously reported procedure [18], RNA samples from 10 patients treated with either sorafenib (sorafenib group, 10 patients) or lenvatinib (lenvatinib group, 10 patients), and 10 control patients without any chemotherapy drug treatment (control group, 10 patients) were extracted and analyzed. At the same time, the edgeR package in R language was performed to analyze the DEGs and DEMs between the 2 different treatments. Firstly, we compared the expression levels of all mRNAs of the patients with HCC treated with sorafenib alone and patients with HCC without any chemotherapy. Simultaneously, we compared the expression levels of all mRNAs in patients treated with lenvatinib alone with those without any chemotherapy drugs. The sorafenib-treated patients displayed 466 DEGs, including 57 upregulated genes and 409 downregulated genes, compared with control group (**Figure 1A**). Meanwhile, lenvatinib-treated patients displayed 471 DEGs, including 65 upregulated genes and 406 downregulated genes (**Figure 1B**). By taking the intersection between sorafenib and lenvatinib, 8 genes were selected as the primary significant DEGs for lenvatinib: *FGF4*, *FGF23*, *UNC13C*, *RIMBP2*, *STXBP5L*, *PHOX2B*, *NEUROD4*, and *POU4F2* (**Figure 1E**). *FGF4* and *FGF23* were primary DEGs for 95% of the lenvatinib-treated patients with HCC.

The miRNA expression was analyzed using the same method. Compared with the control group, patients treated with sorafenib alone displayed 12 DEMs, including 6 upregulated miRNAs and 6 downregulated miRNAs (**Figure 1C**); while the lenvatinib-treated group showed 12 differentially expressed DEMs, including 7 upregulated miRNAs and 5 downregulated miRNAs (**Figure 1D**). The miRNAs *has-miR-548ah*, *has-miR-888*, and *has-miR-196a-1* were specific to lenvatinib and were all upregulated (**Figure 1F**). The miRNA *has-miR-548ah* was the primary DEM for 90% of the lenvatinib-treated patients with HCC.

Establishment of miRNA-mRNA Regulatory Network

We utilized the STRING database to construct a PPI network for the 8 genes. The MCODE plug-ins were used to identify significant clustering modules for the gene interactions (**Figure 2A**). At the same time, the 3 candidate miRNAs and 8 mRNAs were further investigated for a regulatory network and visualized with Cytoscape software. Of the miRNAs, *hsa-miR-548* regulated the largest number of target genes, at 4. Meanwhile, the number of target genes regulated by *hsa-miR-888* and *hsa-miR-196a-1* were both 2 (**Figure 2B**). Based on these facts, the 3 DEMs and 8 DEGs were suggested to be the potential hub genes for lenvatinib treatment in patients with HCC.

External In Vitro Experiments Verification of Potential DEGs and DEMs for Lenvatinib

Next, we sought to verify the functions of potential DEGs and DEMs for lenvatinib based on in vitro analysis. The HCC cell line Hep3B2.1-7 was treated with either sorafenib or lenvatinib at 3 μM for 4 days. Compared with that of the untreated control, the expression levels of DEGs (*FGF4*, *FGF23*, *UNC13C*, *RIMBP2*, *STXBP5L*, *PHOX2B*, *NEUROD4*, and *POU4F2*) were decreased based on the RT-PCR measurement (**Figure 2C**); while the activities of DEMs (*has-miR-548ah*, *has-miR-888*, and *has-miR-196a-1*) were enhanced (**Figure 2D**). At the same time, sorafenib treatment did not show significant differences for the DEGs and DEMs of lenvatinib (data not shown). These results were consistent with the results of the differential expression analysis (**Figure 1**).

Moreover, given the expression level of *FGF4* as a read-out, the mimic transfection of *has-miR-548ah* in Hep3B2.1-7 cells significantly reduced the activity of *FGF4*. Reversely, *has-miR-548ah* inhibitor transfection elevated the activity of *FGF4*. Meanwhile, the *has-miR-548ah* inhibitor attenuated the function of lenvatinib for the expression level of *FGF4* (**Figure 2E**). Collectively, these outcomes suggested that *has-miR-548ah* was a central downstream factor of lenvatinib for *FGF4* gene regulation.

