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Original Article

Interaction of key pathways in sorafenib-treated hepatocellular carcinoma based on a PCR-array

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Abstract: This study aimed to identify the key pathways and to explore the mechanism of sorafenib in inhibiting hepatocellular carcinoma (HCC). The gene expression profile of GSE33621, including 6 sorafenib treated group and 6 control samples, was downloaded from the GEO (Gene Expression Omnibus) database. The differentially expressed genes (DEGs) in HCC samples were screened using the ΔΔCt method with the homogenized internal GAPDH. Also, the functions and pathways of DEGs were analyzed using the DAVID. Moreover, the significant pathways of DEGs that involved in HCC were analyzed based on the Latent pathway identification analysis (LPIA). A total of 44 down-regulated DEGs were selected in HCC samples. Also, there were 84 biological pathways that these 44 DEGs involved in. Also, LPIA showed that Osteoclast differentiation and hsa04664-Fc epsilon RI signaling pathway was the most significant interaction pathways. Moreover, Apoptosis, Toll-like receptor signaling pathway, Chagas disease, and T cell receptor signaling pathway were the significant pathways that interacted with hsa04664. In addition, DEGs such as AKT1 (v-akt murine thymoma viral oncogene homolog 1), TNF (tumor necrosis factor), SYK (spleen tyrosine kinase), and PIK3R1 (phosphoinositide-3-kinase, regulatory subunit 1 (alpha)) were the common genes that involved in the significant pathways. Several pathway interaction pairs that caused by several downregulated genes such as SYK, PI3K, AKT1, and TNF, were identified play curial role in sorafenib treated HCC. Sorafenib played important inhibition roles in HCC by affecting a complicate pathway interaction network.

Keywords: Hepatocellular carcinoma, differentially expressed genes, pathway interaction, sorafenib, latent pathway identification analysis

Introduction

Hepatocellular carcinoma (HCC) is the sixth most common malignancies worldwide that characterized by powerful invasion ability, easy to metastasis and poor prognosis [1]. About 60,000 million cases will diagnose as HCC very year, in which the majority cases are from Chinese people [2]. Treatment methods such as surgery and liver transplantation are benefit to the HCC patients in early stage with the 5-year survival rate of 60%-70% [3]. However, there were no useful treatment methods on HCC patients in later stage due to the complicate mechanism of HCC metastasis and invasion [4]. Therefore, exploring several therapeutic targets for HCC will drive to improve the understanding of HCC metastasis mechanism.

Previous study revealed that the signaling transduction system played crucial roles in HCC development [5]. It has been demonstrated that there were four molecular pathways that driving crucial roles in HCC metastasis and invasion. For instance, overexpression of Ras in Ras-MAPKK (Ras-mitogen-activated protein kinase) pathway down-regulates the expression of tumor suppressor Sprouty and Spred-1 in HCC [6]. Aberrant activation of PI3K/Akt/mTOR (phosphatidylinositol 3-kinase/protein kinase B/mammalian target of rapamycin) pathway is associated with HCC progression [7] and mutation of PI3K contributes to the Akt hyperactivation that leading to a poor HCC prognosis [8]. The activated Wnt/β-catenin pathway results in the β-catenin phosphorylation and inhibition of β-catenin degradation, and results in the combination between β-catenin and TCF (transcription factor) in cells, so as to stimulate the transcription of downstream target genes in HCC [9]. Xie *et al.* reported that the genetic polymorphisms of several key molecules in JAK/STAT

(Janus kinase/signal transducers and activators of transcription) signaling pathway is associated with HCC susceptibility, such as IL-6, STAT3 and mTOR [10].

Sorafenib is a cancer treated drug that is useful for many cancers, such as thyroid cancer [11] and non-small cell lung cancer [12]. It has been reported that sorafenib is the first peroral multi-kinase inhibitor that functioning as a molecular target drug for HCC treatment in recent years [13]. For example, Liu *et al.* proved that sorafenib inhibited the cell proliferation and induced the cell apoptosis of HCC via inhibiting the activates of Raf-1, β-Raf kinase and tyrosine kinase receptor to block the Raf/MEK/ERK signaling pathway and VEGF signaling pathway [14]. Also, Gedaly *et al.* reported that sorafenib inhibits the HCC cell proliferation via blocking Ras/MAPK and PI3K/Akt/mTOR pathways [15]. Although many studies have reported the useful treatment of sorafenib in HCC. However, the mechanism of sorafenib in inhibiting HCC metastasis and invasion remains largely unknown due to the complicate signal transduction system in HCC.

