Oncologie

Tech Science Press

DOI: 10.32604/oncologie.2022.020357

ARTICLE

Exploration of Combinational Therapeutic Strategies for HCC Based on TCGA HCC Database

Dong Yan[1,#], Chunxiao Li[2,#], Yantong Zhou[2], Xue Yan[1], Weihua Zhi[1], Haili Qian[2,*] and Yue Han[1,*]

[1]Department of Interventional Therapy, National Cancer Center, National Clinical Research Center for Cancer, Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

[2]State Key Laboratory of Molecular Oncology, National Cancer Center, National Clinical Research Center for Cancer, Cancer Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

[*]Corresponding Authors: Haili Qian. Email: qianhaili001@163.com; Yue Han. Email: doctorhan@163.com

[#]These authors contributed equally to this work

Received: 18 November 2021 Accepted: 14 February 2022

ABSTRACT

Hepatocellular carcinoma (HCC) is one of the most deadly types of cancer. Sorafenib is currently the only available first-line molecular targeted drug approved by the FDA for HCC. However, primary and secondary resistance is often encountered with treatment with sorafenib. Genomic alterations found in HCC represent potential targets to develop new drugs or new combinational strategies against this type of cancer. Here we analyzed genomic alterations from the TCGA database of HCC samples and the corresponding targeted drugs available to the clinic to identify candidate drugs that might hold promise when used in combination with sorafenib. Our results revealed that IL6, JAK1, LEPR and RAF1 related pathways were commonly altered in HCC, which have targeted drugs available in medical practice. Fourteen genes with available targeting drugs were frequently altered in HCC. The pathways and gene targets with the respective targeted drugs warrant further evaluation in clinical trials to determine their therapeutic value in the treatment of HCC, alone or in combination with sorafenib. In summary, the analysis of TCGA, identified a series of pathways with targeted drugs available that were altered in HCC. Combination treatment with specific targeted drugs, depending on the altered pathways found in individuals may provide a better treatment strategy that will ultimately improve individual patient survival.

KEYWORDS

Hepatocellular carcinoma; TCGA database; sorafenib; combinational treatment; targeting drug

Nomenclature

CNA	Copy Number Alterations
HCC	Hepatocellular Carcinoma
NGS	Next-Generation Sequencing
TCGA	The Cancer Genome Atlas
FDA	Food and Drug Administration
RTK	Receptor-Tyrosine Kinase

1 Introduction

Liver cancer is one of the most common malignancies and is notorious for its high mortality. It ranks as the sixth most common cancer worldwide [1]. Hepatocellular carcinoma (HCC) is the most common subtype of liver cancer and is associated with hepatitis virus B, alcohol consumption, and exposure to some other chemicals [2,3].

While great progress in targeted therapy has been achieved in the treatment of a series of cancer types, few targeting reagents have been proven to be effective in hampering HCC development. Among the few available drugs, sorafenib is the most important first-line treatment approved by the FDA for HCC and is widely accepted by clinicians and patients due to its efficacy in improving the outcomes of HCC patients [4–6].

The exploration of drug targets and subsequent drug development depends largely on the identification of the altered presence of certain molecules that have an essential role in cancer cell survival and proliferation. However, the process of identifying potential driving molecular events of carcinogenesis was difficult and inefficient before the widespread use of genomic sequencing techniques (next-generation sequencing, NGS). Thanks to the development of NGS, a large number of genomic alterations have been revealed in the last decade [7,8]. For HCC, several studies have outlined an overview of genomic abnormalities, including EEF1A1, SMARCA4, and LZTR1 genes [9,10].

As the dominant clinically available targeting reagents for HCC, sorafenib and regorafenib, acting on Raf/mitogen-activated protein kinase/extracellular signal-regulated kinase (RAF/MEK/ERK) and VEGF signaling pathways, did not result in remarkable improvement in the patient's outcome as other targeting reagents did in breast cancer or lung cancer [11–13]. The accumulating clinical and biological databases of cancer disease provide reliable source to dig out and verify new clues for cancer diagnosis and treatment [14]. In June 2017, the TCGA program published new findings of global genomic alteration of the receptor tyrosine kinase (RTK) in HCC samples and suggested that abnormally overexpressed RTK pathways may influence the sensitivity of HCC to RTK inhibitors [9]. Unfortunately, this paper did not disclose the detailed VEGFR status in HCC. Given the key role of VEGFR in sorafenib treatment, it is advisable to phenotype HCC patients according to the genomic molecular signatures of the HCC tissues to improve outcomes in selected HCC patients. We also noticed the frequent occurrence of structurally abnormal chromosomes, which might contain critical gene targets for successful targeted therapy.

Currently, for HCC patients not eligible for curative treatments, solafenib is one of the few first-line systematic treatments. In the SHARP (A Phase III Study of Sorafenib in Patients with Advanced Hepatocellular Carcinoma) trial, sorafenib produced a response in only 43% of the patients, with an overall survival benefit of 10.7 months *vs*. 7.9 months of best supportive care only [4]. Therefore, further efforts are urgently needed to develop new targets for monotherapy or combination therapy. We globally screened genomic abnormalities as potential targets of small molecular inhibitors. The screening strategy uncovered candidates for other therapeutic options.