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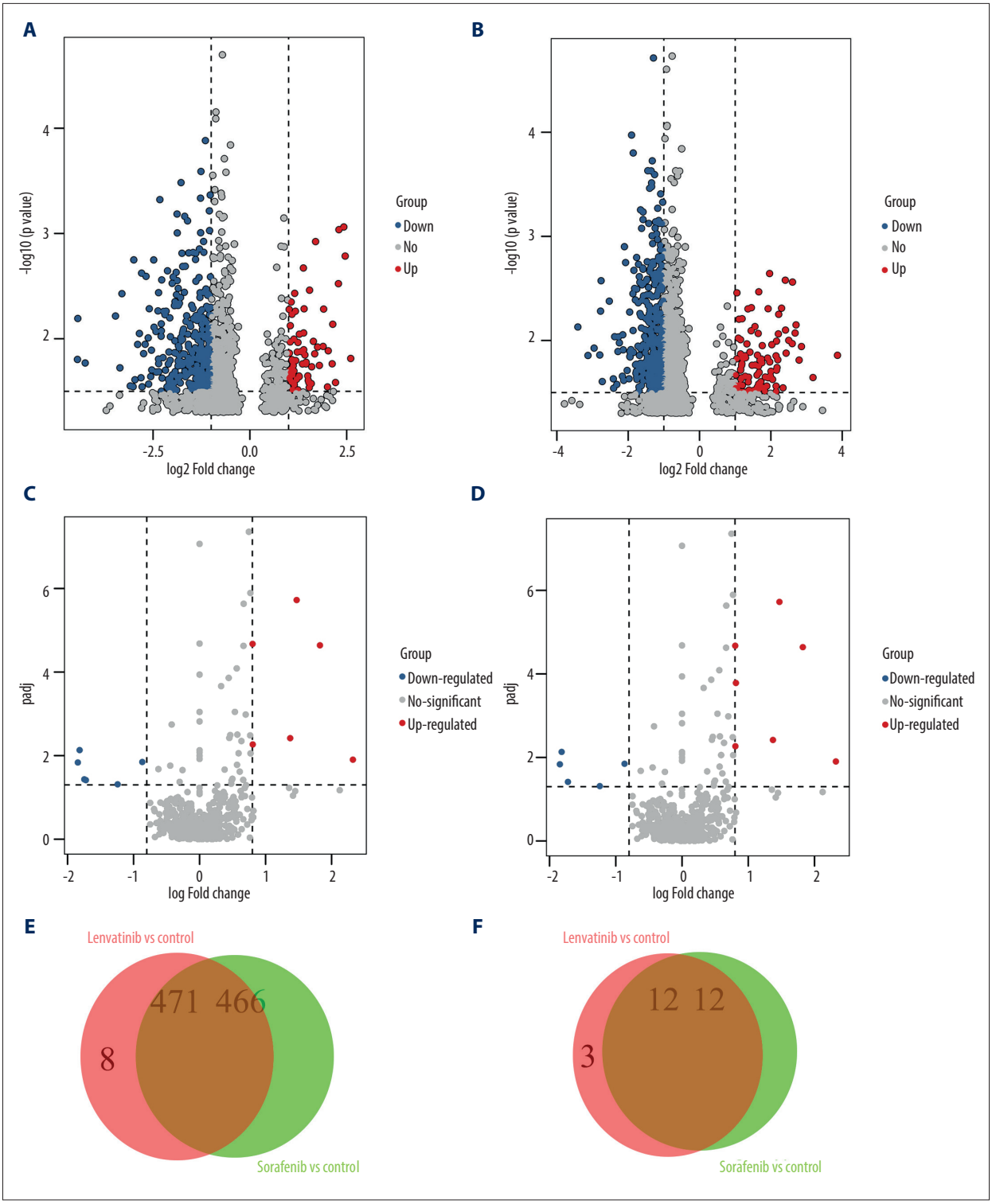

