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Research article

Bioinformatics analysis revealed hub genes and pathways involved in sorafenib resistance in hepatocellular carcinoma

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Abstract: Hepatocellular carcinoma (HCC) is increasingly known as a serious, worldwide public health concern. Sorafenib resistance is the main challenge faced by many advanced HCC patients. The specific mechanisms of sorafenib resistance remind unclear. In the current study, GEO2R was conducted to identify differentially expressed genes (DEGs) between sorafenib-resistant samples and the control group by using RNA-sequence analysis and analyzing dataset GSE109211. Next, protein-protein interaction (PPI) network was built to explore key targets proteins in sorafenib-resistant HCC. Furthermore, gene ontology (GO) analysis was used to research the underlying roles of key proteins. Moreover, the Kaplan-Meier survival analysis was performed to display the effect of key proteins on overall survival in HCC. Western blotting was performed to detected resistance-related proteins and CCK-8 assay was employed to measured cell viability. In the present research, 164 sorafenib resistance-related DEGs in HCC were identified by using RNA-sequence analysis and analyzing the dataset GSE109211. GO analysis revealed DEGs were involved in regulating multiple biological processes and molecular functions. DYNLL2, H2AFJ, SHANK2, ZWILCH, CDC14A, IFT20, MTA3, SERPINA1 and TCF4 were confirmed as key genes in this process. Moreover, our study showed Akt signaling was aberrantly activated and inhibition of Akt signaling enhanced anti-tumor capacity of sorafenib in sorafenib-resistant HCC cells. Identification of the DEGs in sorafenib resistant HCC cells may further provide the new insights of underlying sorafenib-resistant mechanisms and offer latent targets for early diagnosis and new therapies to improve clinical efficacy for sorafenib-resistant HCC patients.

Keywords: biomarkers; sorafenib resistance; gene expression profiling; prognosis; Akt signaling

1. Introduction

Hepatocellular carcinoma (HCC) is the third most common malignancy in mortality [1], surgical therapy and liver transplantation were mostly used for early-stage HCC treatment [2, 3]. And most patients would have missed the best treatment period when they were diagnosed with advanced stage HCC [4]. Sorafenib, one of the first‐line targeted therapy agents for advanced HCC, is a multi‐kinases inhibitor including platelet-derived growth factor receptor (PDGFR)-β, RET/PTC, FLT-3, KIT, RET, vascular endothelial growth factor receptors (VEGFRs) and downstream intracellular serine/threonine kinases in the MAPK cascade [5–7]. Sorafenib inhibited HCC progression by suppressing cell growth and angiogenesis [6]. However, one of clinical challenges for HCC treatment is that most advanced patients would acquire resistance during treatment with sorafenib [8]. Therefore, understanding the mechanisms underlying sorafenib resistance were of great significance for searching for novel therapeutic strategies for sorafenib-resistant HCC patients.

Previous studies had investigated that a few signaling pathways participated in regulating sorafenib resistance in HCC, such as EGFR signaling, PI3K/Akt pathway, Autophagy and Epithelial-mesenchymal transition (EMT) [9–12]. PI3K/Akt signaling pathway has a pivotal impact on modulating sorafenib resistance in HCC [10]. Morgensztern et al [13] found silencing of Akt could enhance the sensitivity of HCC cells to sorafenib-induced apoptosis. p21, a potent cyclin-dependent kinase inhibitor, can arrest cell-cycle by interacting with different stimuli such as CDK, p53, PCNA, E2F1, K1F, STAT3 AP4, CDX2, MYC, DNA repair process, proteasomes and ER-α [14]. Another study indicated that p-STAT3 up-regulation could be responsible for sorafenib resistance [15]. In agree with above study, our results also indicated that the expression levels of p-Akt, p-STAT3, cyclinD1 and c-Myc were remarkably increased in sorafenib-resistant HCC cells compared with sensitive HCC cells.

In this study, we conducted RNA-sequence analysis as well as bioinformatics analysis to comprehensively identify DEGs between sorafenib-resistant SK-Hep1-SR cells and sensitive SK-Hep1 cells. PPI analysis was conducted to identify key genes related to this process in HCC. At the same time, the expression levels of sorafenib resistance-related DEGs were evaluated between normal sample tissues and liver cancer samples by using TCGA database. A Kaplan-Meier analysis was used to assess the prognostic value of sorafenib resistance-related genes in HCC. We also evaluated whether Akt signaling inhibition could enhance the sensitivity of sorafenib to sorafenib-resistant HCC cells. These findings from this study may make contributions that provided potential biomarkers and targets to improve clinical efficacy for sorafenib-resistant HCC patients.

2. Material and methods

2.1. Microarray data

GSE109211 dataset was used to identify sorafenib resistance-related DEGs in HCC. GSE109211 dataset contained 67 samples treated with sorafenib and 73 samples treated with Placebo totally.

2.2. DEG analysis

GEO2R (http://www.ncbi.nlm.nih.gov/geo/geo2r/) was conducted to identify DEGs between sorafenib group and the placebo group by utilizing GSE109211 dataset. The cut-off criteria of a

statistically significant difference was defined as |log2FC| ≥ 1.5 and P < 0.01. A heat map was constructed by the GEO2R.

2.3. Functional and pathway enrichment analysis

GO enrichment analysis of the selected DEGs was conducted to use the Database of Annotation Visualization and Integrated Discovery (DAVID; http://david.abcc.ncifcrf.gov/). DAVID database contains three category analysis including molecular function, biological processes and cellular compounds [16]. P value < 0.05 was selected to represent a statistical significance.

2.4. PPI network analysis

The Search Tool for the Retrieval of Interacting Genes (STRING; http://string-db.org/) was utilized to construct PPI network analysis of the selected DEGs. STRING database gives the proven interactions of the identified DEGs. The information of interacted DEGs (the combined score > 0.4) was download from STRING and then Cystoscope (http://www.cytoscape.org/) was performed to build the PPI network [17]. The criteria were number of nodes > 4 and MCODE score > 3.

2.5. TCGA Data processing

mRNA HTSeq-Counts data of Liver hepatocellular carcinoma (LIHC) was obtained from The Cancer Genome Atlas (TCGA) database (https://cancergenome.nih.gov/). LIHC dataset contains 371 liver cancer samples (361 Hepatocellular Carcinoma, 7 Hepatocholangiocarcinoma and 3 Fibrolamellar Carcinoma) and 50 normal samples. The Variance Stabilizing Transformation (VST) function of the R package DESeq2 was used to normalize the HTSeq-Counts expression data. The R package ggplot2 was performed to draw the boxplots between LIHC normal samples and tumor samples, and t-test was conducted to assess the statistical significance. P values < 0.05 represent significantly difference.

2.6. Survival analysis

The web tool Kaplan-Meier plotter (KM plotter, http://kmplot.com/analysis/index.php?p=service&cancer=liver_rnaseq) was used to predict the genes on overall survival in HCC [18]. According to the median expression of the interesting genes, patients were separated to two groups and statistically conducted the survival rate the hazard ratio (HR) with 95% confidence intervals. The survival analysis plot displayed the log rank P value appropriately.

2.7. RNA-sequence analysis

The RNA sequence analysis was conducted by building RNA-seq library through the specifications of Illumina preparation Kit (San Diego, CA). TBS380 was chose to maintain the quality of RNA-seq library and the Illumina HiSeq 4000 was utilized to sequence (2 × 150bp read length). The raw data were analyzed and DEGs were identified on Majorbio I-Sanger Cloud Platform (www.i-sanger.com), which is a free online platform.

2.8. Cell culture and reagents

SK-Hep1 cells were purchased from the cell bank of Chinese Academy of Science (Shanghai, China), which were cultured in DMEM (Hyclone) supplemented with ten percent of FBS (Gibco) and maintained in 5% CO_2 at 37 ℃. Sorafenib resistant cell line SK-Hep1-SR was induced by concentration gradient and successfully obtained after 8 months.

