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Network analysis of liver cancer: a system biology approach

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ABSTRACT

Aim: Determining critical dysregulated proteins in liver cancer was the main aim of this study.
Background: Liver cancer is a common health problem characterized by difficulties in early diagnosis and rapid progression. Due to the lack of targeted drugs and the other features of the disease, the survival rate for patients is extremely low.
Methods: The related dysregulated proteins for liver cancer were retrieved from the STRING database. The queried proteins were included in a network by Cytoscape software, and the central nodes of the network were enriched via gene ontology.
Results: Among 11 introduced central nodes (GAPDH, TP53, EGFR, MYC, INS, ALB, IL6, AKT1, VEGFA, CDH1, and HRAS), HRAS and AKT1 were highlighted as critical dysregulated proteins which can be considered as possible biomarkers.
Conclusion: Analysis revealed that AKT1, HRAS and the related biochemical pathways (especially "HIF-1 signaling pathway") are the possible diagnostic and therapeutic agents of liver cancer.

Keywords: Liver cancer, AKT1, HRAS, Biomarker, Gene ontology.

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Introduction

Liver cancer, a common human cancer, is a disease with poor diagnosis and rapid progress, which causes high mortality rates and difficult treatment (1). Many efforts using proteomics and genomics to explore the molecular mechanism of liver cancer led to introducing some dysregulated genes and proteins in liver cancer (2-4). In high throughput methods such as proteomics and genomics, large numbers of dysregulated proteins or genes identify which express the gene expression pattern in the studied samples (5, 6).

Network analysis is useful for organizing and analyzing many proteins, genes, or metabolites. In this method, the queried proteins are linked together to construct a network (7). Network analysis of dysregulated proteins in many diseases is led to the discovery of many critical proteins in the studied disorders (8, 9).

Protein network analysis explores patterns of connection between elements of the network. In such analysis, the proteins interacting directly with many other nodes are known as hub nodes. It is proposed that the hub nodes play an essential role in the network and are participated in the possible dysregulated biological

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processes (10). Human cancer is a common disorder that is studied via network analysis. Researchers introduce many central proteins for different types of cancers (11). M Zamanian Azodi et al. published a document about common colon and breast cancer features using protein-protein interaction network analysis (12).

Biochemical pathway analysis is the other analytical method to identify disease-dysregulated pathways. The explored dysregulated pathways provide new insight into the molecular mechanism of diseases and make it possible for the drug targets to be determined. Biochemical pathway analysis is used to assess molecular features of different types of diseases (13).

TB Nguyen et al. published data about network analysis of liver cancer. Based on this document, several hub genes, such as TOP2A, RRM2, NEK2, CDK1, and CCNB1, are introduced for a network of liver cancer. This investigation used data from the Gene Expression Omnibus database (14). M Zamanian Azodi et al. reported information about the prevention effects of physical exercises on liver cancer via network analysis (15).

In the present study, the well-established data from the STRING database related to liver cancer analyzes via network analysis to find the potent central protein considering 4 centrality parameters: degree, betweenness centrality, closeness centrality, and stress. The central proteins were enriched via gene ontology to identify the main dysregulated biochemical pathways.

Methods

STRING database includes "protein query", "PubMed query", and "disease query". The number of 100 proteins that are related to liver cancer were extracted from the "disease query" of the STRING database (16) and were interacted via undirected edges by Cytoscape software version 3.7.2 (17). The network was analyzed by the "NetworkAnalyzer" application of Cytoscape and visualized based on degree value. The distribution of degree value,

