

Original Article

Detection and screening of small molecule agents for overcoming Sorafenib resistance of hepatocellular carcinoma: a bioinformatics study

Jinli Lv^{1,2}, Bo Zhu¹, Liang Zhang¹, Qichao Xie¹, Wenlei Zhuo¹

¹Institute of Cancer, Xinqiao Hospital, Third Military Medical University, Chongqing 400037, China; ²Department of General Surgery, The 153th Central Hospital of PLA, Zhengzhou 450007, Henan, China

Received November 28, 2014; Accepted February 3, 2015; Epub February 15, 2015; Published February 28, 2015

Abstract: Sorafenib, a novel orally-available multikinase inhibitor blocking several crucial oncogenic signaling pathways, presented survival benefits and became the first-line drug for treatment of patients with Hepatocellular carcinoma (HCC). However, the acquired resistance to Sorafenib resulted in limited benefits. In this study, we aimed to explore possible agents that might overcome Sorafenib resistance by bioinformatics methods. The gene expression profiles of HCC-3sp (acquired Sorafenib-resistance) and HCC-3p (Sorafenib-sensitive) cell line were downloaded from Gene Expression Omnibus (GEO) database. Then, the differentially expressed genes (DEGs) were selected using dChip software. Furthermore, Gene Ontology (GO) and pathway enrichment analyses were performed by DAVID database. Finally, the Connectivity Map was utilized to predict potential chemicals for reversing Sorafenib resistance. Consequently, a total of 541 DEGs were identified, which were associated with cell extracellular matrix, cell adhesion and binding-related items. KEGG pathway analysis indicated that 8 dysfunctional pathways were enriched. Finally, several small molecules, such as pregnenolone and lomustine, were screened out as potential therapeutic agents capable of overcoming Sorafenib resistance. The data identified some potential small molecule drugs for treatment of Sorafenib resistance and offered a novel strategy for investigation and treatments of HCC.

Keywords: Sorafenib resistance, differentially expressed genes, dysfunctional pathway, function enrichment analysis, hepatocellular carcinoma

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies with complex molecular mechanisms [1]. Most HCC patients are diagnosed at its late stage. However, the conventional chemotherapies are usually ineffective treatments for them, leading to poor outcome. Sorafenib, an orally multikinase inhibitor targeting a series of molecular pathways mediated by PDGFR, VEGFR, or Raf kinase, presented dramatically therapeutic effect on patients with HCC, including reduction of recurrence risk and prolongation of overall survival [2]. Sorafenib has been proved to be the standard first-line therapeutic agent for advanced HCC by the US Food and Drug Administration (FDA). However, the acquired resistance eventually occurred in most of patients with initial response to Sorafenib, which seriously offset

its clinical benefit and became a severe clinical challenge for HCC treatment.

It is still not fully understood about the molecular mechanism related to acquired Sorafenib-resistance. Previous studies have described that various dysfunctional signal pathways were involved in this progress. For instance, several abnormal expression and/or activation of signaling molecules such as ERK [3], Met/Akt [4], CD44 [5], TGF- β [5], SETD4 [6] and HIF-2 α [7] were included. Besides, recent evidence suggests that Sorafenib resistance of HCC cells may be associated with altered autophagy mechanism [8]. Moreover, epithelial-mesenchymal transition (EMT) contributed to not only Sorafenib resistance but also cell proliferation, metastasis and recurrence of HCC [9]. In addition, recent reports revealed that HCC stem cells might play important roles in Sorafenib

Sorafenib-resistance and hepatocellular cancer

Table 1. The most significant up-regulated and down-regulated DEGs (Top 15)

	Probe set	Gene symbol	Fold change	P value
Up-regulated	7961693	ldhb	3.74	0.000002
	7971077	postn	3.52	0.003723
	8056257	Fap	3.02	0.000012
	7917182	ELTD1	2.98	0.000714
	7961514	MGP	2.94	0.002494
	8127563	col12a1	2.83	0.000193
	8112980	edil3	2.76	0.000093
	8163257	lpar1	2.71	0.000127
	8059905	Col6a3	2.69	0.000005
	8021081	Slc14a1	2.65	0.000027
Down-regulated	8017766	Apoh	-3.99	0.000033
	8103326	FGG	-3.70	0.000045
	8149521	FGL1	-3.64	0
	7957023	LYZ	-3.51	0
	8097910	fgb	-3.50	0.000006
	8084648	AHSG	-3.42	0
	8098439	epcam	-3.33	0.000037
	7926061	ITIH2	-3.33	0.000001
	8082797	TF	-3.17	0.00001
	8103311	FGA	-3.16	0.00043

resistance [10]. Taking together, these data demonstrated that various factors induced Sorafenib-resistance in HCC through multiple pathways. Therefore, it is difficult to treat Sorafenib-resistant HCC patients by interrupting single molecular or pathway.

Recently, numerous approaches in the attempt to overcome Sorafenib resistance have been reported, such as a combination with 2-Methoxyestradiol [11], knockout of ADAM10 [12] and inhibition of Akt [13]. However, the treatment effectiveness was limited due to the unclear pathogenesis. In fact, many investigations mainly concentrated on a certain target. Since mechanisms of Sorafenib resistance involved multiple alterations in cellular and molecular levels, traditional therapeutic approaches targeting any single gene were insufficient to illuminate the molecular mechanism. Thus, it is critical to explore gene variation in Sorafenib resistance by more powerful genome-wide technologies, which might help clarify the nature of Sorafenib resistance and then develop effective treatment strategies.