2.9. Cell proliferation assay

Cell counting kit-8 (CCK-8) was purchased from Beyotime (China). Sorafenib tosylate was obtained from MedChemExpress (USA). MK2206 was acquired from Selleck Chemicals Biotechnology (USA). Absorbance was measured at 450 nm with microplate reader (Tecan, Switzerland).

2.10.Western blotting analysis

Cells were lysed in RIPA buffer (Sigma) added with protease inhibitors (Sigma) and PMSF (Sigma). Cells lysates were subjected to western blot analysis by utilizing a 10% or 15% acrylamide gel. The primary antibodies against Akt (#4691), Phospho-Akt (Ser-473, #4060), c-Myc (#13987), Phospho-STAT3(Tyr-705, #9145), p21(#2947) were from Cell Signaling Technology (USA). The antibody against STAT3 (ab68153) was from Abcam (UK). The antibodies against β-actin (sc-47778), cyclinD1 (sc-753) were from Santa Cruz Biotechnology (USA).

2.11.Statistical analysis

Statistical comparisons between two groups of data were conducted by utilizing t-test according to the test condition. Mann-Whitney U test was used for the non-parametric cohort data. P value < 0.05 was selected as statistical significance with a 95% confidencial level.

3. Results

3.1. Identification of sorafenib resistance-related DEGs in HCC

GSE109211 dataset was used to confirm sorafenib resistance-related DEGs in HCC which contained 67 samples treated with sorafenib (Sor) and 73 samples treated with placebo (Plac). 404 genes were up-regulated and 167 genes were down-regulated in sorafenib treated HCC samples compared to placebo group. Hierarchical clustering of the DEGs was shown in Figure 1A.

Parental SK-Hep1 cells and sorafenib-resistant SK-Hep1-SR cells were treated with 16 μM sorafenib, their cell viability was 16.7% and 75.7%, respectively. The finding demonstrated that SK-Hep1-SR was less sensitive to sorafenib. This result implied that the sorafenib-resistant cell line SK-Hep1 was successfully constructed (Figure S1). RNA-sequence analysis was conducted to identify DEGs between parental SK-Hep1 and sorafenib-resistant SK-Hep1-SR cell lines. As shown in Figure 1B, 1629 genes were found to be dysregulated in SK-Hep1-SR cells compared to SK-Hep1 cells, including 886 up-regulated and 743 down-regulated genes. Hierarchical clustering of the DEGs was shown in Figure 1B. By combing these analyses together, we identified 164 sorafenib resistance-related DEGs in HCC including 121 up-regulated (Figure 1C) and 43 down-regulated (Figure 1D) sorafenib resistance-related DEGs.

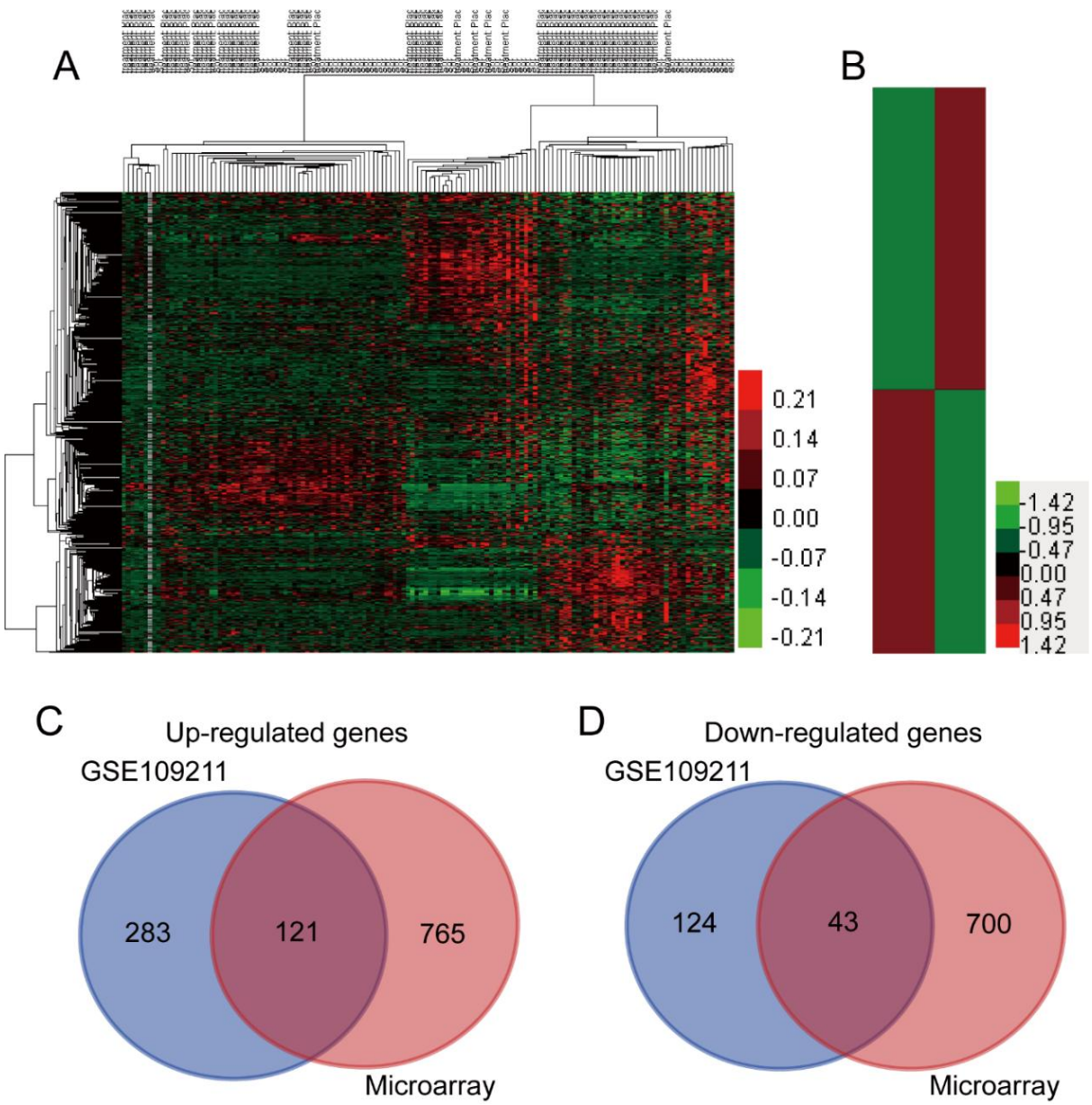

Figure 1. Identification of sorafenib resistance-related DEGs in HCC. (A) The heatmap shows that the DEGs between 67 samples incubated with Sorafenib and 73 are Placebo by using public GSE109211 dataset. (B) The heatmap shows that the DEGs between SK-Hep1 cells and SK-Hep1-SR cells. (C-D) The Venn diagram describes the dysregulated genes between public GSE109211 dataset and our mRNA microarray data.

3.2. Bioinformatics analysis of sorafenib resistance-related DEGs in HCC

We next performed bioinformatics analysis for these DEGs by using DAVID system. These results displayed that DEGs were associated with cilium assembly, ER to Golgi vesicle-mediated transport, mitotic nuclear division, adult behavior, cilium morphogenesis, cell division, maintenance of organ identity, regulation of mitotic cell cycle, platelet aggregation, regulation of cAMP metabolic process, spermatogenesis, positive regulation of I-κB kinase/ NF-κB signaling, intraciliary transport involved in cilium morphogenesis, sister chromatid cohesion (Figure 2A). Moreover, we also found

sorafenib resistance-related DEGs in HCC were involved in regulating multiple molecular functions, including protein binding, metal ion binding, protein homodimerization activity, ion channel binding, identical protein binding, cadherin binding involved in cell-cell adhesion, GKAP/Homer scaffold activity, histone deacetylase activity (Figure 2B).