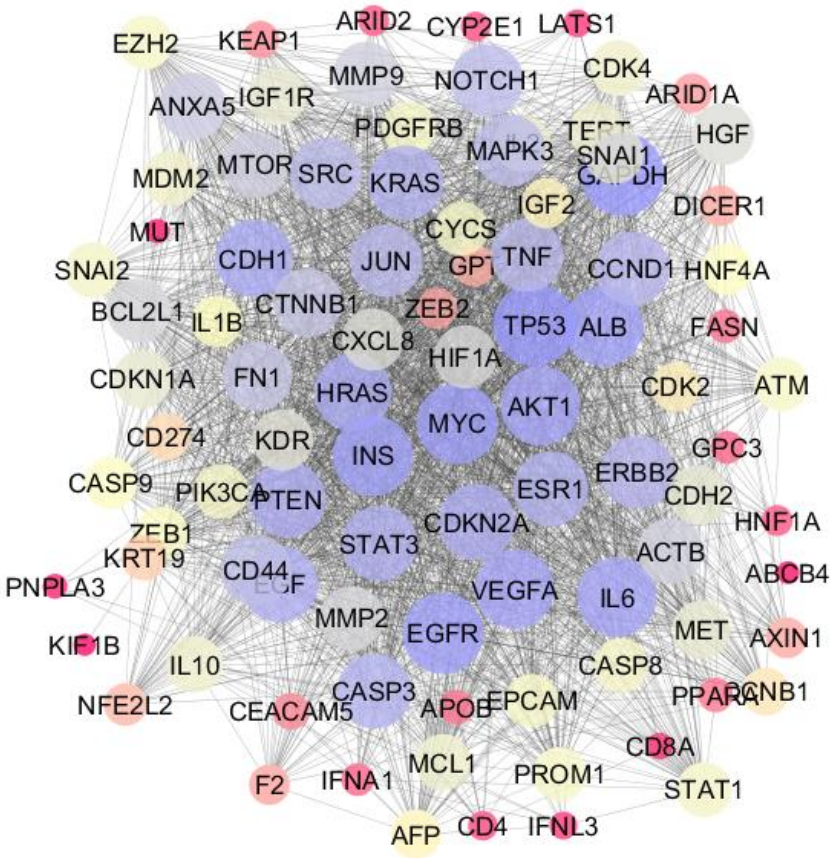

Figure 1. Main connected component subnetwork of liver cancer. The nodes are layout based on degree value. Red to blue refers to node degree increment.

betweenness centrality, and closeness centrality for the network nodes was plotted. Since centrality parameters can differentiate the nodes of the constructed network, the network was invested based on degree values, betweenness and closeness centralities, and stress. To find the central nodes of the network, 20% of nodes based on degree value, betweenness centrality, closeness centrality, and stress were identified in the four groups of nodes. Common proteins between the four groups were determined as central proteins. Gene ontology analysis was applied for the central proteins via Cytoscape software's ClueGO application (version 2.5.7) (18). The biochemical pathways were extracted from KEEG (Kyoto Encyclopedia of Genes and Genomes) and were classified based on kappa score.

Results

A network including a main connected component and 6 isolated proteins was constructed. 2359 edges connected a number of 94 nodes. The network was visualized by degree value (Figure 1). To understand the property of the created network, plots of degree value, betweenness centrality, and closeness centrality distribution are provided and shown in Figures 2-4. The finding indicates that there are a few numbers of nodes that can be considered central nodes.

The top 20 nodes based on degree value were identified as hub proteins. Like hub proteins, 20 nodes considering betweenness centrality were determined as bottlenecks. The number of 15 common hubs and bottlenecks were selected as hub-bottlenecks. The 11 hub-bottlenecks were common, with the nodes considered as top nodes based on closeness centrality and stress. These