Microarray is a high-throughput tool for carrying out global gene expression profiles efficiently, which has been widely used to explore the

mechanisms underlying some diseases. Previous reports successfully described the gene expression profile of acquired Sorafenib-resistant hepatocellular carcinoma cells by using this tool [14]. Thus, to better understand the intrinsic mechanisms and develop effective drugs, it is necessary to perform further research about gene expression profile of acquired Sorafenib resistance. Therefore, in the present study, using computational bioinformatics methods, public microarray data were downloaded for identifying differentially expressed genes (DEGs) between acquired Sorafenib-resistant and -sensitive hepatocellular carcinoma cells. The functions of DEGs were further investigated by Gene Ontology (GO) annotation and pathway enrichment. In addition, candidate small molecules for reversing Sorafenib-resistance were screened by CMAP. We aimed to better understand the molecular mechanisms of Sorafe-

nib resistance and develop new potential therapeutic drugs for its reversion.

Methods

Identification of differentially expressed genes (DEGs) from public microarray data

The public gene expression profile (GSE26391) was downloaded from the Gene Expression Omnibus (GEO) database (<http://www.ncbi.nlm.nih.gov/geo/>) for obtaining the DEGs in acquired Sorafenib-resistant HCC cells compared to Sorafenib-sensitive HCC cells. This dataset was uploaded by Van et al in 2011 [14], containing HCC cell line HCC-3p (Sorafenib-sensitive) and HCC-3sp (acquired Sorafenib-resistance), which were isolated from one HCC patient. Then, the dataset was analyzed by dChip software (v.2011.01) (<http://www.hsph.harvard.edu/>). T test was used to identify Sorafenib resistance-related DEGs between HCC-3sp and HCC-3p cells, with a threshold of P -value < 0.05 and fold-change ≥ 1.5 .

Functional enrichment analysis of DEGs

The functional enrichment analysis of the DEGs was performed by the Database for Annotation,

Sorafenib-resistance and hepatocellular cancer

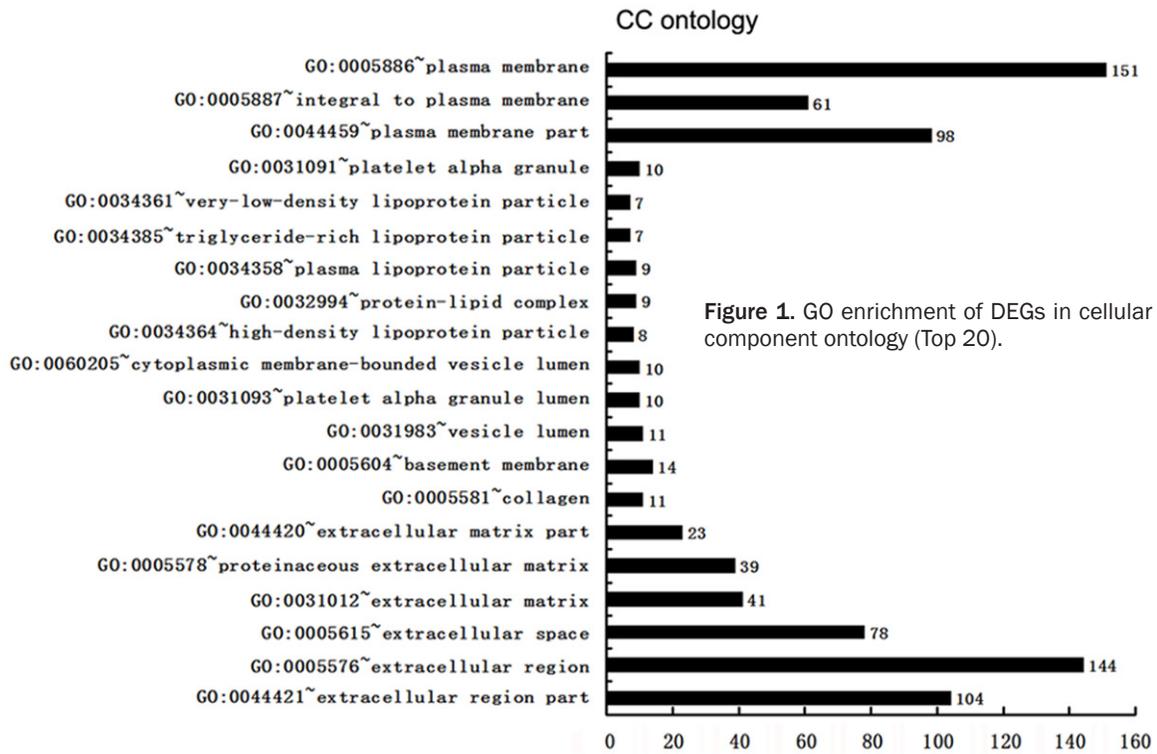


Figure 1. GO enrichment of DEGs in cellular component ontology (Top 20).