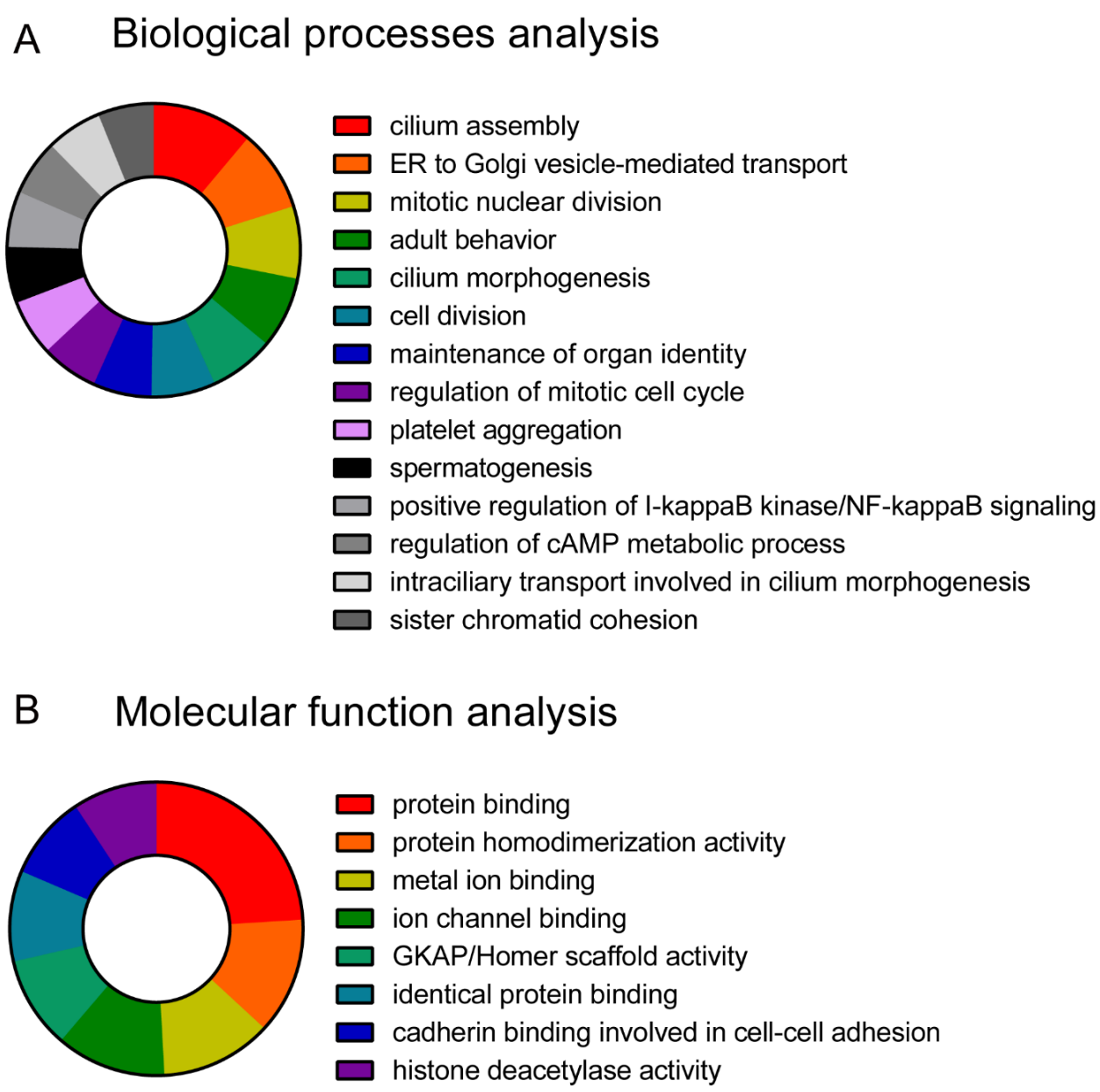

Figure 2. Bioinformatics analysis of sorafenib resistance-related DEGs in HCC. (A) The pie chart shows the biological processes analysis of dysregulated genes. (B) The pie chart shows the molecular functions analysis of dysregulated genes.

3.3. PPI network analysis identifies key genes involved in sorafenib resistance-related DEGs in HCC

PPI network was constructed to understand the relationship among DEGs in HCC using STRING database. As shown in Figure 3, this network included 107 nodes and 134 edges. DEGs with degrees > 4 were identified as key genes in this network. Key genes included DYNLL2 (degree

= 10), H2AFJ (degree = 6), SHANK2 (degree = 5), ZWILCH (degree = 5), CDC14A (degree = 4), IFT20 (degree = 4), MTA3 (degree = 4), SERPINA1 (degree = 4), and TCF4 (degree = 4).

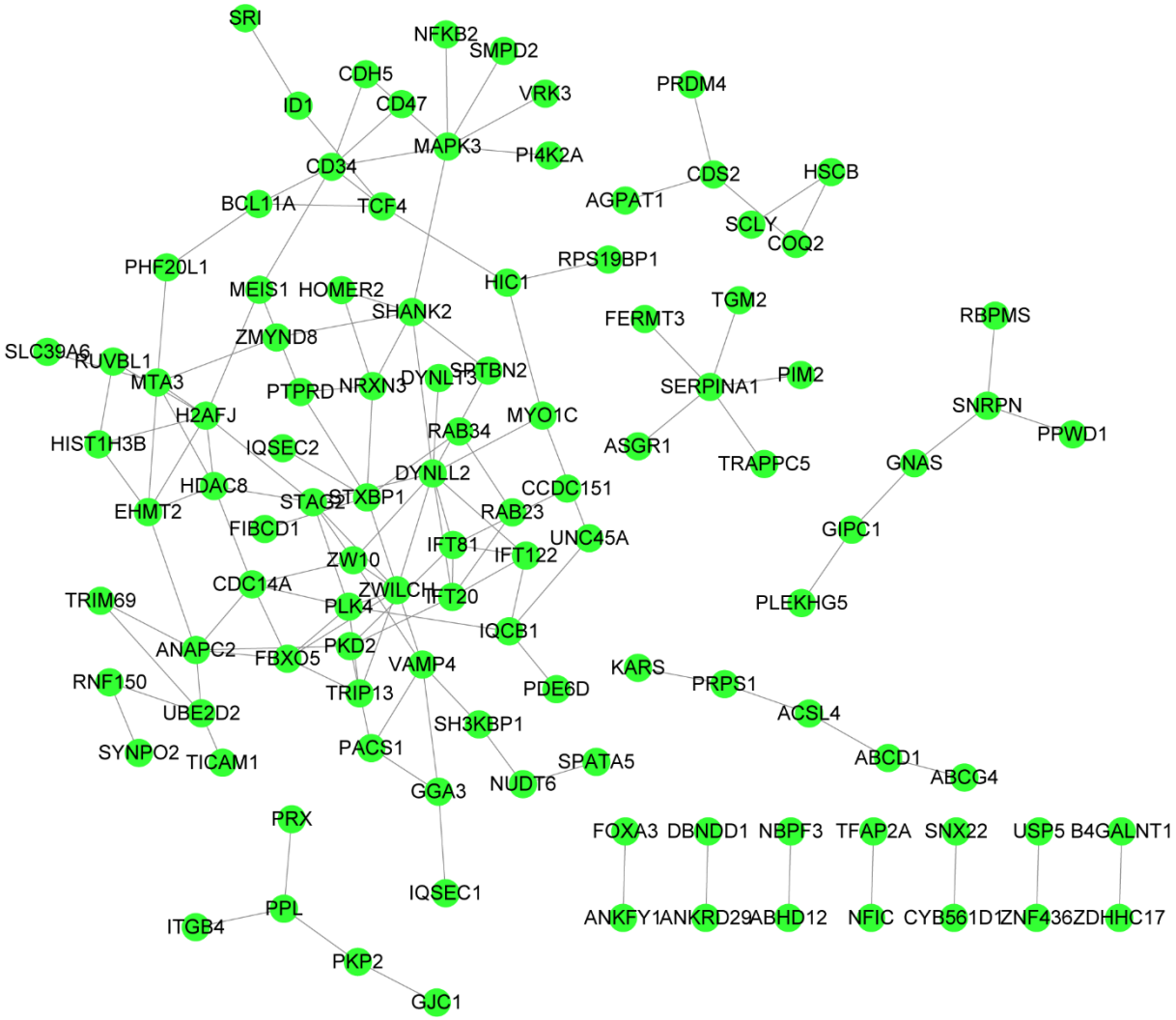

Figure 3. PPI network analysis identifies key genes involved in sorafenib resistance-related DEGs in HCC. Analysis of the interaction of dysregulated proteins via PPI network. The green nods represent the proteins in PPI network.