There is still much to learn about the application of sorafenib. We performed variation screening on the RTK pathway and genome-wide analysis in TCGA liver cancer data and combined it with the expression level of VEGFR2, the main target of sorafenib, to screen for drug-related variants in an attempt to provide more accurate target information correlated with sorafenib and RTK pathway abnormalities.

2 Materials and Methods

2.1 Dataset Used in the Analysis

A dataset including a total of 364 cases of TCGA Liver Hepatocellular Carcinoma with data on CNA, mRNA, and clinical information was obtained from https://media.githubusercontent.com/media/cBioPortal/datahub/master/public/lihc_tcga.tar.gz. The data were analyzed for genomic abnormalities associated with hepatocellular carcinoma treatment significance.

2.2 Methods Used in the Analysis

GO and KEGG pathway cluster analyses were performed with DAVID 6.8 [15]. Genetic alteration of the case set was extracted with CBioPortal online tools [16,17]. Genomic variation analysis and heatmap construction were performed with R. All samples were sorted by VEGFR2 mRNA expression as the row and the genome location as the column, and the CNV value was represented by the color of the heatmap. Survival analysis was performed using KMPLOT online tools [18].

The boxplot figure was generated with R 3.4.1 software. Venny 2.1 was used to identify overlapping subsets of gene symbols. The survival curves were plotted with Prism 7. Gene interaction network analysis was visualized by the STRING 10 platform [19] to construct a gene interaction network, and then Cytoscape 3.5.1 was employed [19,20]. By analyzing the topological characteristics of this gene interaction network, the highest-linked genes were screened for discussion.

Statistical analysis was conducted with SPSS 19 statistical software. Sample analysis of variance using the Kruskal–Wallis test was used to check for differences in the samples. $P < 0.05$ was considered statistically significant.

3 Results

3.1 Genomic Targets for RTK Family Inhibitors in Hepatocellular Carcinoma

RTK family-dominated pathways represent the main signaling pathway contributing to cancer growth [21]. Therefore, abnormalities in the RTK family pathway are considered the main driving force for cancer development and progression. We first explored the genomic alterations in RTK networks in the hepatocellular carcinoma database. We found a series of gene mutations in RTK-related pathways. By pathway enrichment analysis, we noticed a significant somatic mutational signature in this patient panel. In addition to the EGFR pathway, which has been well defined in the development of hepatocellular carcinoma, other pathways, such as the interleukin-2 (IL-2) and insulin receptor pathways, were also frequently mutated targets (Fig. 1).

To illustrate the potential effect of these gene mutations, we classified the mutations in terms of the influence on their protein products to shed light on the potential future drug development and treatment regimen design. There were 59 genes involved in the RTK pathway with mutations. We put these genes into the KEGG database to match the corresponding drugs available in clinical practice. We retrieved 5 drugs corresponding to four RTK pathway targets, including IL6, JAK1, LEPR, and RAF1 (the target genes and their corresponding clinical drugs are shown in Table 1).