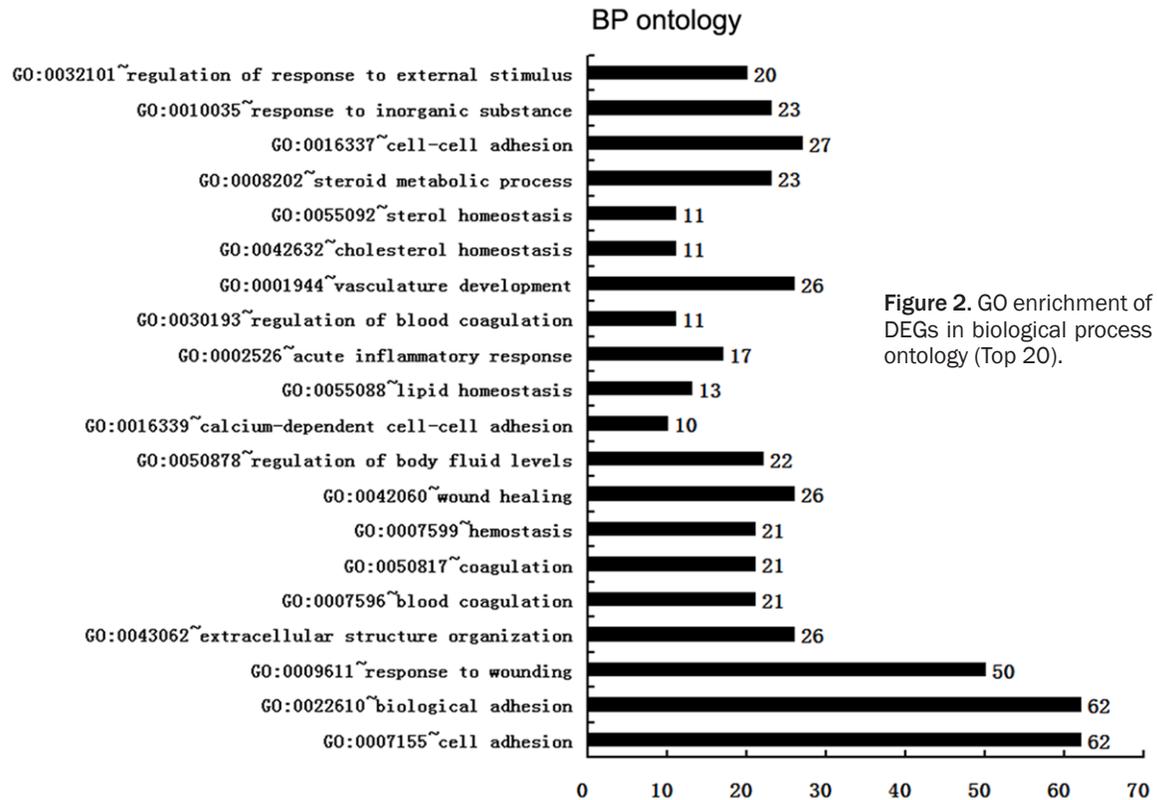


Figure 2. GO enrichment of DEGs in biological process ontology (Top 20).

Visualization and Integrated Discovery (DAVID) database, including gene ontology (GO) func-

tion analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. In GO

Sorafenib-resistance and hepatocellular cancer

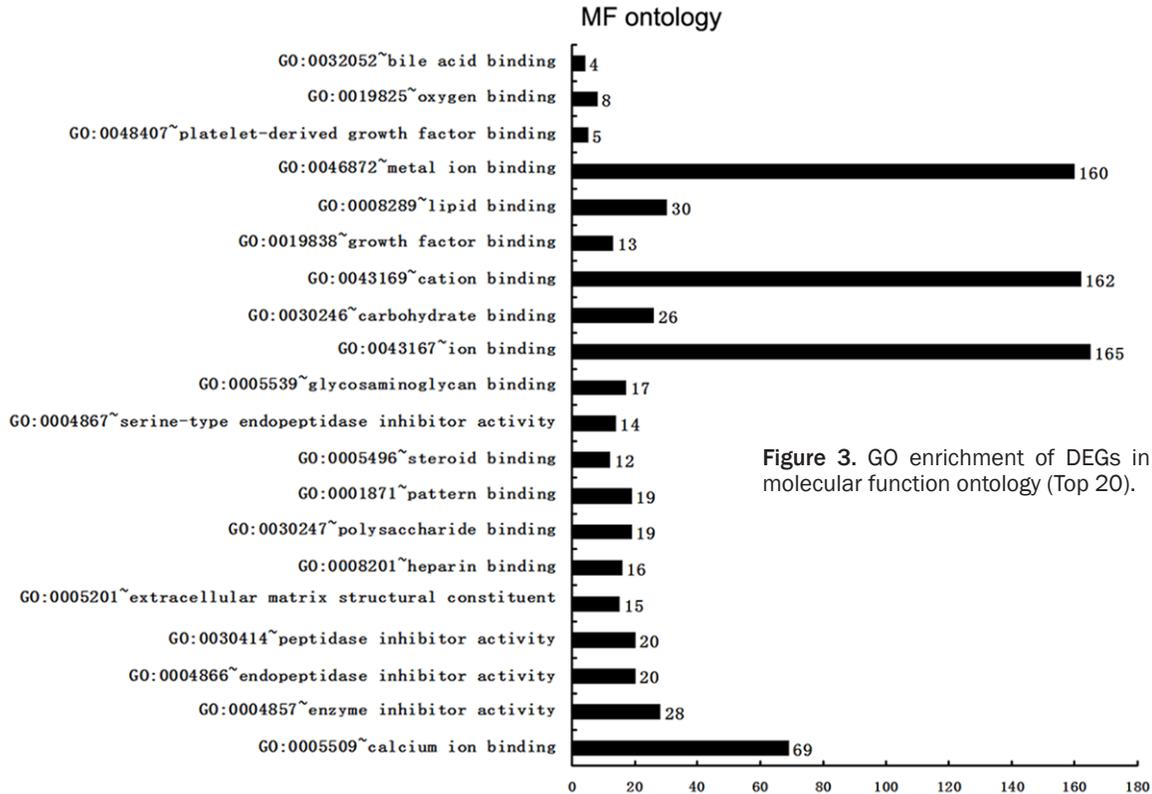


Figure 3. GO enrichment of DEGs in molecular function ontology (Top 20).