3.4. Sorafenib resistance-related genes are dysregulated in HCC samples

We also evaluated the expression levels of sorafenib resistance-related DEGs between normal samples and hepatocellular carcinoma by analyzing TCGA dataset. As shown in Figure 4, our results showed H2AFJ, ANAPC2, MTA3, HDAC8, ZWILCH, PLK4, IQCB1, GGA3, CD34, and EHMT2 were significantly overexpressed in HCC samples, however, CDH5, PPL, DYNLL2 and SERPINA1 were markedly decreased in HCC samples compared with normal samples. These data further indicated that sorafenib resistance-related DEGs were involved in the development of HCC.

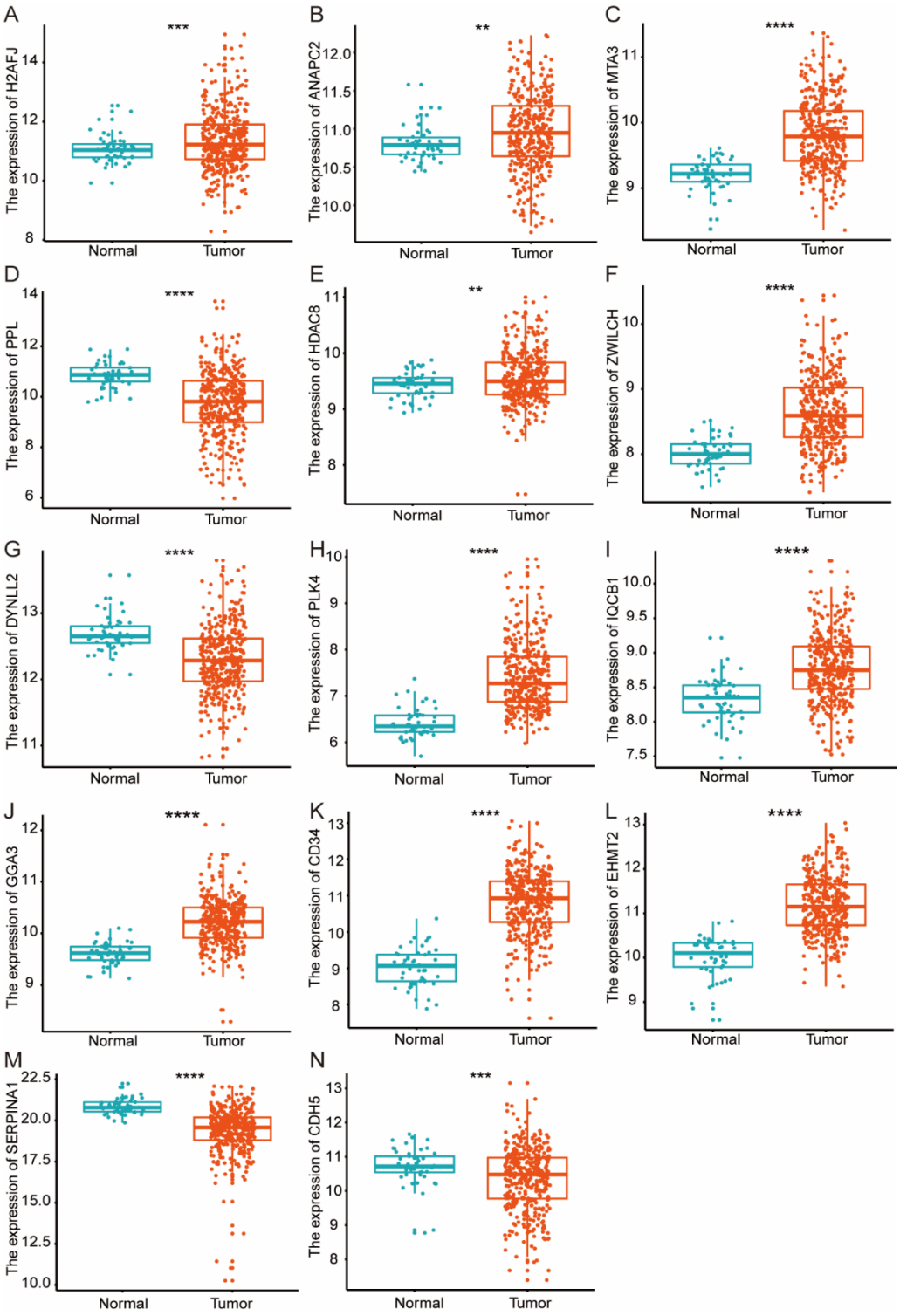

Figure 4. Sorafenib resistance-related genes are dysregulated in HCC samples. (A-M) H2AFJ (A), ANAPC2 (B), MTA3 (C), HDAC8 (E), ZWILCH (F), PLK4 (H), IQCB1 (I), GGA3 (J), CD34 (K), EHMT2 (L) are up-regulated while PPL (D), DYNLL2 (G), SEROINA1 (M), CDH5 (N) are down-regulated in HCC tissues by using TCGA dataset. $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***), $p < 0.0001$ (****).

3.5. Sorafenib resistance-related genes are correlated to the overall survival time

To obtain the prognostic value of sorafenib resistance-related genes in HCC, we conducted Kaplan-Meier analysis. The median expression of candidates in all HCC samples were selected as cutoff to divide HCC samples as high- and low- groups. As shown in Figure 5, we observed higher expression of EHMT2, GGA3, IQCB1 and PLK4 were correlated to shorter overall survival time in HCC patients. However, HCC patients with higher expression of CDH5, ANAPC2 and CD34 had longer overall survival time than HCC patients with lower CDH5, ANAPC2 and CD34 expression (Figure 5).