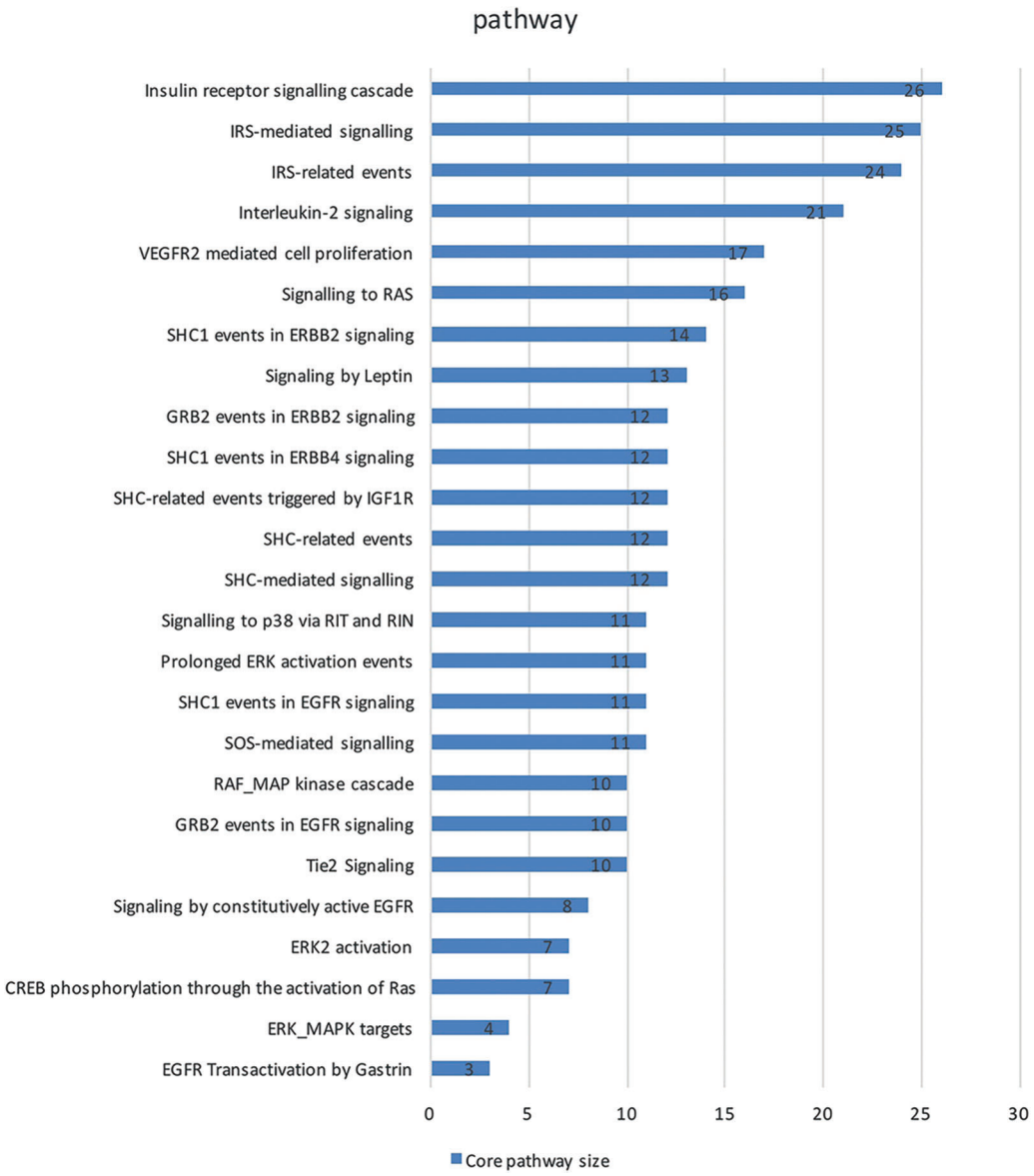

Figure 1: GO-BP enrichment of gene functions that carry mutations from RTK pathways

Table 1: Altered pathway targets with available targeting drugs

RTK targets	Available targeting drugs
IL6	Siltuximab
JAK1	Ruxolitinib, Tofacitinib
LEPR	Metreleptin
RAF1	Sorafenib

3.2 VEGFR2-Associated Genomic and Epigenomic Heterogeneity May Undermine Solafenib Efficacy

Using CNV data from TCGA, we retrieved a large range of genomic alterations. We found that in the entire cohort of patients, four genomic loci exhibited frequent copy number alterations, including amplification of segments in chromosomes 1q and 8q and deletion of regions in chromosomes 8p and 17p, carrying many oncogenes and tumor suppressor genes (Fig. 2). The mixed presence of CNVs further complicated the complexity of hepatocellular carcinoma biology and was attributed to the clinical hardness of manipulation. The segment CNVs comprised genes controlling multiple physical and pathological functions, such as the cell cycle, cellular metabolism, and cellular damage repair, etc.

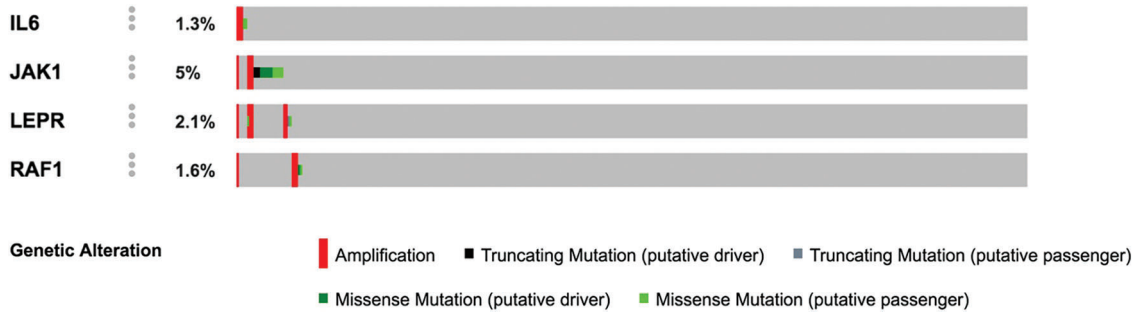

Figure 2: Patterns of genetic alteration of the four gene targets involved in HCC

Given the prominent presence of VEGFR2 signaling mutations in the RTK pathways mentioned above and their value as direct targets of sorafenib drugs, we combined CNV and RNA-seq data to investigate the detailed genomic abnormalities accompanying VEGFR2 status alterations. We classified the hepatocellular carcinoma patients according to the transcriptional levels of VEGFR2 in their tumors. A lower VEGFR2 mRNA level was significantly associated with more frequent genomic abnormalities of fragment amplification in parts of chromosomes 2, 3, 12, and 21, as well as fragment deletion in chromosomes 4, 11, 16, and 17 (Fig. 3). Overall, a higher VEGFR2 level resulted in more normal genomic integrity. From this observation, we can speculate that higher VEGFR2 levels are powerful enough to drive the development and progression of hepatocellular carcinoma.