analysis, the categories include cellular component (CC), biological process (BP), and molecular function (MF) terms, and P -value < 0.01 was regarded as statistically significant differences. In KEGG pathways analysis, enriched pathways were identified according to the hypergeometric distribution with a P -value of < 0.01 .

Connectivity Map analysis

CMAP (The Connectivity Map, <http://www.broad.mit.edu/cmap/>) database contains more than 7,000 expression signatures involving 6100 small molecules as treatment-control pairs. By comparing queried expression signatures, CMAP has previously been applied to explore the mechanisms of drug action, as well as to identify new potential drugs [15, 16]. The DEGs, divided into down-regulated and up-regulated groups, were submitted to CMAP for analysis. Consequently, the enrichment scores that ranged from -1 to 1 were calculated. Small molecules with negative enrichment scores, implying the ability of agents to reverse the expression direction of query genes, were chosen as potential drugs for treatment of Sorafenib resistance. To further identify the potential drugs with the capability of overcoming

Sorafenib resistance, more strict criteria were utilized by limiting the number of repeat experiments to more than 4 times, setting the proportion of effective rate to $> 50\%$, and using a threshold of P -value < 0.05 .

Results

Identification of DEGs between Sorafenib-sensitive and acquired Sorafenib resistant HCC cells

Based on the public microarray data set GSE-26391, the dChip Software was utilized to analyze the gene expression profiles and identify the DEGs between Sorafenib-sensitive HCC-3p cells and acquired Sorafenib-resistant HCC-3sp cells with the described criteria. As a result, a total of 541 DEGs were selected, including 276 up-regulated and 265 down-regulated DEGs. The top ten down-regulated DEGs and up-regulated DEGs were listed in **Table 1**.

Functional annotation and pathway enrichment of DEGs

To investigate the altered biological function of the DEGs, the DEGs were clustered through

Sorafenib-resistance and hepatocellular cancer

Table 2. The enriched KEGG pathway of DEGs

Term	Count	P value
hsa04610: Complement and coagulation cascades	27	1.14E-20
hsa04512: ECM-receptor interaction	19	6.37E-10
hsa04510: Focal adhesion	24	7.24E-07
hsa00980: Metabolism of xenobiotics by cytochrome P450	12	7.93E-06
hsa00140: Steroid hormone biosynthesis	9	2.10E-04
hsa00982: Drug metabolism	10	3.52E-04
hsa05412: Arrhythmogenic right ventricular cardiomyopathy	10	1.59E-03
hsa00590: Arachidonic acid metabolism	8	3.86E-03

Table 3. Six therapeutic small molecule agents with potential abilities to overcome Sorafenib resistance of hepatocellular carcinoma

CMAp name	Mean	N	Enrichment	P value	Specificity	Percent non-null
pregnenolone	-0.284	4	-0.733	0.01007	0.0255	50
bretylum tosilate	-0.409	4	-0.728	0.01118	0.0165	50
lomustine	-0.422	4	-0.715	0.01333	0.0986	50
chlorambucil	-0.496	4	-0.675	0.02493	0.0714	75
Prestwick-1100	-0.479	4	-0.653	0.03378	0.0311	75
carisoprodol	-0.381	4	-0.63	0.04579	0.0248	50

Gene Ontology (GO) analysis in DAVID, with $P < 0.01$. The enriched GO terms, divided into CC, BP and MF ontologies, were illustrated as **Figures 1-3**.

In the CC ontology, we found that the majority of enriched categories were associated with extracellular construction, such as extracellular region (144 genes), extracellular matrix (41 genes), collagen (11 genes) and basement membrane (14 genes). The second enriched CC GO terms were membrane-related categories such as plasma membrane (151 genes) and vesicle lumen (11 genes). In addition, the third enriched CC GO terms included protein-lipid complex (9 genes), plasma lipoprotein particle (9 genes), high-density lipoprotein particle (8 genes), and other similar protein-lipid related items.

In the BP ontology, the most significant GO categories were adhesion related items such as cell adhesion (62 genes), biological adhesion (62 genes), cell-cell adhesion (27 genes) and extracellular structure organization (26 genes). Besides, the other enriched categories were associated with coagulation, including blood coagulation (21 genes) and hemostasis (21 genes). Another enriched categories comprised vasculature development (27 genes), steroid

metabolic process (23 genes) and lipid homeostasis (13 genes).

In the MF ontology, the binding-related items constitute the majority of enriched GO categories, including calcium ion binding (69 genes), polysaccharide binding (19 genes), steroid binding (12 genes), ion binding (165 genes), metal ion binding (160 genes), growth factor binding (13 genes), lipid binding (30 genes) and cation binding (162 genes). The other enriched MF ontologies were enzyme related items, such as enzyme inhibitor activity (28 genes), endopeptidase inhibitor activity (20

genes), and peptidase inhibitor activity (20 genes).