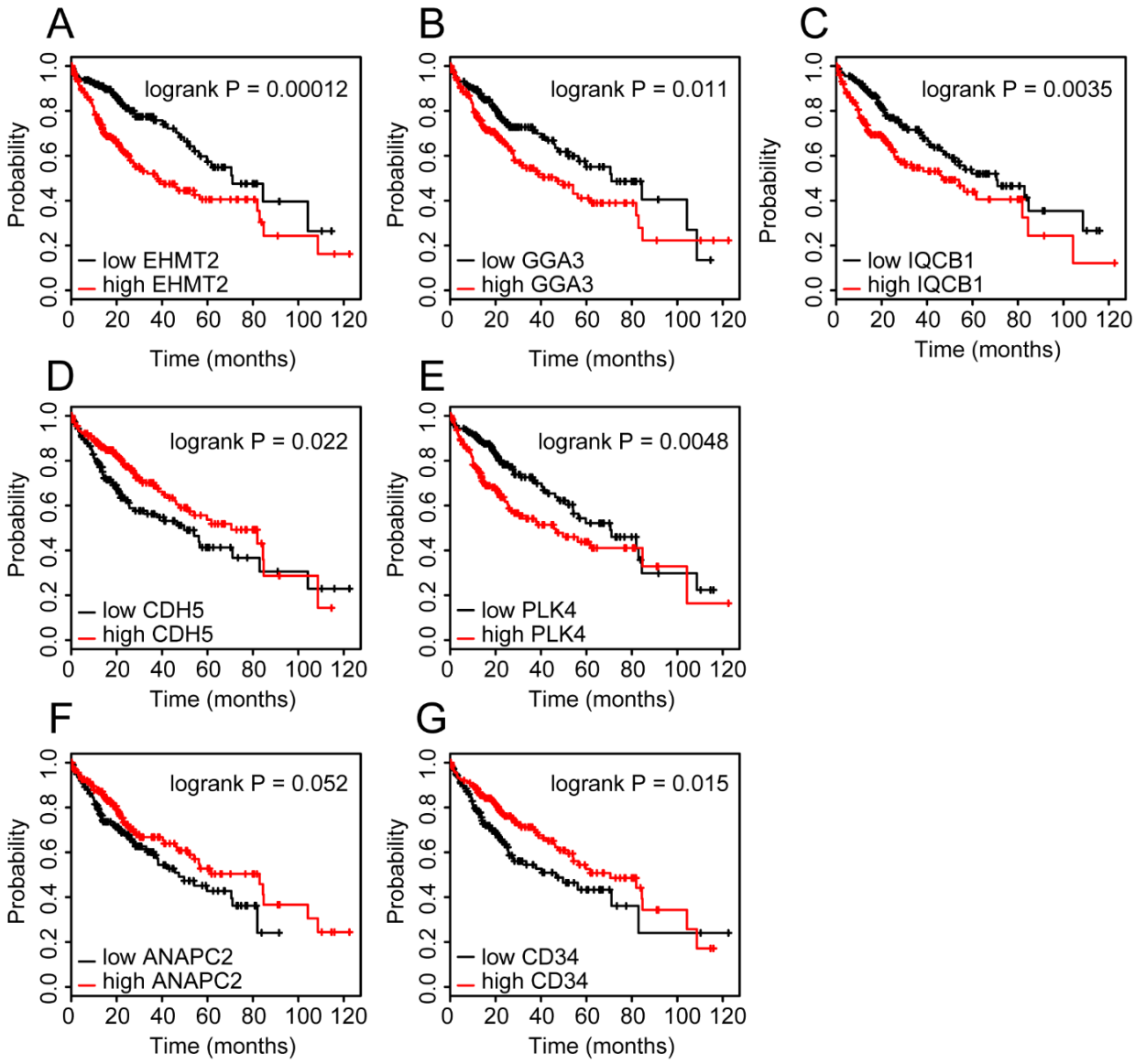

Figure 5. Sorafenib resistance-related genes are correlated to the overall survival time. (A-G) The Kaplan–Meier curve analysis displays higher expression levels of EHMT2 (A), GGA3 (B), IQCB1 (C), PLK4 (E) are significantly with shorter overall survival time and higher expression levels of CDH5 (D), ANAPC2 (F), CD34 (G) are significantly associated with longer survival time in HCC.

3.6. Akt signaling is activated in sorafenib-resistant HCC cells

Furthermore, we detected the levels of several important signaling regulators in sorafenib-resistant HCC cells, including Akt, STAT3, cyclinD1, p21 and c-Myc (Figure 6A). The results showed the phosphorylation levels of Akt and STAT3, c-Myc and cyclinD1 were markedly increased while the expression levels of p21 was decreased in SK-Hep1-SR cells were in comparison with parental SK-Hep1 cells (Figure 6B). Moreover, we indicated that sorafenib could activate the Akt phosphorylation levels in SK-Hep1-SR (Figure 6C-D). Taken together, these results showed that Akt signaling could participate in sorafenib resistance.

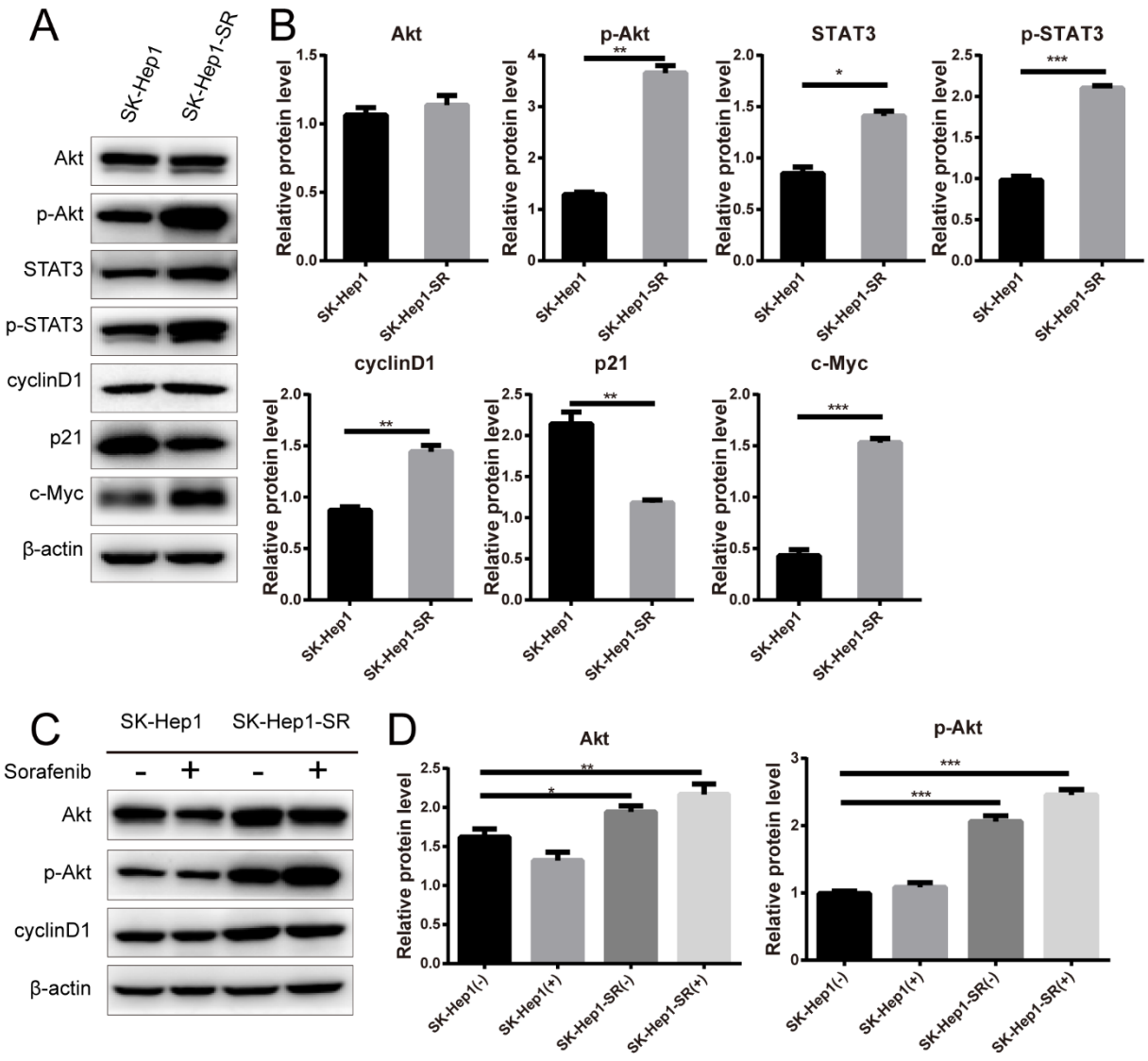

Figure 6. Akt signaling is activated in sorafenib-resistant SK-Hep1-SR cells. (A) SK-Hep1 and SK-Hep1-SR cells were grown to 80% confluence and gathered for western blotting analysis. (B)The relative protein levels were determined by Image J software. (C) SK-Hep1 and SK-Hep1-SR cells were treated with the absence or presence of sorafenib (5 μM) for 48 h and harvested cells which were subjected to immunoblotting. (D) The relative protein levels were determined by Image J software. Data represented three independent experiments. $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***).