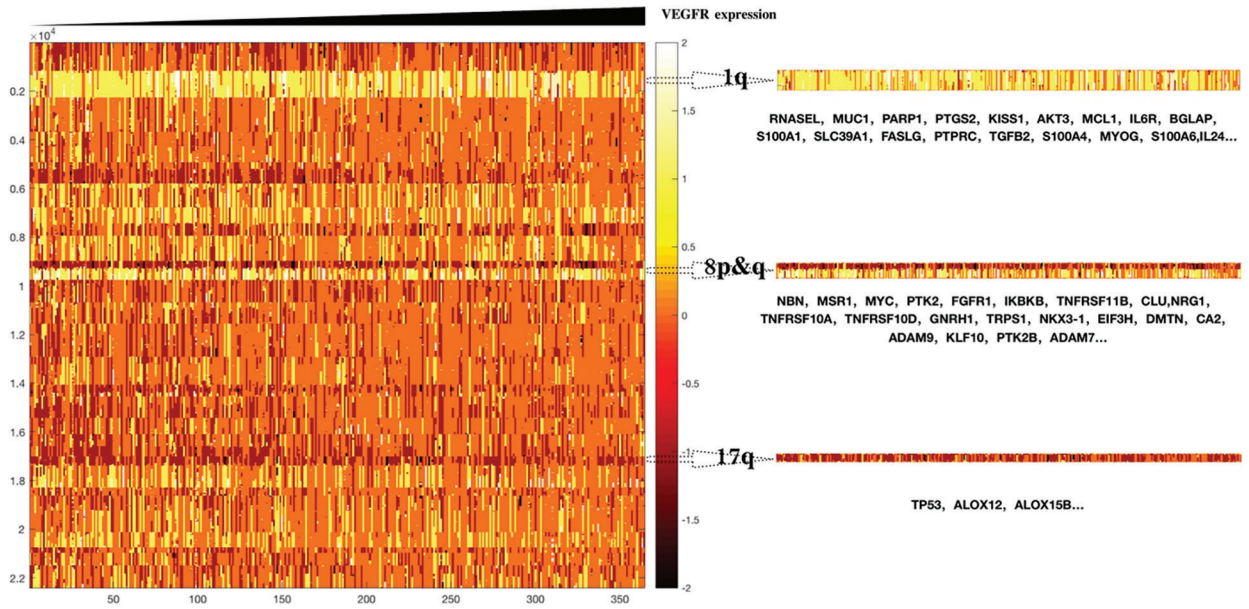

Figure 3: Heatmap showing the genomic abnormality frequency along with VEGFR2 transcriptional level escalation. Each column represents a sample, and each row represents a gene. Samples were sorted by the mRNA expression of VEGFR2, and genes were sorted by their genome location

We also performed an analysis on the association of VEGFR2 levels with pathological parameters. We found that VEGFR2 was significantly associated with pathological grade but was not significantly associated with parameters such as age, sex, clinical stage, or other variables (Fig. 4).

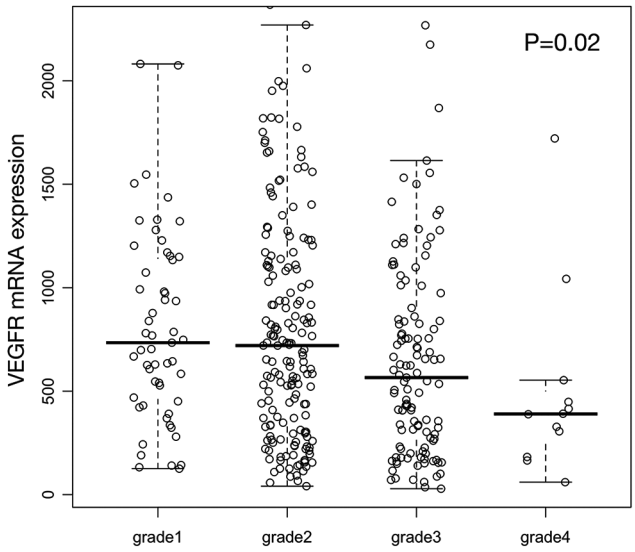

Figure 4: Association between VEGFR2 level and pathological grade. Plots of the negative association of VEGFR2 level with pathological grade of hepatocellular carcinomas

3.3 Gene Targets Involved in Genomic Alterations Ensure the Possibility of Combinational Treatment Strategies with Sorafenib in HCC

Accumulation of gene abnormalities from large fragmental alterations may lead to a complex signaling interaction during carcinogenesis. To elucidate the potential synergistic contribution of gene abnormalities from fragmental genomic alterations, we identified the genes frequently enclosed in the altered genomic region. A total of 1554 genes comprised the hepatocellular carcinoma-associated genomic fragment alterations, among which 14 genes were defined as targets with currently available drugs based on the KEGG database (Fig. 5A and Table 2). We plotted the survival curve for these 14 target genes according to the transcriptomes and the survival data of the cases. As a result, we found that the transcriptional expression of ADRA1A, SERPINC1, and SQLE was associated with survival outcomes (Fig. 5B).

3.4 Network Analysis Revealed the Main Candidates That May Be Targeted in HCC

Among these 1554 candidate genes, 42 genes were reported to be related to cancer development and advancement (Fig. 6A). Through gene network analysis of the 42 cancer-related genes, we constructed a gene–gene interaction model to show the knot point targets in the network (Fig. 6B). In this network, the most recognized genes include TP53, Myc, PARP1, FASLG, PTGS2, etc., which are also located on the central knots of the interaction map. Among the key knot genes, PARP1 is the clear target of olaparib, which was approved by the FDA for usage in ovarian cancer (there are several other drugs targeting PARP1, such as rucaparib and niraparib). PTGS2, with another symbol of COX2, is a molecule widely participating in various oncogenic behaviors [22]. Its selective inhibitors, such as celecoxib and meloxicam, have also been approved in clinical practice.