Furthermore, a total of 8 dysfunctional pathways were enriched via the KEGG pathway analysis, including complement and coagulation cascades (27 genes), ECM-receptor interaction (19 genes), focal adhesion (24 genes), metabolism of xenobiotics by cytochrome P450 (12 genes), steroid hormone biosynthesis (9 genes), drug metabolism (10 genes), arrhythmogenic right ventricular cardiomyopathy (10 genes), and arachidonic acid metabolism (8 genes) (**Table 2**).

Sorafenib-resistant HCC cell signature-specific drug screening from CMAp database

The DEGs, including 276 up-regulated and 265 down-regulated DEGs, were analyzed by CMAp tool. As results, 646 small molecule chemicals with negative enrichment scores were predicted, which indicated a potential to reverse Sorafenib-resistance signature. By further filtering with the described criteria, six remained agents were screened out as the most promising therapeutic small-molecule candidates to overcome Sorafenib resistance of HCC, including pregnenolone, bretylum tosilate, lomustine, chlorambucil, Prestwick-1100, and carisoprodol (**Table 3**).

Discussion

Acquired Sorafenib-resistance is a huge challenge for treating patients with HCC. Therefore, it is very necessary to explore the mechanism of Sorafenib resistance, and develop possible treatment strategies for it. Through gene expression profiling by microarray technology, the key genes associated with drug resistance could be discovered, which could be further utilized to explore novel diagnostic and therapeutic strategies. In this study, we identified DEGs of Sorafenib-resistant HCC cells. Then, we analyzed their functions by using GO annotation and pathway enrichment. Finally, some small molecule agents that had potential to overcome Sorafenib-resistance were screened out by CMAP tool. These results not only explored possible mechanisms and candidate drugs for Sorafenib-resistance, but also present novel strategies for HCC therapy.

The dChip is a powerful software for probe-level and high-level analysis of gene expression microarrays. Using this tool, we obtained 541 DEGs between Sorafenib-resistant and -sensitive HCC cells, including 276 up-regulated and 265 down-regulated DEGs. Among them, we found that the majority of the top 10 up-regulated and down-regulated DEGs were associated with extracellular matrix, such as *Postn* and *Fap*, which might reflect the extracellular matrix-related signal pathways participated in Sorafenib-resistance. Up to date, a series of studies have demonstrated the relationship between the DEGs and tumor development or drug resistance. *Ldhd* (Lactate dehydrogenase B), the top one up-regulated DEG, was an essential gene for the growth of KRAS-dependent lung adenocarcinomas [17] and triple-negative breast cancer [18]. *Postn*, a secreted ECM protein, was capable of enhancing proliferation, angiogenesis, invasion and metastasis and improving survival through induction of EMT (epithelial-mesenchymal transition) in some types of cancers, such as breast cancer [19] and gastric cancer [20]. In addition, evidence revealed that *Postn* could promote drug resistance of pancreatic cancer [21] and ovarian cancer [22]. *Fap*, fibroblast activation protein, could suppress the bortezomib-induced apoptosis in myeloma cells through β -catenin signaling pathway [23] and induce the resistance to doxorubicin or Fas-induced apoptosis [24]. *MGP*, an extracellular matrix protein, par-

ticipates in the cell attachment and spreading [25] and contributes to chemoresistance of ovarian cancer [26]. Furthermore, according to reports, other DEGs, such as *col4a2*, *Col6a3*, *EDIL3*, *Epcam* and *Ipar1* were also related to tumor progression or drug resistance [27-29]. Collectively, these results highlight that the DEGs might contribute to Sorafenib-resistance through various mechanisms. We hypothesized that the DEGs not only have a potential to be biomarkers for distinguishing or predicting Sorafenib-resistance, but also might be used as targets for Sorafenib-resistance treatment. However, the mechanisms remain unclear at present and further verification experiments are needed in the future.

Through GO annotation in DAVID database, we analyzed the biological function of the DEGs. In CC oncology, we discovered that the majority of the DEGs were enriched in extracellular construction-related items such as extracellular matrix and basement membrane. Besides, the second enriched CC GO terms were cellular membrane-related ontologies such as plasma membrane and vesicle lumen, and the third enriched CC GO terms were protein-lipid related items. The results reflect that complex cellular components, especially extracellular construction, might contribute to Sorafenib-resistance, which is in accordance with the results of the majority of DEGs showed in **Table 1**. The majority of DEGs products are extracellular construction and cell-surface protein, which participate in tumor microenvironment. As we know, tumor microenvironment might play crucial roles in the therapeutic resistance process [30], including Sorafenib-resistance of cancers [31]. Furthermore, most of the DEGs enriched in BP ontologies were adhesion-related items such as cell adhesion and extracellular structure organization. These data were concordant with the results of CC oncology enrichment. Previous studies have shown that multifarious microenvironmental factors and focal adhesion signaling play crucial roles in therapy resistance of cancers [32]. In addition, In MF portion, the most significant enriched ontologies were binding-related items, including ion binding, lipid binding, steroid binding and growth factor binding, while the second enriched ontologies were enzyme-related items. These data reflected that the DEGs may affect the binding between growth factor, ion, lipid and extracellular matrix, and then influ-

ence enzyme activity that might result in Sorafenib-resistance. It has been reported that ATP-binding cassette (ABC) transporters play crucial roles in chemoresistance of cancer cells via promoting efflux of drugs from cells [33]. Accordingly, DEGs might lead to Sorafenib-resistance through binding-related mechanisms, which is worth evaluating in further studies.