Using the gene expression profile of GSE33621 [16], Heindryckx *et al.* proved that inhibition of placental growth factor would be benefit for the therapeutic strategy of HCC metastasis and invasion [17]. In this study, we used microarray analysis to screen the differentially expressed genes (DEGs) in the Sorafenib treated HCC samples. Comprehensive bioinformatics analysis was used to identify the significant pathways that involved in HCC. Our study aimed to identify the significant pathways in HCC metastasis and invasion and explain the role of sorafenib in HCC treatment.

Methods

Microarray data and data preprocessing

The gene expression profile of GSE33621 [16] was downloaded from the GEO (Gene Expression Omnibus) database in NCBI (http://www.ncbi.nlm.nih.gov/geo/) which is the biggest completely public storage, based on the platform of GPL1126 SuperArray GEArray Q series Human Cancer PathwayFinder Gene Array. The platform includes a total of 96 genes that involved in 6 cancer related pathways. The study contains 12 samples which are examined with 6 from sorafenib treated group and 6 from control group.

The single or Multi-Gene qPCR assays in RT2 Profiler PCR Array Data Analysis V3.5 online software [18] was used to preprocess the CEL files.

DEG screening

GAPDH (glyceraldehyde-3-phosphate dehydrogenase) was chosen as the homogenized internal gene. ΔΔCt method [19] was used to screen the DEGs from the normalized profile data with *P*-value < 0.05 and Fold change = $2^{-\Delta\Delta Ct}$, in which ΔCt stands for the expression value of normalized GAPDH while ΔΔCt stands for the case group expression value minus control group expression value.

Latent pathway identification analysis (LPIA)

The LPIA, developed by Pham *et al.* [20], was a method for identification the interactions of pathways associated with DEGs. A significant interaction represented a strong correlation between pathways and disease. The process of LPIA showed as follows:

Step 1: the GO BP (biological process) terms (named G) and KEGG pathways (named P) of DEGs were identified using the clusterProfiler [21] in R; Step 2: a bipartite network was constructed between G and P, one edge of the node was G and the other edge of node was P, edge represents one gene participated in both G and P, the weight of edge was determined by two factors, (1) the relative overlap of G and P was calculated using the Jaccard, (2) mean expression value stand for the expression value of each DEG. The weight formula was shown as follows:

$$w_{GP} = \left| \frac{G \cap P}{G \cup P} \right| \times med\{D_{E_X} : x \in G \cup P\}$$

Whereaze, $\left| \frac{G \cap P}{G \cup P} \right|$ stands for the Jaccard similarity coefficient of G and P, DE represents the expression value of DEG. $G \cup P$ stands for the total DEGs associated with G and P; Step 3: based on the bipartie network, pathways that connected with at least one BP term were chosen to construct the pathway network. The weight formula of edge was $A_{ij} = \sum_{k=1}^{G} w_{G_k P_i} \times w_{G_k P_j}$;

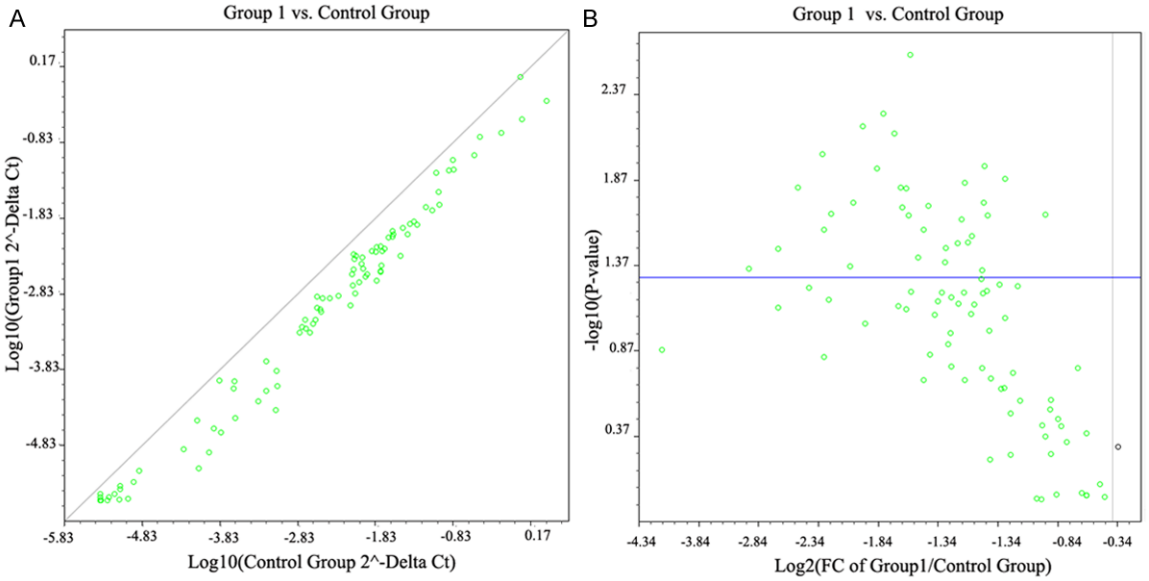

Figure 1. The scatter diagram and volcanic figure of Ct values in case and control group. A: The left figure stands for the Ct of log 10 *P*-value; B: The right figure stands for the Ct of log 2 fold change.