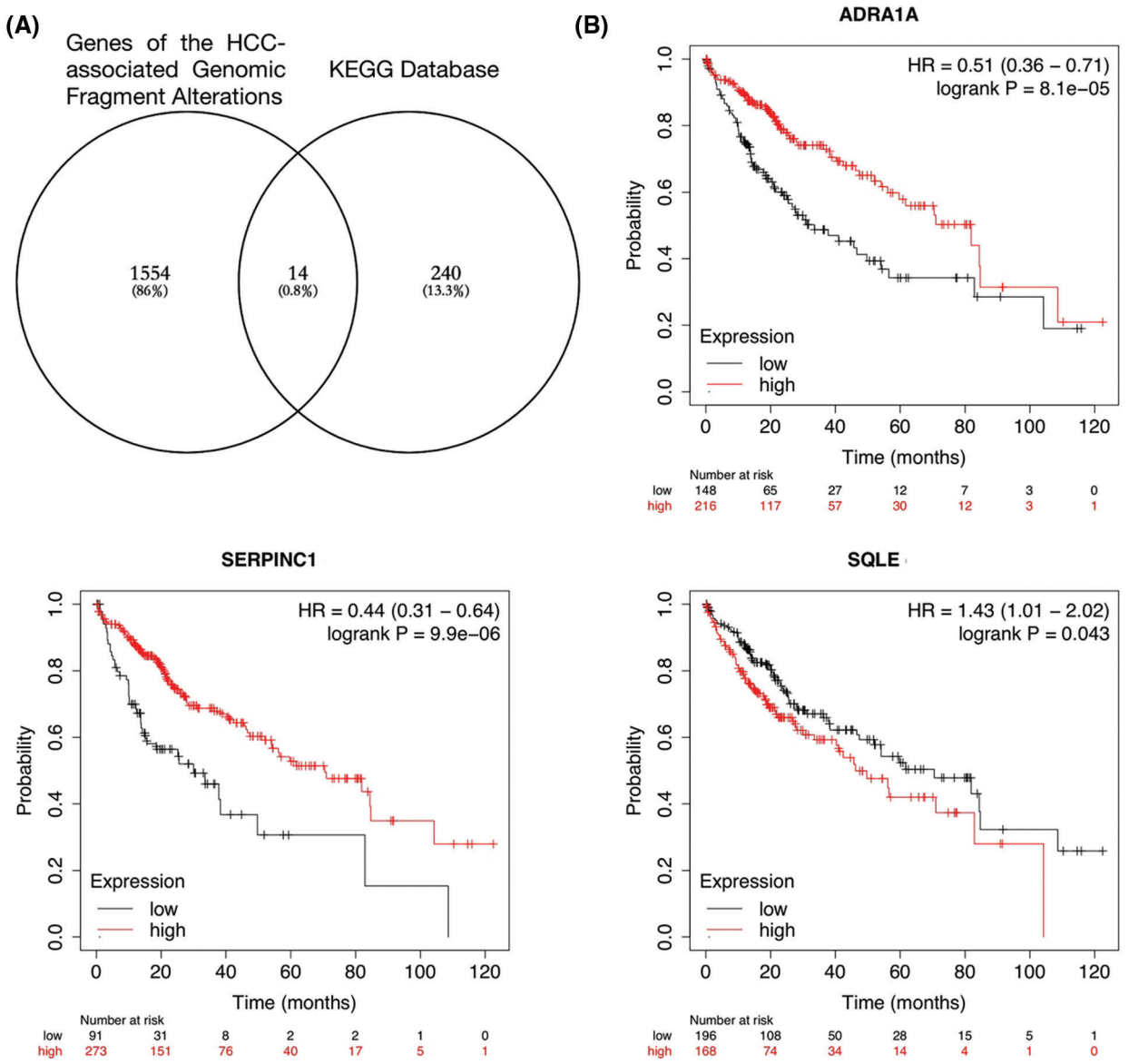

Figure 5: The genomic alteration-involved gene targets with available drugs based on the KEGG database (A); survival curve plotting of ADRA1A, SERPINC1, and SQLE (B)

Table 2: The list of gene targets with clinical drugs available which are frequently involved in genomic alterations in HCC

Gene targets	Available drugs
ADRA1A	Alfuzosin
REN	Aliskiren
ADORA1	Aminophylline
CA2	Brinzolamide
PTGS2	Etodolac

(Continued)

Table 2 (continued)

Gene targets	Available drugs
SERPINC1	Fondaparinux
SV2A	Levetiracetam
ADRB3	Mirabegron
SQLE	Naftifine
PARP1	Olaparib
LPL	Omega-3-acid
OPRK1	Pentazocine
GLP2R	Teduglutide
IL6R	Tocilizumab