Figure 1. The differential analysis of mRNA and miRNA. (**A, B**) Volcano diagram of differentially expressed genes (DEGs) for Sorafenib treatment and Lenvatinib treatment, respectively. The horizontal axis is the log2FC value, while the vertical axis is -log10 (*P* value). Red color represents upregulation, green represents downregulation, and black represents no significant difference respectively. (**C, D**) The volcano diagram of differentially expressed miRNAs (DEMs). (**E, F**) The Venn diagrams of DEGs and DEMs for Sorafenib treatment and Lenvatinib treatment, respectively.

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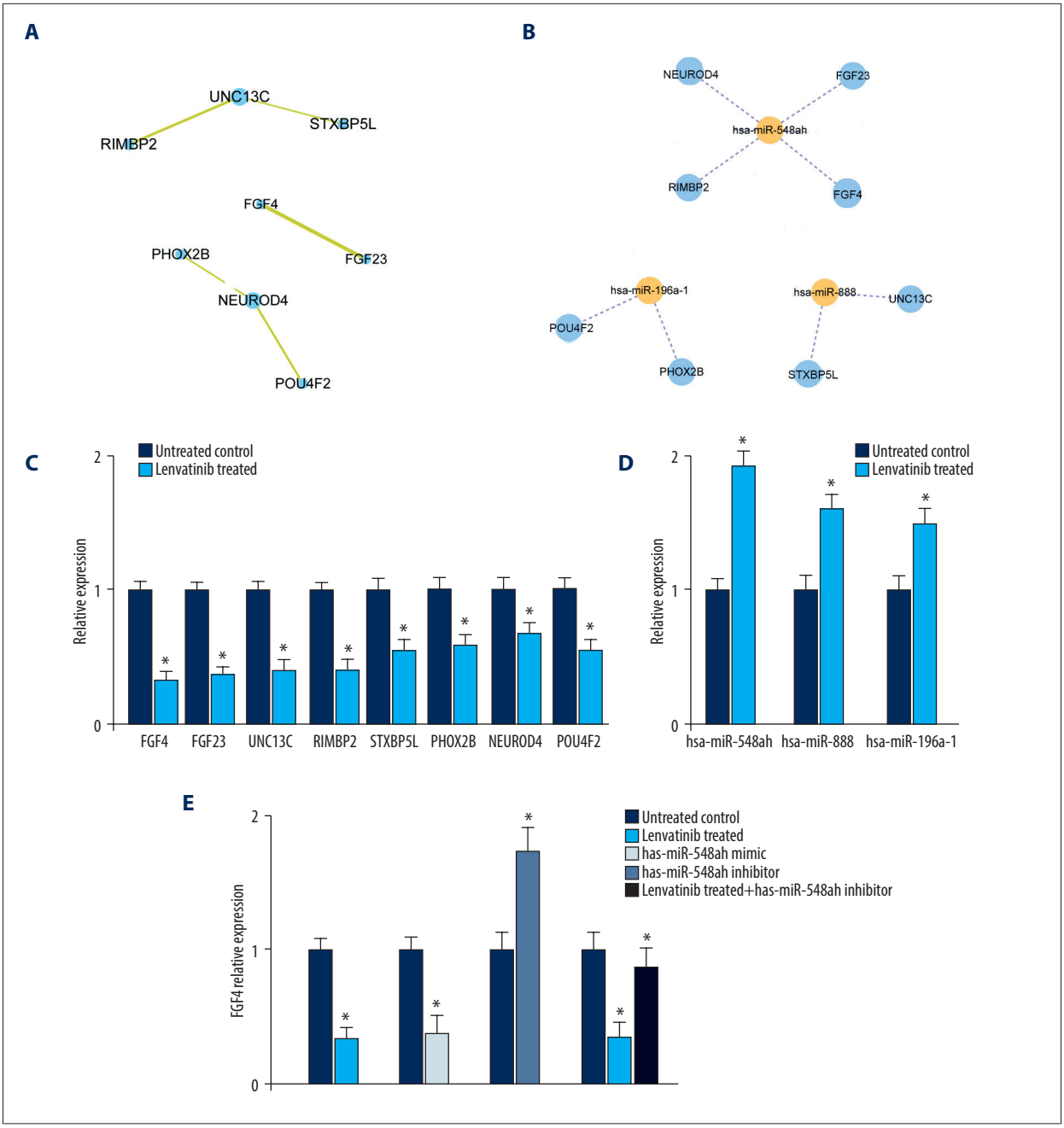