Pathway analysis may reflect more precise biological function of genes than GO analysis. In the present study, 8 pathways were enriched. Among them, the adhesion related pathways, including ECM-receptor interaction and focal adhesion, have been reported to facilitate drug resistance of cancers. These enriched pathways corresponded to the results of GO analysis. Then, the other enriched pathways were the metabolism related pathways, including metabolism of xenobiotics by cytochrome P4-50, steroid hormone biosynthesis, drug metabolism and arachidonic acid metabolism. It is easily comprehensible that metabolism is responsible for drug-resistant phenotype development of cancers. As we know, sphingolipid metabolism and glutathione metabolism have been reported to play important roles in multi-drug resistance of cancers [34, 35]. In addition, another enriched pathway was complement and coagulation cascades. A recent study found up-regulated expression of membrane-bound complement restriction proteins (mCRPs) CD55, CD46 and CD59 in head and neck cancer, which enable cancer cells to escape complement-dependent cytotoxicity and antibody-dependent killing, hence enhancing the survival ability of cancer cells [36]. Therefore, we hypothesized that the DEGs enriched in metabolism-related pathways might confer Sorafenib-resistance to HCC, which deserves further research.

In the present study, several molecule chemicals with highly significant negative scores were identified, which have a potential to treat HCC with Sorafenib-resistance. Among the chemicals, three have been applied in cancer treatment. Pregnenolone, an endogenous steroid hormone, has been demonstrated to treat metastatic prostate cancer [37]. Lomustine, an alkylating nitrosourea compound used in chemotherapy, exhibits anticancer activities against some cancer types, especially brain tumors [38]. Chlorambucil, a chemotherapy drug, was used in the treatment of chronic lymphocytic

leukemia and non-Hodgkin lymphoma [39]. In addition, other chemicals were usually used in other non-oncologic diseases. Bretylium tosylate, an antiarrhythmic agent, was also traditionally utilized as an inhibitor of sympathetic transmission [40]. Carisoprodol, a carbamic acid ester, was used as a centrally acting skeletal muscle relaxant [41]. Nevertheless, no report has been retrieved to prove the abilities of overcoming Sorafenib-resistance of the above chemicals, although they showed negative enrichment scores in CMAP analysis. Our study only provides the preliminary clues, and further evaluations for their potential pharmacological effects are still needed.

In conclusion, the study provides some primary research and analysis about the mechanism of Sorafenib-resistance of HCC. A series of important DEGs and dysfunctional pathways participating in Sorafenib-resistance were discovered. Moreover, some small molecule drugs that have a potential to reverse Sorafenib-resistance were screened out. This study may imply an efficient strategy for research and treatment of Sorafenib-resistant HCC. However, further investigations are still required to validate the results.

Acknowledgements

This work was supported by the special foundation for the 1130 Project of Xinqiao Hospital of Third Military Medical University (2012).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Wenlei Zhuo or Dr. Bo Zhu, Institute of Cancer, Xinqiao Hospital, Third Military Medical University, Chongqing 400037, China. E-mail: zhuowenlei@tmmu.edu.cn (WLZ); zhubosyd@21cn.com (BZ)

References

- [1] Makarova AS and Lazarevich NL. Deregulation of signaling pathways involved in sorafenib resistance of hepatocellular carcinoma. *Klin Lab Diagn* 2013; 66-68, 34-67.
- [2] Page AJ, Cosgrove DC, Philosophe B and Pawlik TM. Hepatocellular carcinoma: diagnosis, management, and prognosis. *Surg Oncol Clin N Am* 2014; 23: 289-311.
- [3] Chen S, Wang Y, Ruan W, Wang X and Pan C. Reversing multidrug resistance in hepatocellular carcinoma cells by inhibiting extracel-