Step 4: random walk method [22] was used to calculate the interactive significance of each pathway pair, and the significant interactions was selected. The transfer matrix of random walk method was $T_{ij} = \frac{A_{ij}}{\sum_{j=1}^{N_p} A_{ij}}$ Whereas N_p stands for the total pathways in network, T_{ij} stands for the probability of one pathway from Pi to Pj.

Then samples were repeated using the bootstrap method [23] from step 1 to step 4, and then the significant *p*-value of pathway interaction was obtained.

Results

Data preprocessing and DEGs screening

The Ct value of DEGs was shown in **Figure 1**. A total of 44 down-regulated DEGs were selected using the ΔΔCt method with *P*-value < 0.05 (**Table 1**). There were 84 biological pathways that these 44 DEGs involved in, such as AKT1 (v-akt murine thymoma viral oncogene homolog 1), ERBB2 (v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)), FAS (Fas (TNF receptor superfamily, member 6)), SYK (spleen tyrosine kinase), TNF (tumor necrosis factor), TP53 (tumor protein p53), and PIK3R1 (phosphoinositide-3-kinase, regulatory subunit 1 (alpha)).

Latent pathway identification analysis (LPIA)

The interaction network of pathways associated with the selected 44 DEGs was shown in **Figure 2**. There were 2775 interaction pairs in this constructed interaction network. Besides, the significant interaction pairs of pathways with the top 10 weight were shown in **Table 2**. Interaction pair between hsa04380-Osteoclast differentiation and hsa04664-Fc epsilon RI signaling pathway was the most significant interaction pathway with weight = 10.4131309, which was caused by the down-regulation of SYK (**Table 1**). Also, there were 6 pathways that interacted with hsa04664 among the pathways with top 10 weights, such as hsa04210-Apoptosis, hsa04620-Toll-like receptor signaling pathway, hsa05142-chagas disease, and hsa04660-T cell receptor signaling pathway (**Table 2**).

In addition, pathways were interacted via the DEGs involved in the relevant pathways. DEGs such as AKT1, TNF, SYK, and PIK3R1 were the genes involved in hsa04664-Fc epsilon RI signaling pathway, FAS, TNF, SYK, AKT1, TP53, and PIK3R1 were the genes involved in hsa04210-Apoptosis, PIK3R1, AKT1, and TNF