Figure 2. Establishment of protein–protein interaction (PPI) and miRNA-mRNA regulatory network. (**A**) The construction of PPI network, in which each dot represents a node. (**B**) The miRNA-mRNA regulatory network. The orange node is miRNA and the blue node is mRNA. The solid line represents positive regulation, and the dotted line represents negative regulation respectively. (**C**) The RT-PCR investigations of differentially expressed genes. (**D**) The RT-PCR investigations of differentially expressed miRNAs. (**E**) The expression levels of *FGF4* for different treatments in Hep3B2.1-7 cells.

Comparison of Clinical Tumor Markers and Hepatobiliary Function Between the 2 Drugs

In addition to the molecular mechanism network, we further compared the clinical manifestations of the 2 treatments for advanced patients with HCC. There was no obvious difference in the first month between sorafenib and lenvatinib treatment. However, there were significant differences in alpha-fetoprotein, carbohydrate antigen 19-9, alanine aminotransferase, aspartate aminotransferase, total bilirubin, and direct bilirubin among patients in the lenvatinib group ($P<0.05$) from the third month, which suggested that lenvatinib had a better

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Table 1. Comparison of tumor markers and hepatobiliary function between 2 groups.

Group	AFP		CA19-9		ALT		AST	
	S	L	S	L	S	L	S	L
Start point	>1000	>1000	129.6±31.6	127.1±32.3	47.3±7.8	51.2±7.8	52.2±9.1	54.7±7.9
1 month	>1000	>1000	118.7±30.3	101.5±24.4	31.2±5.8	26.0±5.7	47.4±8.1	41.3±6.1
3 months	582.3±91.8	395.4±67.2*	58.7±17.4	10.1±3.2*	30.0±6.6	24.1±3.6*	34.1±6.8	27.3±4.3*
6 months	60.4±16.8	17.9±5.2*	42.8±8.9	5.77±1.6*	23.4±5.4	14.1±3.1*	31.7±6.9	26.5±5.2*

Group	TBIL		DBIL		CHE	
	S	L	S	L	S	L
Start point	27.8±6.3	30.7±6.5	12.3±4.2	13.1±4.1	6017.7±593.8	6143.7±896.4
1 month	21.2±5.3	22.5±7.3	9.7±3.1	8.1±3.2	5713.8±896.3	5611.8±916.8
3 months	18.8±4.2	13.5±2.6*	7.1±2.1	4.7±1.2*	5412.2±981.7	5321.8±897.9
6 months	16.8±3.8	12.1±3.1*	6.7±2.5	4.3±1.6*	5198.8±917.4	5233.9±898.3

S indicates sorafenib treatment and L indicates lenvatinib treatment. * Represents $P<0.05$ compared with sorafenib treatment group. AFP – alpha-fetoprotein; CA19-9 – carbohydrate antigen 19-9; ALT – alanine aminotransferase; AST – aspartate aminotransferase; TBIL – total bilirubin; DBIL – direct bilirubin; CHE – cholinesterase.

Table 2. Comparison of safety and adverse reactions of the 2 groups (case%).