Sorafenib-resistance and hepatocellular cancer

- ular signal-regulated kinase/mitogen-activated protein kinase signaling pathway activity. *Oncol Lett* 2014; 8: 2333-2339.
- [4] Chen W, Wu J, Shi H, Wang Z, Zhang G, Cao Y, Jiang C and Ding Y. Hepatic stellate cell coculture enables sorafenib resistance in Huh7 cells through HGF/c-Met/Akt and Jak2/Stat3 pathways. *Biomed Res Int* 2014; 2014: 764981.
- [5] Fernando J, Malfettone A, Cepeda EB, Vilarasa-Blasi R, Bertran E, Raimondi G, Fabra A, Alvarez-Barrientos A, Fernandez-Salguero P, Fernandez-Rodriguez CM, Giannelli G, Sancho P and Fabregat I. A mesenchymal-like phenotype and expression of CD44 predict lack of apoptotic response to sorafenib in liver tumor cells. *Int J Cancer* 2015; 136: E161-72.
- [6] Li GM, Wang YG, Pan Q, Wang J, Fan JG and Sun C. RNAi screening with shRNAs against histone methylation-related genes reveals determinants of sorafenib sensitivity in hepatocellular carcinoma cells. *Int J Clin Exp Pathol* 2014; 7: 1085-1092.
- [7] Zhao D, Zhai B, He C, Tan G, Jiang X, Pan S, Dong X, Wei Z, Ma L, Qiao H, Jiang H and Sun X. Upregulation of HIF-2 α induced by sorafenib contributes to the resistance by activating the TGF- α /EGFR pathway in hepatocellular carcinoma cells. *Cell Signal* 2014; 26: 1030-1039.
- [8] Fischer TD, Wang JH, Vlada A, Kim JS and Behrens KE. Role of autophagy in differential sensitivity of hepatocarcinoma cells to sorafenib. *World J Hepatol* 2014; 6: 752-758.
- [9] Xia H, Ooi LL and Hui KM. MicroRNA-216a/217-induced epithelial-mesenchymal transition targets PTEN and SMAD7 to promote drug resistance and recurrence of liver cancer. *Hepatology* 2013; 58: 629-641.
- [10] Hashimoto N, Tsunedomi R, Yoshimura K, Watanabe Y, Hazama S and Oka M. Cancer stem-like sphere cells induced from de-differentiated hepatocellular carcinoma-derived cell lines possess the resistance to anti-cancer drugs. *BMC Cancer* 2014; 14: 722.
- [11] Ma L, Li G, Zhu H, Dong X, Zhao D, Jiang X, Li J, Qiao H, Ni S and Sun X. 2-Methoxyestradiol synergizes with sorafenib to suppress hepatocellular carcinoma by simultaneously dysregulating hypoxia-inducible factor-1 and -2. *Cancer Lett* 2014; 355: 96-105.
- [12] Zhang W, Liu S, Liu K, Ji B, Wang Y and Liu Y. Knockout of ADAM10 enhances sorafenib anti-tumor activity of hepatocellular carcinoma in vitro and in vivo. *Oncol Rep* 2014; 32: 1913-1922.
- [13] Zhai B, Hu F, Jiang X, Xu J, Zhao D, Liu B, Pan S, Dong X, Tan G, Wei Z, Qiao H, Jiang H and Sun X. Inhibition of Akt reverses the acquired resistance to sorafenib by switching protective autophagy to autophagic cell death in hepatocellular carcinoma. *Mol Cancer Ther* 2014; 13: 1589-1598.
- [14] van Zijl F, Mall S, Machat G, Pirker C, Zeillinger R, Weinhaeusel A, Bilban M, Berger W and Mikulits W. A human model of epithelial to mesenchymal transition to monitor drug efficacy in hepatocellular carcinoma progression. *Mol Cancer Ther* 2011; 10: 850-860.
- [15] Huang P, Cao K and Zhao H. Screening of critical genes in lung adenocarcinoma via network analysis of gene expression profile. *Pathol Oncol Res* 2014; 20: 853-858.
- [16] Isozaki Y, Hoshino I, Akutsu Y, Hanari N, Mori M, Nishimori T, Murakami K, Akanuma N, Toyozumi T, Takahashi M, Suito H, Takeshita N, Maruyama T, Suzuki A, Nakayama T and Matsubara H. Screening of Alternative Drugs to the Tumor Suppressor miR-375 in Esophageal Squamous Cell Carcinoma Using the Connectivity Map. *Oncology* 2014; 87: 351-363.
- [17] McClelland ML, Adler AS, Deming L, Cosino E, Lee L, Blackwood EM, Solon M, Tao J, Li L, Shames D, Jackson E, Forrest WF and Firestein R. Lactate dehydrogenase B is required for the growth of KRAS-dependent lung adenocarcinomas. *Clin Cancer Res* 2013; 19: 773-784.
- [18] McClelland ML, Adler AS, Shang Y, Hunsaker T, Truong T, Peterson D, Torres E, Li L, Haley B, Stephan JP, Belvin M, Hatzivassiliou G, Blackwood EM, Corson L, Evangelista M, Zha J and Firestein R. An integrated genomic screen identifies LDHB as an essential gene for triple-negative breast cancer. *Cancer Res* 2012; 72: 5812-5823.
- [19] Xu D, Xu H, Ren Y, Liu C, Wang X, Zhang H and Lu P. Cancer stem cell-related gene periostin: a novel prognostic marker for breast cancer. *PLoS One* 2012; 7: e46670.
- [20] Liu Y and Liu BA. Enhanced proliferation, invasion, and epithelial-mesenchymal transition of nicotine-promoted gastric cancer by periostin. *World J Gastroenterol* 2011; 17: 2674-2680.
- [21] Baril P, Gangeswaran R, Mahon PC, Caulee K, Kocher HM, Harada T, Zhu M, Kalthoff H, Crnogorac-Jurcevic T and Lemoine NR. Periostin promotes invasiveness and resistance of pancreatic cancer cells to hypoxia-induced cell death: role of the beta4 integrin and the PI3k pathway. *Oncogene* 2007; 26: 2082-2094.
- [22] Januchowski R, Zawierucha P, Rucinski M and Zabel M. Microarray-based detection and expression analysis of extracellular matrix proteins in drug-resistant ovarian cancer cell lines. *Oncol Rep* 2014; 32: 1981-1990.
- [23] Zi FM, He JS, Li Y, Wu C, Wu WJ, Yang Y, Wang LJ, He DH, Yang L, Zhao Y, Zheng GF, Han XY, Huang H, Yi Q and Cai Z. Fibroblast activation