Table 1. Information of differentially expressed genes

Gene Symbol	Description	Fold Change	*P*-value
AKT1	v-akt murine thymoma viral oncogene homolog 1	0.147624	0.02642
APAF1	apoptotic peptidase activating factor 1	0.297302	0.005349
BAX	BCL2-associated X protein	0.386891	0.047011
BCL2L1	BCL2-like 1	0.366021	0.048627
CCNE1	cyclin E1	0.34151	0.036517
CDC25A	cell division cycle 25 homolog A (S. pombe)	0.251739	0.010465
CDK2	cyclin-dependent kinase 2	0.371131	0.048012
CDKN2A	cyclin-dependent kinase inhibitor 2A (melanoma, p16, inhibits CDK4)	0.266093	0.026237
CFLAR	CASP8 and FADD-like apoptosis regulator	0.267943	0.023552
CHEK2	protein kinase CHK2-like; CHK2 checkpoint homolog (S. pombe); similar to hCG1983233	0.283221	0.016826
ERBB2	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)	0.0960547	0.026726
ETS2	v-ets erythroblastosis virus E26 oncogene homolog 2 (avian)	0.275476	0.01593
FAS	Fas (TNF receptor superfamily, member 6)	0.0960547	0.016608
HTATIP2	Short Chain Dehydrogenase/Reductase Family	0.147624	0.002546
ITGA1	integrin, alpha 1	0.408951	0.016446
ITGA2	integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor)	0.31864	0.02997
ITGA3	integrin, alpha 3 (antigen CD49C, alpha 3 subunit of VLA-3 receptor)	0.34151	0.020447
ITGB1	integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12)	0.178006	0.007464
ITGB3	integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61)	0.41466	0.00515
ITGB5	integrin, beta 5	0.291183	0.027195
JUN	jun oncogene	0.356013	0.026579
MAP2K1	mitogen-activated protein kinase kinase 1	0.251739	0.029829
MDM2	Mdm2 p53 binding protein homolog (mouse)	0.363493	0.021195
MET	met proto-oncogene (hepatocyte growth factor receptor)	0.332171	0.006027
MMP1	matrix metallopeptidase 1 (interstitial collagenase)	0.246558	0.025416
MMP2	matrix metallopeptidase 2 (gelatinase A, 72kDa gelatinase, 72kDa type IV collagenase)	0.125869	0.003765
MTA2	P53 Target Protein In Deacetylase	0.432269	0.000882
MTSS1	Metastasis Suppressor Protein 1	0.373712	0.019675
NME1	non-metastatic cells 1, protein (NM23A) expressed in; NME1-NME2 readthrough transcript; non-metastatic cells 2, protein (NM23B)	0.295248	0.042317
PIK3R1	phosphoinositide-3-kinase, regulatory subunit 1 (alpha)	0.222211	0.001802
PLAU	plasminogen activator, urokinase	0.162668	0.012227
PLAUR	plasminogen activator, urokinase receptor	0.233258	0.006666
PNN	SR-Like Protein	0.173139	0.011982
RB1	retinoblastoma 1	0.0559391	0.002672
S100A4	Leukemia Multidrug Resistance	0.307786	0.019805
SERPINB5	serpin peptidase inhibitor, clade B (ovalbumin), member 5	0.0595399	0.036155
SYK	spleen tyrosine kinase	0.0871715	0.006901
TGFBR1	transforming growth factor, beta receptor 1	0.303549	0.02148
TNF	tumor necrosis factor (TNF superfamily, member 2)	0.126745	0.017021
TP53	tumor protein p53	0.192109	0.002516
EPDR1	Ependymin Related Protein 1	0.295248	0.035598
B2M	beta-2-microglobulin	0.248273	0.000825
HPRT1	hypoxanthine phosphoribosyltransferase 1	0.417544	0.032709
HGDC	(R)-2-hydroxyglutaryl-CoA dehydratase subunit alpha	0.400535	0.045343