Group	Hypertension	Hand–foot skin reaction	Diarrhea	Nausea	Fatigue	Frequent urination	Dizziness
S	21 (30.0%)	10 (14.3%)	19 (27.1%)	2 (2.9%)	2 (0.4%)	2 (0.4%)	6 (8.6%)
L	18 (45.0%)*	4 (10.0%)*	16 (40.0%)*	1 (2.5%)	1 (0.0%)	1 (0.0%)	4 (10.0%)

S indicates sorafenib treatment and L indicates lenvatinib treatment. * Represents $P<0.05$ compared with sorafenib treated group.

therapeutic effect than sorafenib (**Table 1**). During the observation period, the cholinesterase levels of the sorafenib and lenvatinib groups remained similar ($P>0.05$).

Comparison of Clinical Adverse Reactions Between the 2 Drugs

Adverse effects of both drugs occurred frequently. To address the advantages and disadvantages of the 2 treatments in terms of adverse effects, we further investigated the adverse events associated with the treatments, including hand-foot skin reaction, arterial hypertension, diarrhea, fatigue, nausea, frequent urination, and dizziness, for patients with advanced HCC. As shown in **Table 2**, no serious adverse events occurred for the patients in the 2 groups. However, patients in the sorafenib group developed hypertension and diarrhea less frequently than the lenvatinib group. At the same time, the frequency of hand-foot skin reaction in patients treated with lenvatinib was relatively lower ($P<0.05$). There were no significant differences in nausea, fatigue, frequent urination, and dizziness in this study ($P>0.05$).

Discussion

The mortality of HCC was reported to be the third among all solid tumors, just behind carcinomas of the lung and colon [24]. Sorafenib was previously the first choice for HCC in the clinical setting. However, owing its adverse effects and drug resistance, the overall outcomes of sorafenib have been greatly restricted [25]. Lenvatinib was firstly initiated in 2008 as a multitargeted RTK inhibitor capable of inhibiting multiple kinases in a nanomole concentration (half the maximal (Baune et al., 2018) inhibiting concentration, 4-100 nM) [26]. Showing angiogenesis inhibition in animal experiments, lenvatinib was subsequently developed as an orally available TKI in solid tumors, including thyroid cancer, hepatocellular carcinoma, and renal cell carcinoma, as a single agent or in combination [27,28]. Since lenvatinib was used in the treatment of advanced HCC, its advantages over sorafenib have gradually emerged. However, it is worth noting that lenvatinib is not specific for HCC treatment and its underlying molecular mechanisms remain unclear. In this study, we developed a comprehensive specific expression

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profile for lenvatinib treatment. Different from previous reports, the results hypothesized potential DEGs and DEMs for lenvatinib treatment compared with sorafenib. In respect to the 2 different drug administrations, we have proposed several new insights.