Sorafenib-resistance and hepatocellular cancer

- protein protects bortezomib-induced apoptosis in multiple myeloma cells through beta-catenin signaling pathway. *Cancer Biol Ther* 2014; 15: 1413-1422.
- [24] Joyner DE, Trang SH, Aboulaia AJ, Damron TA and Randall RL. FAP-associated desmoid invasiveness correlates with in vitro resistance to doxorubicin. *Fam Cancer* 2009; 8: 569-580.
- [25] Nishimoto SK and Nishimoto M. Matrix gla protein binds to fibronectin and enhances cell attachment and spreading on fibronectin. *Int J Cell Biol* 2014; 2014: 807013.
- [26] Januchowski R, Zawierucha P, Rucinski M, Nowicki M and Zabel M. Extracellular matrix proteins expression profiling in chemoresistant variants of the A2780 ovarian cancer cell line. *Biomed Res Int* 2014; 2014: 365867.
- [27] Feng MX, Ma MZ, Fu Y, Li J, Wang T, Xue F, Zhang JJ, Qin WX, Gu JR, Zhang ZG and Xia Q. Elevated autocrine EDIL3 protects hepatocellular carcinoma from anoikis through RGD-mediated integrin activation. *Mol Cancer* 2014; 13: 226.
- [28] Wei JS, Johansson P, Chen L, Song YK, Tolman C, Li S, Hurd L, Patidar R, Wen X, Badgett TC, Cheuk AT, Marshall JC, Steeg PS, Vaque Diez JP, Yu Y, Gutkind JS and Khan J. Massively parallel sequencing reveals an accumulation of de novo mutations and an activating mutation of LPAR1 in a patient with metastatic neuroblastoma. *PLoS One* 2013; 8: e77731.
- [29] Lai KK, Shang S, Lohia N, Booth GC, Masse DJ, Fausto N, Campbell JS and Beretta L. Extracellular matrix dynamics in hepatocarcinogenesis: a comparative proteomics study of PDGFC transgenic and Pten null mouse models. *PLoS Genet* 2011; 7: e1002147.
- [30] Borriello L and DeClerck YA. Tumor microenvironment and therapeutic resistance process. *Med Sci (Paris)* 2014; 30: 445-451.
- [31] Nguyen TV, Sleiman M, Moriarty T, Herrick WG and Peyton SR. Sorafenib resistance and JNK signaling in carcinoma during extracellular matrix stiffening. *Biomaterials* 2014; 35: 5749-5759.
- [32] Eke I and Cordes N. Focal adhesion signaling and therapy resistance in cancer. *Semin Cancer Biol* 2014; 31C: 65-75.
- [33] Wang YJ, Zhang YK, Kathawala RJ and Chen ZS. Repositioning of Tyrosine Kinase Inhibitors as Antagonists of ATP-Binding Cassette Transporters in Anticancer Drug Resistance. *Cancers (Basel)* 2014; 6: 1925-1952.
- [34] Truman JP, Garcia-Barros M, Obeid LM and Hannun YA. Evolving concepts in cancer therapy through targeting sphingolipid metabolism. *Biochim Biophys Acta* 2014; 1841: 1174-1188.
- [35] Ramsay EE and Dilda PJ. Glutathione S-conjugates as prodrugs to target drug-resistant tumors. *Front Pharmacol* 2014; 5: 181.
- [36] Kesselring R, Thiel A, Pries R, Fichtner-Feigl S, Brunner S, Seidel P, Bruchhage KL and Woltenberg B. The complement receptors CD46, CD55 and CD59 are regulated by the tumour microenvironment of head and neck cancer to facilitate escape of complement attack. *Eur J Cancer* 2014; 50: 2152-2161.
- [37] Goyal J and Antonarakis ES. Clinical Evaluation of Abiraterone in the Treatment of Metastatic Prostate Cancer. *Clin Med Insights Urol* 2013; 2013: 1-14.
- [38] van den Bent MJ, Brandes AA, Taphoorn MJ, Kros JM, Kouwenhoven MC, Delattre JY, Bernsen HJ, Frenay M, Tijssen CC, Grisold W, Sipos L, Enting RH, French PJ, Dinjens WN, Vecht CJ, Allgeier A, Lacombe D, Gorlia T and Hoang-Xuan K. Adjuvant procarbazine, lomustine, and vincristine chemotherapy in newly diagnosed anaplastic oligodendroglioma: long-term follow-up of EORTC brain tumor group study 26951. *J Clin Oncol* 2013; 31: 344-350.
- [39] Hillmen P, Gribben JG, Follows GA, Milligan D, Sayala HA, Moreton P, Oscier DG, Dearden CE, Kennedy DB, Pettitt AR, Nathwani A, Varghese A, Cohen D, Rawstron A, Oertel S and Pocock CF. Rituximab plus chlorambucil as first-line treatment for chronic lymphocytic leukemia: Final analysis of an open-label phase II study. *J Clin Oncol* 2014; 32: 1236-1241.
- [40] Brain KL and Cunnane TC. Breylium abolishes neurotransmitter release without necessarily abolishing the nerve terminal action potential in sympathetic terminals. *Br J Pharmacol* 2008; 153: 831-839.
- [41] Spence MM, Shin PJ, Lee EA and Gibbs NE. Risk of injury associated with skeletal muscle relaxant use in older adults. *Ann Pharmacother* 2013; 47: 993-998.