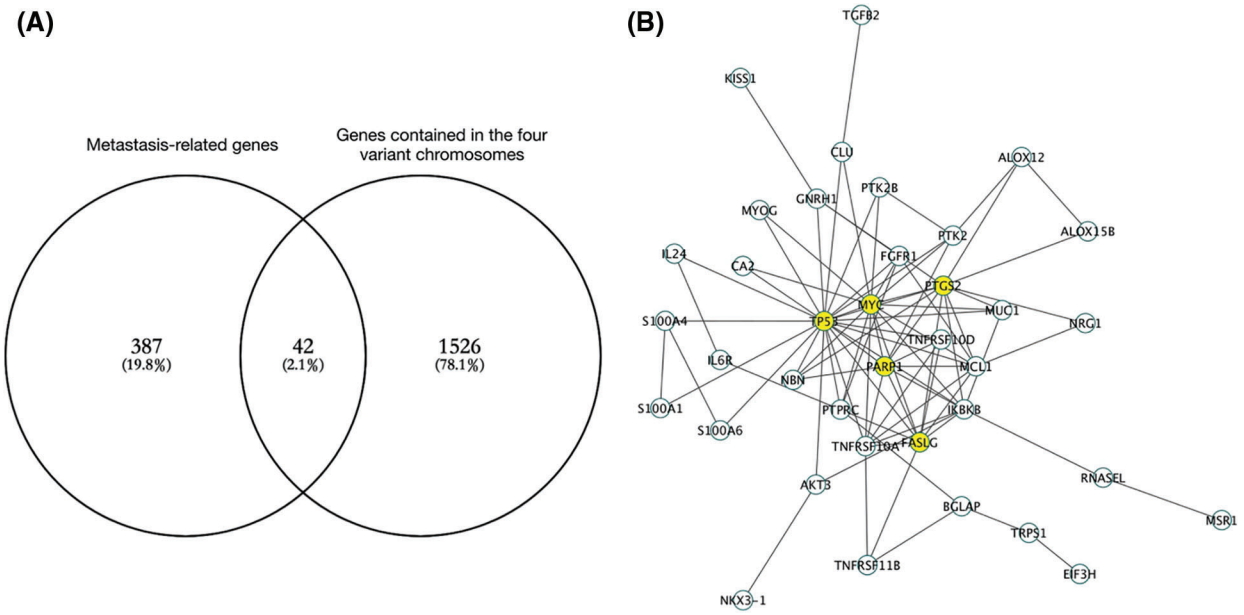

Figure 6: Integrated analysis of the gene targets involved in genomic alterations in HCC. Forty-two genes have been reported to be related to cancer development and advancement (A). B: The interaction network of cancer-related genes extracted from large fragmental genomic alterations (OS = overall survival) (B)

4 Discussion

For HCC, as well as other main types of malignancy, genomic alteration serves as an initiating force or subsequent promoting or accelerating force after epigenetic modifications to drive cancer development. For example, EGFR mutation and EML-ALK fusion variants made a major contribution to lung cancer development and progression [23]. In contrast, the identification of driving gene mutation signatures was relatively unclear in HCC. EGFR is one of the few genes that have been reported to be closely related to HCC development, and abnormally elevated EGFR signaling was proven to be able to induce measurable treatment benefits in patients receiving EGFR-targeted therapy. Among the EGFR inhibitor family members, sorafenib is typically used in HCC treatment. However, HCC patients treated with sorafenib only extended their survival span from 7.9 to 10.7 months on average [4]. Therefore, it is urgent to explore new gene alterations of HCC that play a major role in the progression of HCC, and develop drugs to counter them.

Along these lines, we analyzed the genomic alteration signatures of hepatocellular carcinoma based on the newly released HCC genomic and transcriptional datasets from the TCGA database, which consisted of genomic and transcriptional information of 363 cases of HCC samples [7,8]. Interestingly, we revealed some remarkable points that are valuable for clinical applications and further drug development. This set of genomic data has been explored by the Cancer Genome Atlas Research Network. The authors reported an overview of the genomic events accumulated in HCC, but they did not reveal the treatment- and drug-development-related genomic alteration profile, leaving the data underexplored for their clinical value.

By bioinformatic investigation, we enriched a series of gene sequence and structure alterations that occurred with high frequency in HCC. Gene alterations in the RTK family involve 59 genes, among which only 4, namely, IL6, TAK1, LEPR, and RAF1, have been evaluated for their drugability [24,25]. Only RAF1 is a target of the well-defined EGFR inhibitor sorafenib, supporting the potential value of sorafenib in treating hepatocellular carcinoma as well as highlighting the urgent shortage of candidate drugs available to clinical practice against HCC. IL-6 is a cytokine originally found to be a key regulator in inflammation, and B cell maturation has been linked to a wide variety of inflammation-related diseases, including cancers [26]. It was reported that IL-6 did not play a direct role in affecting cancer proliferation and invasion but could promote cancer cell apoptosis [27]. There have also been various data showing the therapeutic value of the IL-6-related STAT3 pathway in HCC treatment [28]. In our analysis, the biological nature of IL-6 mutation was not revealed; therefore, it is difficult to propose a detailed mechanism through which IL-6 is involved in hepatocellular carcinoma development. JAK1, a membrane protein with protein-tyrosine kinase (PTK) activity, is able to phosphorylate STAT proteins (which are important signal transducers and transcription activators) and other essential oncogenic behaviors [29]. Its active role in the development and progression of hepatocellular carcinoma is well recognized. Yang et al. reported the mutation of JAK1 as a predictor of the sensitivity of hepatocellular carcinoma to treatment with JAK/STAT3 inhibition, confirming the value of JAK2 mutation in hepatocellular carcinoma management [30].