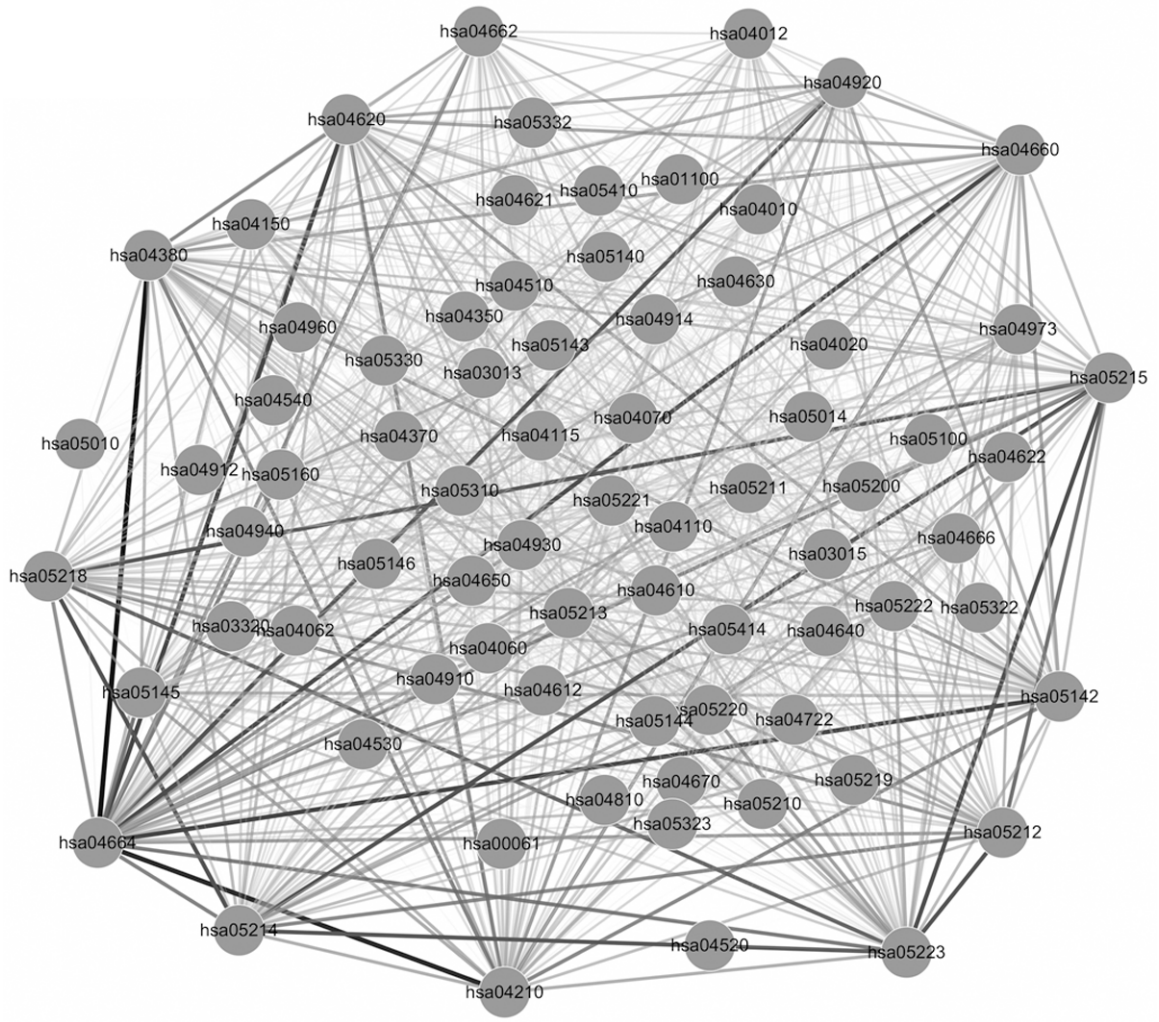

Figure 2. Interaction network of pathways. Edge stands for the interaction between two pathways, the thickness of edge stands for the size of interaction weight.

were involved in hsa04660-T cell receptor signaling pathway, and TNF and AKT1 were involved in hsa04920-Adipocytokine signaling pathway (Table S1).

Discussion

Hepatocellular carcinoma (HCC) is the sixth most common malignancies worldwide that characterized by powerful invasion ability, easy to metastasis and poor prognosis [1]. The mechanism of sorafenib in inhibiting HCC metastasis and invasion has not been fully reported. In this study, we analyzed the significant pathways that involved in the sorafenib treated HCC to illustrate the mechanism of sorafenib in inhibiting HCC. Hsa04380-Osteoclast differentiation and hsa04664-Fc epsilon RI signaling pathway associated with the down-regulated SYK was the most significant interaction pathway pair. Additionally, hsa04210-Apoptosis, hsa04660-T cell receptor signaling pathway, and hsa04920-Adipocytokine signaling pathway, associated with the DEGs such as AKT1, TNF, and PIK3R1 were the important pathways in HCC.

SYK is a member of the family of non-receptor type Tyr protein kinases that widely expressed in hematopoietic cells and mediates the cellular responses including proliferation, differentiation and phagocytosis [24]. Yuan *et al.* proved that loss of SYK mRNA was highly correlated with SYK methylation and then contributed to the metastasis of HCC and resulted to the poor treatment [25]. Also, the precious study

Table 2. Pathway interaction pairs with the top 10 weight

pathway	pathway	weight
hsa04380~Osteoclast differentiation	hsa04664~Fc epsilon RI signaling pathway	10.4131309
hsa04210~Apoptosis	hsa04664~Fc epsilon RI signaling pathway	9.899374049
hsa04620~Toll-like receptor signaling pathway	hsa04664~Fc epsilon RI signaling pathway	9.151907933
hsa05142~Chagas disease (American trypanosomiasis)	hsa04664~Fc epsilon RI signaling pathway	9.090325321
hsa04660~T cell receptor signaling pathway	hsa04664~Fc epsilon RI signaling pathway	8.8833853
hsa05215~Prostate cancer	hsa05223~Non-small cell lung cancer	8.823397413
hsa05215~Prostate cancer	hsa05214~Glioma	8.698434482
hsa05218~Melanoma	hsa05214~Glioma	8.664112555
hsa04920~Adipocytokine signaling pathway	hsa04664~Fc epsilon RI signaling pathway	8.594524769
hsa05223~Non-small cell lung cancer	hsa05212~Pancreatic cancer	8.568905067

revealed that Fc epsilon RI signal mediated the tyrosine phosphorylation of SYK in rat tumor mast cells [26], and SYK was a critical factor in immune receptor signaling [27]. Our data showed that SYK involved in Fc epsilon RI signaling pathway was downregulated in sorafenib treated HCC, we speculated that the downregulated SYK enhanced the interaction activity of Fc epsilon RI signaling pathway with other pathways. In addition, Zou *et al.* reported that osteoclasts with mutation of tyrosine kinase SYK failed to organize the cytoskeleton, suggested its essential role for osteoclast function [28]. Osteoclast differentiation factor is involved in the bone metastasis of cancer [29]. Therefore, SYK may involve in osteoclast differentiation. Also, Ikeda *et al.* firstly reported that hepatocyte-derived cells from HCC cells had the potential for osteoclastogenesis [30]. In this study, Fc epsilon RI signaling was interacted with osteoclast differentiation pathway, suggesting the important inhibition role of sorafenib in HCC metastasis by downregulating SYK and then affecting the two pathways.