One striking finding of the present study was that 3 DEMs, *has-miR-548ah*, *has-miR-888*, and *has-miR-196a-1*, displayed specific expression patterns in lenvatinib-treated patients with HCC, which were all upregulated. Xing et al suggested that *has-miR-548ah* is significantly upregulated in peripheral blood mononuclear cells, which are involved in immune tolerance and immune activation stages of chronic hepatitis B in liver disease by targeting IFN-γR1 [29]. The miRNA *has-miR-888* is a multifunctional DEM, which has been shown to participate in the development and formation of HCC, lung cancer, and colorectal cancer [30-32]. In HCC, *has-miR-888* promoted cell migratory and invasive abilities and suppressed the expression of SMAD4 for the HCC process [28]. In addition, *has-miR-196a-1* was demonstrated to promote gastric cancer cell invasion and metastasis by targeting SFRP1 [33]. With the activation of 3 DEMs, it was reasonable to hypothesize that 8 DEGs (*FGF4*, *FGF23*, *UNC13C*, *RIMBP2*, *STXBP5L*, *PHOX2B*, *NEUROD4*, and *POU4F2*) could be repressed in the lenvatinib treatment, which was further supported by our present results (**Figure 2B**). Fibroblast growth factors (FGFs) play a non-redundant autocrine/paracrine function in multiple human cancers. FGF2, FGF4, FGF7, and FGF20 were shown to be representative of paracrine FGFs binding to heparan-sulfate proteoglycan and fibroblast growth factor receptors (FGFRs), while FGF19, FGF21, and FGF23 were indicated as endocrine FGFs binding to Klotho and FGFRs. With other key factors, such as regulatory T cells, cancer-associated fibroblasts, endothelial cells, and myeloid-derived suppressor cells, FGF proteins form a tumor microenvironment for HCC formation [34,35]. UNC13C was found to be a novel tumor suppressor and an essential regulator of the EMT signaling pathway during oral squamous cell carcinoma progression [36]. PHOX2B stands for paired-like homeobox 2B, which is a minimal residual disease marker of neuroblastoma, functioning as a suppressor of neuroblastoma progression [37]. Except for these findings, the relation between these genes and HCC have not yet been fully explored. Therefore, it would be beneficial to further investigate these genes for better evaluating lenvatinib treatment. However, since the targets for lenvatinib were *FGF4*, *FGF23*, and *has-miR-548ah* based on our findings, we hypothesized that the patients with advanced HCC with low expression levels of these targets might display a better respond to lenvatinib. Further investigation is necessary to make a final conclusion.

Lenvatinib was approved by the FDA as a first-line setting for unresectable HCC based on the pivotal trial REFLECT [38]. In this study, a total of 154 sites across 20 countries were involved, enrolling 954 eligible patients. Lenvatinib was taken as 12 mg (>60 kg of body weight) or 8 mg once daily (<60 kg), while sorafenib was taken as 400 mg twice a day. It was reported that the lenvatinib arm had an increased median overall survival of 13.6 months and the sorafenib arm had a median overall survival of 12.3 months [39].

Collectively, in the light of increasing necessity of lenvatinib as a substitution for sorafenib in HCC, we comprehensively summarized the differential expression patterns of DEGs and DEMs between the 2 drug administrations. Interesting, even with the high similarities of chemical structures between the 2 therapies, they still demonstrated several DEGs and DEMs, most of which belonged to the FGF gene family. Together with the external verification of the cellular experiments, it could be suggested that the differences of the 2 chemical compounds were closely associated with FGF-dependent signaling pathways. Additionally, the clinical manifestations and adverse events were also deeply explored in this study. Since the molecular mechanisms underlying the therapies were connected with FGF signaling cassettes, interfering with the signaling axis might be a primary direction to restrict the drug adverse effects. Together, these results offered a meaningful guideline for future clinical HCC chemotherapeutic drug utilization.

Limitations

This study was based on the medical records from a single center and the sample size was limited, which could affect the accuracy of the results. Also, we did not recruit patients with immune-based combinations of drugs for treatment of advanced HCC because the number of patients is small in our hospital and therefore, we could not investigate the differences between single-drug and combined-drug therapy. Moreover, we could not provide a conclusion for long-term clinical manifestations of lenvatinib vs sorafenib for patients with advanced HCC after 3 months of continuous drug treatment. These issues may be worth exploring in the next study.

Conclusions

In this study, we systematically compared the differences between sorafenib and lenvatinib for patients with advanced HCC from genetic and clinical perspectives. Patients treated with lenvatinib developed 8 DEGs (*FGF4*, *FGF23*, *UNC13C*, *RIMBP2*, *STXBP5L*, *PHOX2B*, *NEUROD4*, and *POU4F2*) and 3 DEMs (*has-miR-548ah*, *has-miR-888*, and *has-miR-196a-1*). Simultaneously, the regulatory network of the 3 DEMs and 8 DEGs was established in depth, as we verified the functions of the DEGs and DEMs by external experiments.

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Declaration of Figures' Authenticity

All figures submitted have been created by the authors, who confirm that the images are original with no duplication and have not been previously published in whole or in part.

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