The other 55 genes identified in this analysis provided promising candidates for the development of new targeted drugs for this lethal disease, considering their RTK pathway-connected functions. Most of the identified mutated genes were not well illustrated for their role in hepatocellular carcinoma treatment. Although not all mutations have been well investigated for their effects on protein activity, this mutation gene pool represents potential effective targeting molecules in the future development of drugs against HCC treatment. We also found forty-four mutation-involved genes with cancer-related backgrounds. The functional interaction network of these genes enriched TP53, Myc, and PARP in the key connecting knots. All the genes on the key knots are classical molecules in the process of carcinogenesis.

In this paper, we carried out the data mining of genomic alterations from a large cohort of hepatocellular carcinoma samples, adding more details to the genomic landscape of hepatocellular carcinoma. We also identified genomic abnormalities with targeted drugs available to clinics. This further extended our view on the global network mediating hepatocellular carcinogenesis and guaranteed a highly efficient method for evaluating candidate treatment regimens against this type of cancer. It also shed light on the continued efforts for molecular phenotyping of patients for treatment with sorafenib.

5 Conclusion

This study showed that the frequency of genomic CNVs in cases with low VEGFR2 expression was significantly higher than that in cases with high VEGFR2 expression, which suggests that an important reason for sorafenib treatment failure may be the presence of many CNVs in HCC cells. We also screened and evaluated the most critical tumor-related genes among the common chromosomal aberrations in liver cancer to further explore the target information of liver cancer treatment. The

screening of these genes not only contributes to the overcoming of drug resistance to VEGFR2 targets, but also provides new potential targets for liver cancer research.

Ethics Approval and Informed Consent Statement: Not applicable.

Author Contributions: Yan Dong and Li Chunxiao designed the study, performed the data analysis, results interpretation and wrote the manuscript. Yue Han and Haili Qian reviewed and revised the manuscript. Yantong Zhou, Xue Yan and Weihua Zhi contributed to the data collection and analysis, study design and repared the figures and tables.

Funding Statement: This work was financially supported by grants from the National Key Research and Development Program of China (2021YFF1201302), the National Natural Science Foundation of China (Nos. 81872280, 82073094), the CAMS Innovation Fund for Medical Sciences (CIFMS) (2021-I2M-014), the Open Issue of State Key Laboratory of Molecular Oncology (No. SKL-KF-2021-16), and the Independent Issue of State Key Laboratory of Molecular Oncology (No. SKL-2021-16).

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

References

1. Torre, L. A., Bray, F., Siegel, R. L., Ferlay, J., Lortet-Tieulent, J. et al. (2015). Global cancer statistics. *Journal of Cancer Research and Clinical Oncology, 65(2),* 87–108. DOI 10.3322/caac.21262.
2. Castelli, G., Pelosi, E., Testa, U. (2017). Liver cancer: Molecular characterization, clonal evolution and cancer stem cells. *Cancers, 9(9),* 127. DOI 10.3390/cancers9090127.
3. Llovetm, J. M., Kelley, R. K., Villanueva, A., Singal, A. G., Finn, R. S. et al. (2021). Hepatocellular carcinoma. *Nature Reviews Disease Primers, 7(1),* 6. DOI 10.1038/s41572-020-00240-3.
4. Ray, E. M., Sanoff, H. K. (2017). Optimal therapy for patients with hepatocellular carcinoma and resistance or intolerance to sorafenib: Challenges and solutions. *Journal of Hepatocellular Carcinoma, 4,* 131–138. DOI 10.2147/JHC.
5. Koulouris, A., Tsagkaris, C., Spyrou, V., Pappa, E., Nikolaou, M. et al. (2021). Hepatocellular carcinoma: An overview of the changing landscape of treatment options. *Journal of Hepatocellular Carcinoma, 8,* 387–401. DOI 10.2147/JHC.S300182.
6. Haber, P. K., Puigvehí, M., Castet, F., Lourdusamy, V., Montal, R. et al. (2021). Evidence-based management of hepatocellular carcinoma: Systematic review and meta-analysis of randomized controlled trials (2002–2020). *Gastroenterology, 161(3),* 879–898. DOI 10.1053/j.gastro.2021.06.008.
7. Patch, A. M., Christie, E. L., Etemadmoghadam, D., Garsed, D., Bowtell, D. D. et al. (2015). Whole-genome characterization of chemoresistant ovarian cancer. *Nature, 521(7553),* 489–494. DOI 10.1038/nature14410.
8. The Cancer Genome Atlas Network (2012). Comprehensive molecular characterization of human colon and rectal cancer. *Nature, 487(7407),* 330–337. DOI 10.1038/nature11252.
9. The Cancer Genome Atlas Research Network (2017). Comprehensive and integrative genomic characterization of hepatocellular carcinoma. *Cell, 169(7),* 1327–1341.e23. DOI 10.1016/j.cell.2017.05.046.
10. Jhunjhunwala, S., Jiang, Z., Stawiski, E. W., Gnad, F., Liu, J. et al. (2014). Diverse modes of genomic alteration in hepatocellular carcinoma. *Genome Biology, 15(8),* 436. DOI 10.1186/s13059-014-0436-9.
11. Li, X., Zhang, D., Guan, S., Ye, W., Liu, L. et al. (2017). Efficacy of anti-VEGF agents in the treatment of elderly hepatocellular carcinoma: A systematic review. *Oncotarget, 8(54),* 93179–93185. DOI 10.18632/oncotarget.21452.
12. Bronte, G., Andreis, D., Bravaccini, S., Maltoni, R., Cecconetto, L. et al. (2017). Sorafenib for the treatment of breast cancer. *Expert Opinion on Pharmacotherapy, 18(6),* 621–630. DOI 10.1080/14656566.2017.1309024.
13. Horiike, A., Takeuchi, K., Uenami, T., Kawano, Y., Tanimoto, A. et al. (2016). Sorafenib treatment for patients with RET fusion-positive non-small cell lung cancer. *Lung Cancer, 93,* 43–46. DOI 10.1016/j.lungcan.2015.12.011.