3.7. Inhibition of Akt signaling enhances anti-tumor activity of SK-Hep1-SR cells

MK2206, an effective inhibitor of Akt signaling [19], was tested in our study. As shown in Figure 7A, the phosphorylation levels of Akt in SK-Hep1-SR cells were obviously inhibited in a concentration-dependent manner. Next, we determined whether inhibition of Akt signaling was involved in regulating sorafenib-resistance by using MK2206. As expected, inhibition of Akt signaling could enhance anti-tumor activity of sorafenib as MK2206 concentration increased in sorafenib-resistant SK-Hep1-SR cells (Figure 7B). Meanwhile, when treated with various concentration of sorafenib plus MK2206 (8 μM), the cell viability of SK-Hep1-SR was obviously suppressed (Figure 7C). Overall, these results indicated that inhibition of Akt signaling strengthened sensitivity of sorafenib in drug-resistant SK-Hep1-SR cells.

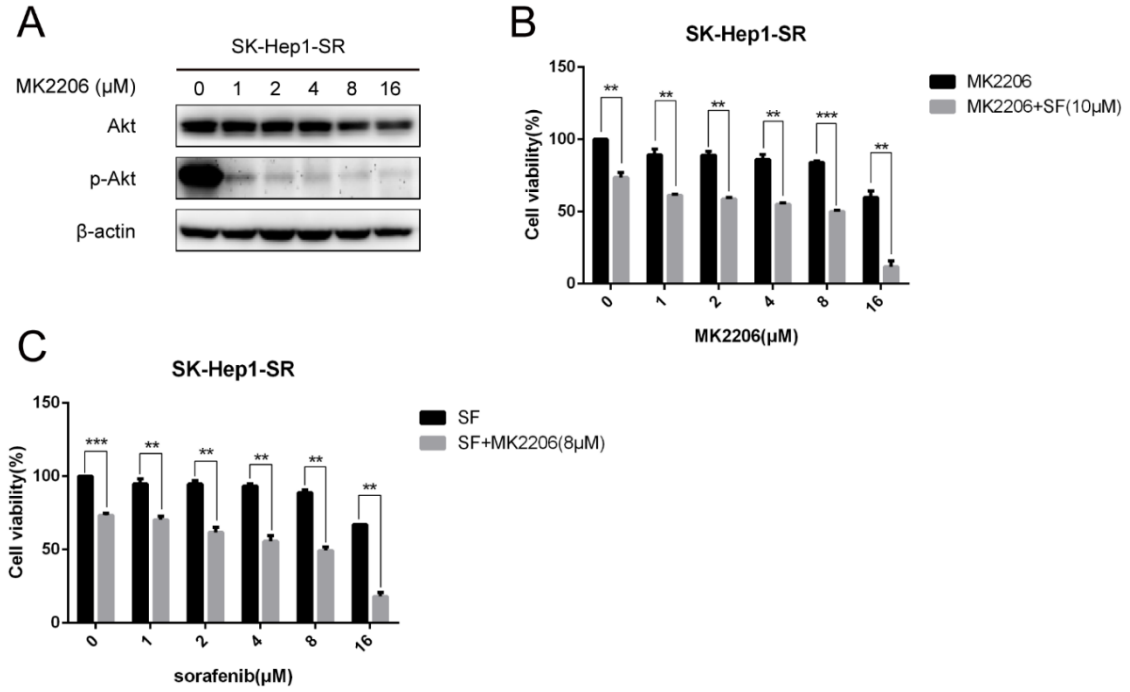

Figure 7. Inhibition of Akt signaling enhances anti-tumor activity of sorafenib in SK-Hep1-SR cells. (A) SK-Hep1-SR cells were treated with the concentration gradient of MK2206 (0, 1, 2, 4, 8, 16 μM) and subjected to immunoblotting. (B) SK-Hep1-SR cells were cultured for 48 h with various concentrations of MK2206 in the presence or absence of sorafenib (10 μM). (C) SK-Hep1-SR cells were incubated with various concentrations of sorafenib in the presence or absence of MK2206 (8 μM). CCK8 assay was used to test cell viability. Data represented three independent experiments. $P < 0.05$ (*), $P < 0.01$ (**), $P < 0.001$ (***).

4. Discussion

HCC has always been one of the knotty problems that plague human health. Because of its insidious onset, many patients are diagnosed at an advanced stage [20]. However, sorafenib resistance is the one of the main obstacles for advanced HCC patients. Many researchers have made efforts in exploring sorafenib resistance in HCC, but the underlying mechanism is still

unclear. There is an urgent need to clarify the mechanism of sorafenib resistance and address the sorafenib-resistant problems.

In this work, we identified 164 sorafenib resistance-related DEGs in HCC by using RNA-sequence analysis and analyzing GSE109211 dataset. Bioinformatics analysis revealed DEGs were involved in regulating multiple biological processes and molecular functions, such as mitotic nuclear division and mitotic cell cycle, which were consistent with previous studies. For example, Huang et al. [21] also found cell cycle and DNA replication were involved in sorafenib-resistant HCC.

Furthermore, PPI network construction was used to identify hub genes in sorafenib-resistant HCC. The hub genes included DYNLL2, H2AFJ, SHANK2, ZWILCH, CDC14A, IFT20, MTA3, SERPINA1, and TCF4. H2AFJ was reported to be related to chemoradiation resistance in colorectal cancer [22]. SHANK2 was found to be involved in liver metastasis of colorectal cancer [23]. ZWILCH played vital roles in composing the mitotic checkpoint, which prevented cells from prematurely exiting mitosis [24]. CDC14A was a key cell cycle regulator [25]. The downstream targets of CDC14A included p53 and CDC25 [26]. Sacristán [27] reported CDC14A regulated G2/M transition by suppressing CDC25 activity and Paulsen [28] reported CDC14A promoted carcinogenesis by binding and dephosphorylating p53. IFT20 was reported to modulate PDGFRα signaling by enhancing the stability of E3 ubiquitin ligases, c-Cbl and Cbl-b [29]. MTA3 was a metastasis regulator [30]. In HCC, MTA3 was up-regulated and correlated with the poor prognosis. SERPINA1 was found to be dysregulated multiple cancers, including lung cancer [31], thyroid cancer [32], breast cancer [33]. SERPINA1 promoted tumor progression in colorectal cancer [34]. TCF4 was a key downstream TF of Wnt signaling [35]. TCF4 was dysregulated in rectal, esophageal, and colorectal Cancer [36–38]. These reports together with our finding provided useful information to understand the mechanisms underlying sorafenib-resistance in HCC.

The most widely used biomarker for the HCC is Alpha-fetoprotein (AFP) [39]. However, the sensitivity and specificity of AFP is low. There was an urgent need to obtain novel biomarkers for HCC. Recently, a series of novel biomarkers were reported in HCC, including CKAP4 [40], Laminin-γ2 [41], miR-20 [42], HMGB3 [43] and KIAA1199 [44]. In this study, we also found that sorafenib resistance-related genes were dysregulated and correlated to the prognosis in the patients with HCC. Our results showed higher expression of EHMT2, GGA3, IQCB1 and PLK4, and lower expression of CDH5, ANAPC2 and CD34 were correlated to shorten overall survival time in HCC patients. Our research may provide novel biomarkers and targets for the diagnosis and treatment of HCC. Interestingly, we found that ANAPC2 and CD34 were overexpressed in HCC, however, high expression of ANAPC2 and CD34 were associated with shorter survival time in HCC patients. These results indicated that ANAPC2 and CD34 might play a critical role in HCC progression. The role of these DEGs and the specific mechanisms involved in sorafenib resistance are required to further study.