AKT1 is one of 3 closely related serine/threonine-protein kinases of AKT kinase that regulate many processed including metabolism, proliferation, cell survival, and angiogenesis [31], while PIK3R1 is a member of PI3-kinases family of lipid kinases capable of phosphorylating the 3'-OH of phosphoinositides [32]. The downregulated SYK suppressed the Raf-1 expression in the downstream MAPK signaling pathway [33, 34] which resulted in the activation of Ras-MAPKK signaling pathway and PI3K/AKT/mTOR pathway [35]. Also, the activated PI3K/AKT/mTOR pathway inhibited the cell growth and proliferation of HCC [36]. Besides, study revealed that the downregulated SYK induced the activation of PI3K [37], and the activated PI3K promoted the AKT/mTOR signaling pathway and NF-κB pathway in HCC [38]. The activated NF-κB weaken the cell proliferation of HCC from the study of Notarbartolo *et al.* [39]. PI3K negatively regulated the TGF-induced cell apoptosis in HCC [40]. Liu *et al.* proved that sorafenib induced HCC cell apoptosis via inhibiting the RAF/MEK/ERK signaling pathway [14]. Thus, the interacted two pathways may involve in HCC cell proliferation. In this study, the downregulated PI3K was involved in Apoptosis pathway that interacted with Fc epsilon RI signaling pathway in sorafenib treated HCC samples, implying the important inhibiting role of sorafenib in HCC via affecting the two interactive pathways.

Meanwhile, study reveals that TNF is an endogenous tumor promoter in HCC [41]. Ormandy *et al.* proved that T cells were gathered in blood of patients with HCC [42]. In this study, TNF that participate in T cell receptor signaling pathway was downregulated in sorafenib treated HCC, indicating that T cell receptor signaling pathway may involve in HCC. On the other hand, Adipose tissue secreted many factors such as leptin and adiponectin [43]. Leptin promotes the invasion and metastasis of HCC by enhancing the cell proliferation and mitosis, which is associated with the activation of PI3K/AKT pathway and ERK pathway [44]. Hence, adipocytokine signaling pathway may be crucial for HCC. Yanawaki *et al.* said that adipocytokine inhibited the TNF-induced vascular inflammation in human endothelial cells [45] and activated adipocytokine signaling pathway was involved in HCC cell invasion [46]. Based on our study, we speculated that sorafenib may inhibit the HCC metastasis by influencing the interacted Adipo-

cytokine signaling pathway and Fc epsilon RI signaling pathway.

In conclusion, our study attempt to identify several interactive pathway pairs associated with sorafenib treated HCC. Sorafenib inhibited HCC progression via downregulating the SYK expression and then affecting the interaction pair of Fc epsilon RI signaling and osteoclast differentiation pathway while inducing HCC cell apoptosis by downregulating PI3K and then influencing apoptosis and Fc epsilon RI signaling pathway. This study may provide theoretic basis for the future exploration of drug target therapy in HCC. However, further experimental studies are still needed to confirm our predicted results.

Disclosure of conflict of interest

None.

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Table S1. Significant differentially expressed genes and their involved pathways

Gene	pathway
E2F1	hsa04110: Cell cycle,
E2F1	hsa05200: Pathways in cancer,
E2F1	hsa05212: Pancreatic cancer,
E2F1	hsa05214: Glioma,
E2F1	hsa05215: Prostate cancer,
E2F1	hsa05218: Melanoma,
E2F1	hsa05219: Bladder cancer,
E2F1	hsa05220: Chronic myeloid leukemia,
E2F1	hsa05222: Small cell lung cancer,
E2F1	hsa05223: Non-small cell lung cancer,
FAS	hsa04010: MAPK signaling pathway,
FAS	hsa04060: Cytokine-cytokine receptor interaction,
FAS	hsa04115: p53 signaling pathway,
FAS	hsa04210: Apoptosis,
FAS	hsa04650: Natural killer cell mediated cytotoxicity,
FAS	hsa04940: Type I diabetes mellitus,
FAS	hsa05010: Alzheimer's disease,
FAS	hsa05200: Pathways in cancer,
FAS	hsa05320: Autoimmune thyroid disease,
FAS	hsa05330: Allograft rejection,
FAS	hsa05332: Graft-versus-host disease,
B2M	hsa04612: Antigen processing and presentation,
ITGB1	hsa04360: Axon guidance,
ITGB1	hsa04510: Focal adhesion,
ITGB1	hsa04512: ECM-receptor interaction,
ITGB1	hsa04514: Cell adhesion molecules (CAMs),
ITGB1	hsa04670: Leukocyte transendothelial migration,
ITGB1	hsa04810: Regulation of actin cytoskeleton,
ITGB1	hsa05130: Pathogenic Escherichia coli infection,
ITGB1	hsa05200: Pathways in cancer,
ITGB1	hsa05222: Small cell lung cancer,
ITGB1	hsa05410: Hypertrophic cardiomyopathy (HCM),
ITGB1	hsa05412: Arrhythmogenic right ventricular cardiomyopathy (ARVC),
ITGB1	hsa05414: Dilated cardiomyopathy,
MMP1	hsa03320: PPAR signaling pathway,
MMP1	hsa05200: Pathways in cancer,
MMP1	hsa05219: Bladder cancer,
MMP2	hsa04670: Leukocyte transendothelial migration,
MMP2	hsa04912: GnRH signaling pathway,
MMP2	hsa05200: Pathways in cancer,
MMP2	hsa05219: Bladder cancer,
PIK3R1	hsa04012: ErbB signaling pathway,
PIK3R1	hsa04062: Chemokine signaling pathway,
PIK3R1	hsa04070: Phosphatidylinositol signaling system,
PIK3R1	hsa04150: mTOR signaling pathway,
PIK3R1	hsa04210: Apoptosis,