14. Wu, W. T., Li, Y. J., Feng, A. Z., Li, L., Huang, T. et al. (2021). Data mining in clinical big data: The frequently used databases, steps, and methodological models. *Military Medical Research, 8(1),* 44. DOI 10.1186/s40779-021-00338-z.
15. Huang, W., Sherman, B. T., Lempicki, R. A. (2009). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nature Protocols, 4(1),* 44–57. DOI 10.1038/nprot.2008.211.
16. Gao, J., Aksoy, B. A., Dogrusoz, U., Dresdner, G., Gross, B. et al. (2013). Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. *Science Signaling, 6(269),* pl1. DOI 10.1126/scisignal.2004088.
17. Cerami, E., Gao, J., Dogrusoz, U., Gross, B. E., Sumer, S. O. et al. (2012). The cBio cancer genomics portal: An open platform for exploring multidimensional cancer genomics data. *Cancer Discovery, 2(5),* 401–404. DOI 10.1158/2159-8290.CD-12-0095.
18. Lánczky, A., Győrffy, B. (2021). Web-based survival analysis tool tailored for medical research (KMplot): Development and implementation. *Journal of Medical Internet Research, 23(7),* e27633. DOI 10.2196/27633.
19. Szklarczyk, D., Franceschini, A., Wyder, S., Forslund, K., Heller, D. et al. (2015). STRING v10: Protein-protein interaction networks, integrated over the tree of life. *Nucleic Acids Research, 43,* D447–D452. DOI 10.1093/nar/gku1003.
20. Shannon, P., Markiel, A., Ozier, O., Baliga, N. S., Wang, J. T. et al. (2003). Cytoscape: A software environment for integrated models of biomolecular interaction networks. *Genome Research, 13(11),* 2498–2504. DOI 10.1101/gr.1239303.
21. Nelson, K. N., Peiris, M. N., Meyer, A. N., Siari, A., Donoghue, D. J. (2017). Receptor tyrosine kinases: Translocation partners in hematopoietic disorders. *Trends in Molecular Medicine, 23(1),* 59–79. DOI 10.1016/j.molmed.2016.11.002.
22. Bornstein, E., Jimeno, A. (2016). Olaparib for the treatment of ovarian cancer. *Drugs of Today, 52(1),* 17–28. DOI 10.1358/dot.2016.52.1.2440714.
23. Romanidou, O., Landi, L., Cappuzzo, F., Califano, R. (2016). Overcoming resistance to first/second generation epidermal growth factor receptor tyrosine kinase inhibitors and ALK inhibitors in oncogene-addicted advanced non-small cell lung cancer. *Therapeutic Advances in Medical Oncology, 8(3),* 176–187. DOI 10.1177/1758834016631531.
24. Qiu, Y., Li, H., Xie, J., Qiao, X., Wu, J. et al. (2021). Identification of ABCC5 among ATP-binding cassette transporter family as a new biomarker for hepatocellular carcinoma based on bioinformatics analysis. *International Journal of General Medicine, 14,* 7235–7246. DOI 10.2147/IJGM.S333904.
25. Xie, J., Li, H., Chen, L., Cao, Y., Hu, Y. et al. (2021). A novel pyroptosis-related lncRNA signature for predicting the prognosis of skin cutaneous melanoma. *International Journal of General Medicine, 14,* 6517–6527. DOI 10.2147/IJGM.S335396.
26. Tsukamoto, H., Fujieda, K., Senju, S., Ikeda, T., Oshiumi, H. et al. (2018). Immune-suppressive effects of interleukin-6 on T-cell-mediated anti-tumor immunity. *Cancer Science, 109(3),* 523–530. DOI 10.1111/cas.13433.
27. Taniguchi, K., Karin, M. (2014). IL-6 and related cytokines as the critical lynchpins between inflammation and cancer. *Seminars in Immunology, 26(1),* 54–74. DOI 10.1016/j.smim.2014.01.001.
28. Hu, M. H., Chen, L. J., Chen, Y. L., Tsai, M., Shiau, S. et al. (2017). Targeting SHP-1-STAT3 signaling: A promising therapeutic approach for the treatment of cholangiocarcinoma. *Oncotarget, 8(39),* 65077–65089. DOI 10.18632/oncotarget.17779.
29. Huynh, J., Etemadi, N., Hollande, F., Ernst, M., Buchert, M. (2017). The JAK/STAT3 axis: A comprehensive drug target for solid malignancies. *Seminars in Cancer Biology, 45(4),* 13–22. DOI 10.1016/j.semcancer.2017.06.001.
30. Yang, S., Luo, C., Gu, Q., Xu, Q., Wang, G. et al. (2016). Activating *JAK1* mutation may predict the sensitivity of JAK-STAT inhibition in hepatocellular carcinoma. *Oncotarget, 7(5),* 5461–5469. DOI 10.18632/oncotarget.6684.