Recent researches have displayed that PI3K/Akt and JAK/STAT signaling pathway participated in regulating sorafenib resistance in HCC. Data from several studies suggested that the suppression of Akt by MK-2206, GDC0068 or LY294002 could overcome sorafenib resistance in HCC [11,45,46]. In this study, we found a few genes involved in modulating AKT signaling, such as PLK4. PLK4 activated PI3K/Akt signaling to regulate epithelial-mesenchymal transition [47]. In addition, we found phosphorylation levels of Akt and STAT3 were remarkably overexpressed in SK-Hep1-SR cells were in comparison with SK-Hep1 cells. Sorafenib could induce the phosphorylation levels of

Akt in SK-Hep1-SR cells, which suggested Akt signaling was markedly activated in sorafenib-resistant HCC cells. Moreover, we discovered inhibition of Akt signaling enhanced anti-tumor compacity of sorafenib in sorafenib-resistant HCC cells. The above results suggested targeting Akt signaling could provide a feasible strategy for the cure of sorafenib-resistant HCC patients. However, the specific molecular mechanisms associated with sorafenib resistance need to further study in detail.

5. Conclusion

In conclusion, we identified 164 sorafenib resistance-related DEGs in HCC by using RNA-sequence analysis and analyzing GSE109211 dataset. Bioinformatics analysis revealed DEGs were involved in regulating multiple biological processes and molecular functions. DYNLL2, H2AFJ, SHANK2, ZWILCH, CDC14A, IFT20, MTA3, SERPINA1, and TCF4 were identified as key genes in this process. We found sorafenib resistance-related genes were dysregulated and correlated to the prognosis in the patients with HCC. Moreover, our study showed Akt signaling was activated and inhibition of Akt signaling enhanced anti-tumor compacity of sorafenib in sorafenib-resistant HCC cells. This present study would provide candidate biomarkers and novel therapeutic methods for sorafenib-resistant HCC patients.

Acknowledgements

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Conflict of interest

The authors declare that they have no conflict of interest.

References

1. L. A. Torre, F. Bray, R. L. Siegel, et al., Global cancer statistics, 2012, *CA. Cancer J. Clin.*, **65**(2015), 87–108.
2. R. Romagnoli, V. Mazzaferro and J. Bruix, Surgical resection for hepatocellular carcinoma: Moving from what can be done to what is worth doing, *Hepatology*, **62** (2015), 340–342.
3. N. F. Esnaola, G. Y. Lauwers, N. Q. Mirza, et al., Predictors of microvascular invasion in patients with hepatocellular carcinoma who are candidates for orthotopic liver transplantation, *J. Gastrointest. Surg.*, **6** (2002), 224–232; discussion 232.
4. S. D'Angelo, M. Secondulfo, R. De Cristofano, et al., Selection and management of hepatocellular carcinoma patients with sorafenib: recommendations and opinions from an Italian liver unit, *Future Oncol.*, **9** (2013), 485–491.
5. L. Adnane, P. A. Trail, I. Taylor, et al., Sorafenib (BAY 43-9006, Nexavar), a dual-action inhibitor that targets RAF/MEK/ERK pathway in tumor cells and tyrosine kinases VEGFR/PDGFR in tumor vasculature, *Meth. Enzymol.*, **407** (2006), 597–612.
6. S. Wilhelm, C. Carter, M. Lynch, et al., Discovery and development of sorafenib: a multikinase

inhibitor for treating cancer, *Nat. Rev. Drug. Discov.*, **5** (2006), 835–844.
7. S. M. Wilhelm, C. Carter, L. Tang, et al., BAY 43-9006 exhibits broad spectrum oral antitumor activity and targets the RAF/MEK/ERK pathway and receptor tyrosine kinases involved in tumor progression and angiogenesis, *Cancer Res.*, **64** (2004), 7099–7109.
8. O. Waidmann and J. Trojan, Novel drugs in clinical development for hepatocellular carcinoma, *Expert Opin. Investig. Drug.*, **24** (2015), 1075–1082.
9. M. J. Blivet-Van Eggelpoel, H. Chettouh, L. Fartoux, et al., Epidermal growth factor receptor and HER-3 restrict cell response to sorafenib in hepatocellular carcinoma cells, *J. Hepatol.*, **57** (2012), 108–115.
10. K. F. Chen, H. L. Chen, W. T. Tai, et al., Activation of phosphatidylinositol 3-kinase/Akt signaling pathway mediates acquired resistance to sorafenib in hepatocellular carcinoma cells, *J. Pharmacol. Exp. Ther.*, **337** (2011), 155–161.
11. B. Zhai, F. Hu, X. Jiang, et al., Inhibition of Akt reverses the acquired resistance to sorafenib by switching protective autophagy to autophagic cell death in hepatocellular carcinoma, *Mol. Cancer Ther.*, **13** (2014), 1589–1598.
12. A. K. Chow, L. Ng, C. S. Lam, et al., The Enhanced metastatic potential of hepatocellular carcinoma (HCC) cells with sorafenib resistance, *PLoS One*, **8** (2013), e78675.
13. D. Morgenszternand H. L. McLeod, PI3K/Akt/mTOR pathway as a target for cancer therapy, *Anticancer Drugs*, **16** (2005), 797–803.
14. A. Parveen, M. S. Akash, K. Rehman, et al., Dual Role of p21 in the Progression of Cancer and Its Treatment, *Crit. Rev. Eukaryot. Gene Expr.*, **26** (2016), 49–62.
15. J. C. Su, P. H. Tseng, S. H. Wu, et al., SC-2001 overcomes STAT3-mediated sorafenib resistance through RFX-1/SHP-1 activation in hepatocellular carcinoma, *Neoplasia*, **16** (2014), 595–605.
16. Z. Xu, Y. Zhou, Y. Cao, et al., Identification of candidate biomarkers and analysis of prognostic values in ovarian cancer by integrated bioinformatics analysis, *Med. Oncol.*, **33** (2016), 130.
17. Y. Guo, Y. Bao, M. Ma, et al., Identification of key candidate genes and pathways in colorectal cancer by integrated bioinformatical analysis, *Int. J. Mol. Sci.*, **18** (2017).
18. B. Győrffy, P. Surowiak, J. Budczies, et al., Online survival analysis software to assess the prognostic value of biomarkers using transcriptomic data in non-small-cell lung cancer, *PloS One.*, **8** (2013), e82241.
19. C. Stottrup, T. Tsang and Y. R. Chin, Upregulation of AKT3 confers resistance to the AKT inhibitor MK2206 in breast cancer, *Mol. Cancer Ther.*, **15** (2016), 1964–1974.
20. A. Forner, J. M. Llovet and J. Bruix, Hepatocellular carcinoma, *Lancet*, **379** (2012), 1245–1255.
21. D. Huang, W. Yuan, H. Li, et al., Identification of key pathways and biomarkers in sorafenib-resistant hepatocellular carcinoma using bioinformatics analysis, *Exp. Ther. Med.*, **16** (2018), 1850–1858.
22. X. Wang, W. M. Ghareeb, X. Lu, et al., Coexpression network analysis linked H2AFJ to chemoradiation resistance in colorectal cancer, *J. Cell. Biochem.*, (2018).
23. K. Kohno, M. Chiba, S. Murata, et al., Identification of natural antisense transcripts involved in human colorectal cancer development, *Int. J. Oncol.*, **37** (2010), 1425–1432.
24. R. Karess, Rod-Zw10-Zwilch: A key player in the spindle checkpoint, *Trends Cell Biol.*, **15** (2005), 386–392.
25. Y. Yu, Z. Kovacevicand D. R. Richardson, Tuning cell cycle regulation with an iron key, *Cell Cycle*, **6** (2007), 1982–1994.