PIK3R1	hsa04370: VEGF signaling pathway,
PIK3R1	hsa04510: Focal adhesion,
PIK3R1	hsa04620: Toll-like receptor signaling pathway,
PIK3R1	hsa04630: Jak-STAT signaling pathway,
PIK3R1	hsa04650: Natural killer cell mediated cytotoxicity,
PIK3R1	hsa04660: T cell receptor signaling pathway,
PIK3R1	hsa04662: B cell receptor signaling pathway,
PIK3R1	hsa04664: Fc epsilon RI signaling pathway,
PIK3R1	hsa04666: Fc gamma R-mediated phagocytosis,
PIK3R1	hsa04670: Leukocyte transendothelial migration,
PIK3R1	hsa04722: Neurotrophin signaling pathway,
PIK3R1	hsa04810: Regulation of actin cytoskeleton,
PIK3R1	hsa04910: Insulin signaling pathway,
PIK3R1	hsa04914: Progesterone-mediated oocyte maturation,
PIK3R1	hsa04930: Type II diabetes mellitus,
PIK3R1	hsa04960: Aldosterone-regulated sodium reabsorption,
PIK3R1	hsa05200: Pathways in cancer,
PIK3R1	hsa05210: Colorectal cancer,
PIK3R1	hsa05211: Renal cell carcinoma,
PIK3R1	hsa05212: Pancreatic cancer,
PIK3R1	hsa05213: Endometrial cancer,
PIK3R1	hsa05214: Glioma,
PIK3R1	hsa05215: Prostate cancer,
PIK3R1	hsa05218: Melanoma,
PIK3R1	hsa05220: Chronic myeloid leukemia,
PIK3R1	hsa05221: Acute myeloid leukemia,
PIK3R1	hsa05222: Small cell lung cancer,
PIK3R1	hsa05223: Non-small cell lung cancer,
PLAU	hsa04610: Complement and coagulation cascades,
PLAUR	hsa04610: Complement and coagulation cascades,
PDGFA	hsa04010: MAPK signaling pathway,
PDGFA	hsa04060: Cytokine-cytokine receptor interaction,
PDGFA	hsa04510: Focal adhesion,
PDGFA	hsa04540: Gap junction,
PDGFA	hsa04810: Regulation of actin cytoskeleton,
PDGFA	hsa05200: Pathways in cancer,
PDGFA	hsa05214: Glioma,
PDGFA	hsa05215: Prostate cancer,
PDGFA	hsa05218: Melanoma,
RB1	hsa04110: Cell cycle,
RB1	hsa05200: Pathways in cancer,
RB1	hsa05212: Pancreatic cancer,
RB1	hsa05214: Glioma,
RB1	hsa05215: Prostate cancer,
RB1	hsa05218: Melanoma,
RB1	hsa05219: Bladder cancer,
RB1	hsa05220: Chronic myeloid leukemia,
RB1	hsa05222: Small cell lung cancer,
RB1	hsa05223: Non-small cell lung cancer,