26. A. Fernandez-Vidal, A. Mazarsand S. Manenti, CDC25A: a rebel within the CDC25 phosphatases family?, *Anticancer Agents Med. Chem.*, **8** (2008), 825–831.
27. M. P. Sacristan, S. Ovejeroand and A. Bueno, Human Cdc14A becomes a cell cycle gene in controlling Cdk1 activity at the G(2)/M transition, *Cell Cycle*, **10** (2011), 387–391.
28. M. T. Paulsen, A. M. Starks, F. A. Derheimer, et al., The p53-targeting human phosphatase hCdc14A interacts with the Cdk1/cyclin B complex and is differentially expressed in human cancers, *Mol. Cancer*, **5** (2006), 25.
29. F. M. Schmid, K. B. Schou, M. J. Vilhelm, et al., IFT20 modulates ciliary PDGFRalpha signaling by regulating the stability of Cbl E3 ubiquitin ligases, *J. Cell. Biol.*, **217** (2018), 151–161.
30. H. Dong, H. Guo, L. Xie, et al., The metastasis-associated gene MTA3, a component of the Mi-2/NuRD transcriptional repression complex, predicts prognosis of gastroesophageal junction adenocarcinoma, *PLoS One*, **8** (2013), e62986.
31. A. M. Houghton, Mechanistic links between COPD and lung cancer, *Nat. Rev. Cancer*, **13** (2013), 233–245.
32. K. Vierlinger, M. H. Mansfeld, O. Koperek, et al., Identification of SERPINA1 as single marker for papillary thyroid carcinoma through microarray meta analysis and quantification of its discriminatory power in independent validation, *BMC. Med. Genomics*, **4** (2011), 30.
33. H. J. Chan, H. Li, Z. Liu, et al., SERPINA1 is a direct estrogen receptor target gene and a predictor of survival in breast cancer patients, *Oncotarget*, **6** (2015), 25815–25827.
34. C. H. Kwon, H. J. Park, J. H. Choi, et al., Snail and serpinA1 promote tumor progression and predict prognosis in colorectal cancer, *Oncotarget*, **6** (2015), 20312–20326.
35. K. Thorsen, F. Mansilla, T. Schepeler, et al., Alternative splicing of SLC39A14 in colorectal cancer is regulated by the Wnt pathway, *Mol. Cell. Proteomics*, **10** (2011), M110002998.
36. M. Nilbert and E. Rambech, Beta-catenin activation through mutation is rare in rectal cancer, *Cancer Genet. Cytogenet.*, **128** (2001), 43–45.
37. S. Yan, C. Zhou, W. Zhang, et al., β-Catenin/TCF pathway upregulates STAT3 expression in human esophageal squamous cell carcinoma, *Cancer Lett.*, **271** (2008), 85–97.
38. M. Takahashi, T. Tsunoda, M. Seiki, et al., Identification of membrane-type matrix metalloproteinase-1 as a target of the beta-catenin/Tcf4 complex in human colorectal cancers, *Oncogene*, **21** (2002), 5861–5867.
39. L. Beneduce, F. Castaldi, M. Marino, et al., Improvement of liver cancer detection with simultaneous assessment of circulating levels of free alpha-fetoprotein (AFP) and AFP-IgM complexes, *Int. J. Biol. Markers*, **19** (2004), 155–159.
40. S. X. Li, L. J. Liu, L. W. Dong, et al., CKAP4 inhibited growth and metastasis of hepatocellular carcinoma through regulating EGFR signaling, *Tumour Biol.*, **35** (2014), 7999–8005.
41. B. J. McMahon, L. Bulkow, A. Harpster, et al., Screening for hepatocellular carcinoma in Alaska natives infected with chronic hepatitis B: a 16-year population-based study, *Hepatology*, **32** (2000), 842–846.
42. M. Abu El Makarem, An overview of biomarkers for the diagnosis of hepatocellular carcinoma, *Hepat. Mon.*, **12** (2012), e6122.
43. W. J. Zheng, M. Yao, M. Fang, et al., Abnormal expression of HMGB-3 is significantly associated with malignant transformation of hepatocytes, *World J. Gastroenterol.*, **24** (2018), 3650–3662.
44. Z. Jiang, X. Zhai, B. Shi, et al., KIAA1199 overexpression is associated with abnormal

expression of EMT markers and is a novel independent prognostic biomarker for hepatocellular carcinoma, *Onco. Targets Ther.*, **11** (2018), 8341–8348.
45. P. Han, H. Li, X. Jiang, et al., Dual inhibition of Akt and c-Met as a second-line therapy following acquired resistance to sorafenib in hepatocellular carcinoma cells, *Mol. Oncol.*, **11** (2017), 320–334.
46. C. H. Wu, X. Wu and H. W. Zhang, Inhibition of acquired-resistance hepatocellular carcinoma cell growth by combining sorafenib with phosphoinositide 3-kinase and rat sarcoma inhibitor, *J. Surg. Res.*, **206** (2016), 371–379.
47. X. Tian, D. Zhou, L. Chen, et al., Polo-like kinase 4 mediates epithelial-mesenchymal transition in neuroblastoma via PI3K/Akt signaling pathway, *Cell Death Dis.*, **9** (2018), 54.

Supplementary

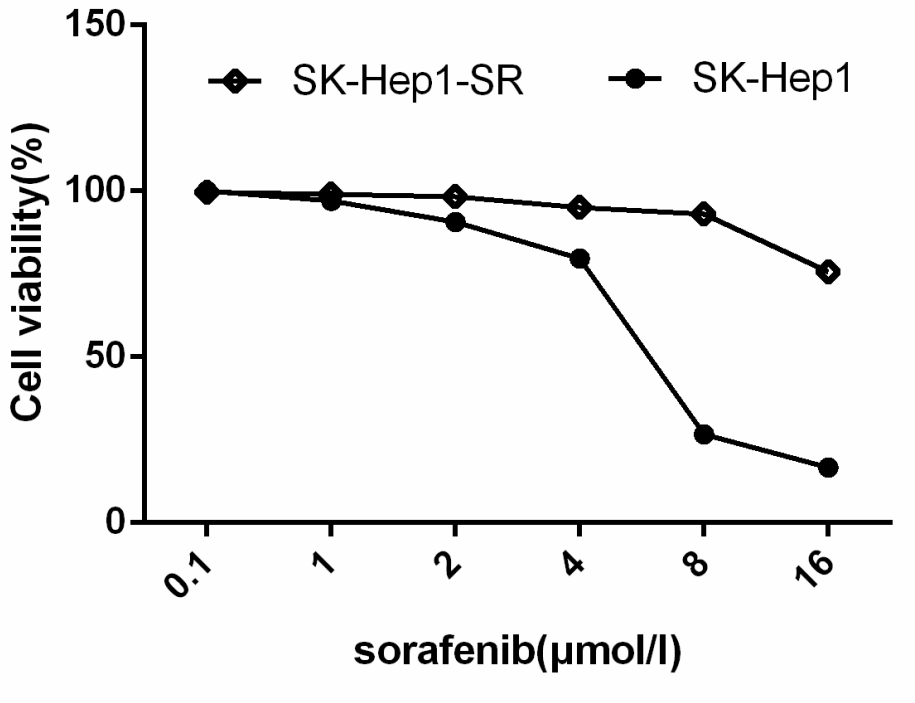

Figure S1. Construction of sorafenib-resistant SK-Hep1-SR cell line. SK-Hep1/SK-Hep1-SR cells were incubated with various concentrations of sorafenib (0.1, 1, 2, 4, 8, 16 μM). CCK8 assay was used to test cell viability. Data represented three independent experiments.