SERPINB5	hsa04115: p53 signaling pathway,
SYK	hsa04650: Natural killer cell mediated cytotoxicity,
SYK	hsa04662: B cell receptor signaling pathway,
SYK	hsa04664: Fc epsilon RI signaling pathway,
SYK	hsa04666: Fc gamma R-mediated phagocytosis,
TNF	hsa04010: MAPK signaling pathway,
TNF	hsa04060: Cytokine-cytokine receptor interaction,
TNF	hsa04210: Apoptosis,
TNF	hsa04350: TGF-beta signaling pathway,
TNF	hsa04620: Toll-like receptor signaling pathway,
TNF	hsa04621: NOD-like receptor signaling pathway,
TNF	hsa04622: RIG-I-like receptor signaling pathway,
TNF	hsa04640: Hematopoietic cell lineage,
TNF	hsa04650: Natural killer cell mediated cytotoxicity,
TNF	hsa04660: T cell receptor signaling pathway,
TNF	hsa04664: Fc epsilon RI signaling pathway,
TNF	hsa04920: Adipocytokine signaling pathway,
TNF	hsa04930: Type II diabetes mellitus,
TNF	hsa04940: Type I diabetes mellitus,
TNF	hsa05010: Alzheimer's disease,
TNF	hsa05014: Amyotrophic lateral sclerosis (ALS),
TNF	hsa05310: Asthma,
TNF	hsa05322: Systemic lupus erythematosus,
TNF	hsa05330: Allograft rejection,
TNF	hsa05332: Graft-versus-host disease,
TNF	hsa05410: Hypertrophic cardiomyopathy (HCM),
TNF	hsa05414: Dilated cardiomyopathy,
TP53	hsa04010: MAPK signaling pathway,
TP53	hsa04110: Cell cycle,
TP53	hsa04115: p53 signaling pathway,
TP53	hsa04210: Apoptosis,
TP53	hsa04310: Wnt signaling pathway,
TP53	hsa04722: Neurotrophin signaling pathway,
TP53	hsa05014: Amyotrophic lateral sclerosis (ALS),
TP53	hsa05016: Huntington's disease,
TP53	hsa05200: Pathways in cancer,
TP53	hsa05210: Colorectal cancer,
TP53	hsa05212: Pancreatic cancer,
TP53	hsa05213: Endometrial cancer,
TP53	hsa05214: Glioma,
TP53	hsa05215: Prostate cancer,
TP53	hsa05216: Thyroid cancer,
TP53	hsa05217: Basal cell carcinoma,
TP53	hsa05218: Melanoma,
TP53	hsa05219: Bladder cancer,
TP53	hsa05220: Chronic myeloid leukemia,
TP53	hsa05222: Small cell lung cancer,
TP53	hsa05223: Non-small cell lung cancer,
AKT1	hsa04010: MAPK signaling pathway,

AKT1	hsa04012: ErbB signaling pathway,
AKT1	hsa04062: Chemokine signaling pathway,
AKT1	hsa04150: mTOR signaling pathway,
AKT1	hsa04210: Apoptosis,
AKT1	hsa04370: VEGF signaling pathway,
AKT1	hsa04510: Focal adhesion,
AKT1	hsa04530: Tight junction,
AKT1	hsa04620: Toll-like receptor signaling pathway,
AKT1	hsa04630: Jak-STAT signaling pathway,
AKT1	hsa04660: T cell receptor signaling pathway,
AKT1	hsa04662: B cell receptor signaling pathway,
AKT1	hsa04664: Fc epsilon RI signaling pathway,
AKT1	hsa04666: Fc gamma R-mediated phagocytosis,
AKT1	hsa04722: Neurotrophin signaling pathway,
AKT1	hsa04910: Insulin signaling pathway,
AKT1	hsa04914: Progesterone-mediated oocyte maturation,
AKT1	hsa04920: Adipocytokine signaling pathway,
AKT1	hsa05200: Pathways in cancer,
AKT1	hsa05210: Colorectal cancer,
AKT1	hsa05211: Renal cell carcinoma,
AKT1	hsa05212: Pancreatic cancer,
AKT1	hsa05213: Endometrial cancer,
AKT1	hsa05214: Glioma,
AKT1	hsa05215: Prostate cancer,
AKT1	hsa05218: Melanoma,
AKT1	hsa05220: Chronic myeloid leukemia,
AKT1	hsa05221: Acute myeloid leukemia,
AKT1	hsa05222: Small cell lung cancer,
AKT1	hsa05223: Non-small cell lung cancer,
ERBB2	hsa04012: ErbB signaling pathway,
ERBB2	hsa04020: Calcium signaling pathway,
ERBB2	hsa04510: Focal adhesion,
ERBB2	hsa04520: Adherens junction,
ERBB2	hsa05200: Pathways in cancer,
ERBB2	hsa05212: Pancreatic cancer,
ERBB2	hsa05213: Endometrial cancer,
ERBB2	hsa05215: Prostate cancer,
ERBB2	hsa05219: Bladder cancer,
ERBB2	hsa05223: Non-small cell lung cancer,