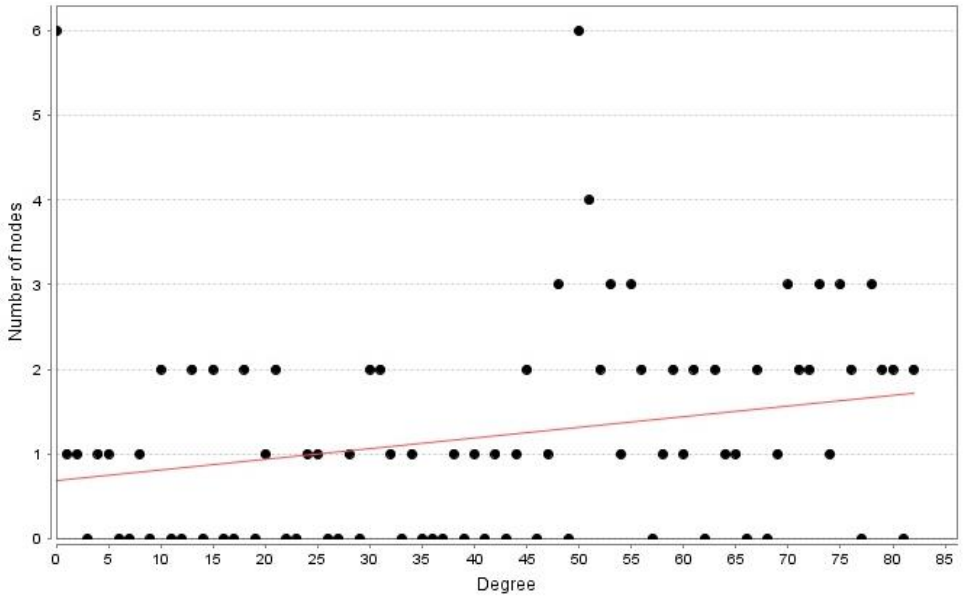

Figure 2. Degree distribution of nodes of liver cancer network. The fitted line is presented in red.

Table 1. Central nodes and the related centrality parameters of liver cancer network

display name	Degree	Betweenness Centrality	Closeness Centrality	Stress
GAPDH	82	0.024	0.894	2858
TP53	82	0.021	0.894	2710
EGFR	80	0.018	0.877	2484
MYC	80	0.015	0.877	2370
INS	79	0.030	0.869	2984
ALB	79	0.018	0.869	2460
IL6	78	0.016	0.861	2336
AKT1	78	0.011	0.861	2212
VEGFA	78	0.011	0.861	2044
CDH1	76	0.010	0.845	1854
HRAS	76	0.008	0.838	1722

11 proteins are well-thought-out as central proteins.

The 11 central proteins and their centrality properties are shown in Table 1. The number of 21 biochemical pathways related to the central nodes are identified via gene ontology. The determined pathways are shown in Figures 5 and 6. The introduced pathways are grouped into 3 clusters: bladder cancer, prostate cancer, and HIF-1 signaling pathway.

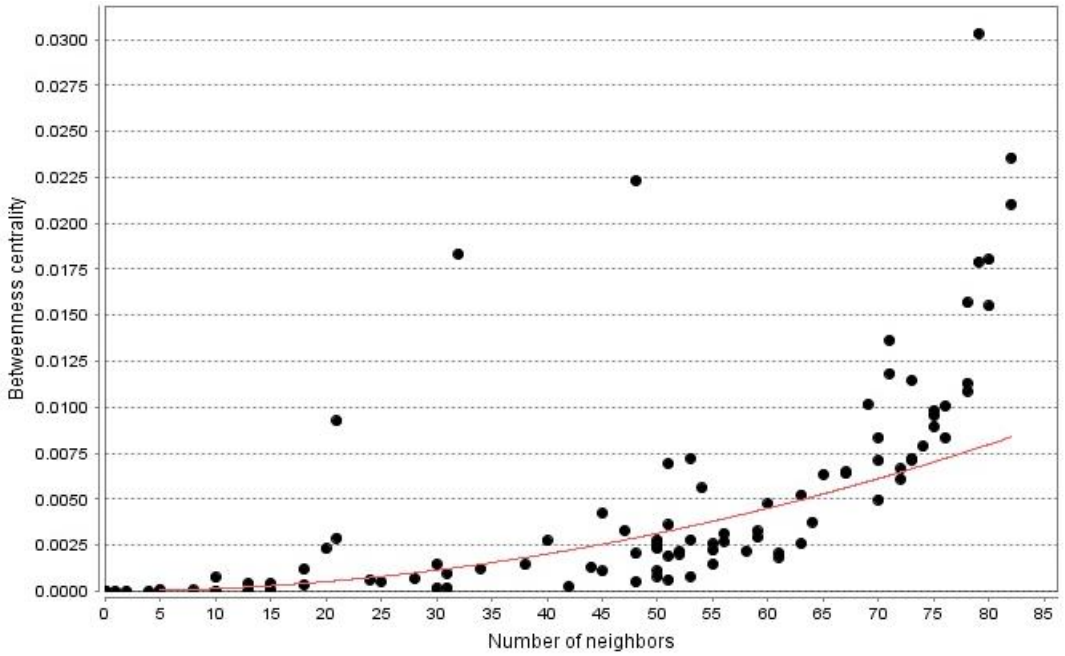

Figure 3. Betweenness centrality distribution of nodes of liver cancer network. The fitted line is presented in red. Centrality amounts are reported as unnormalized data.

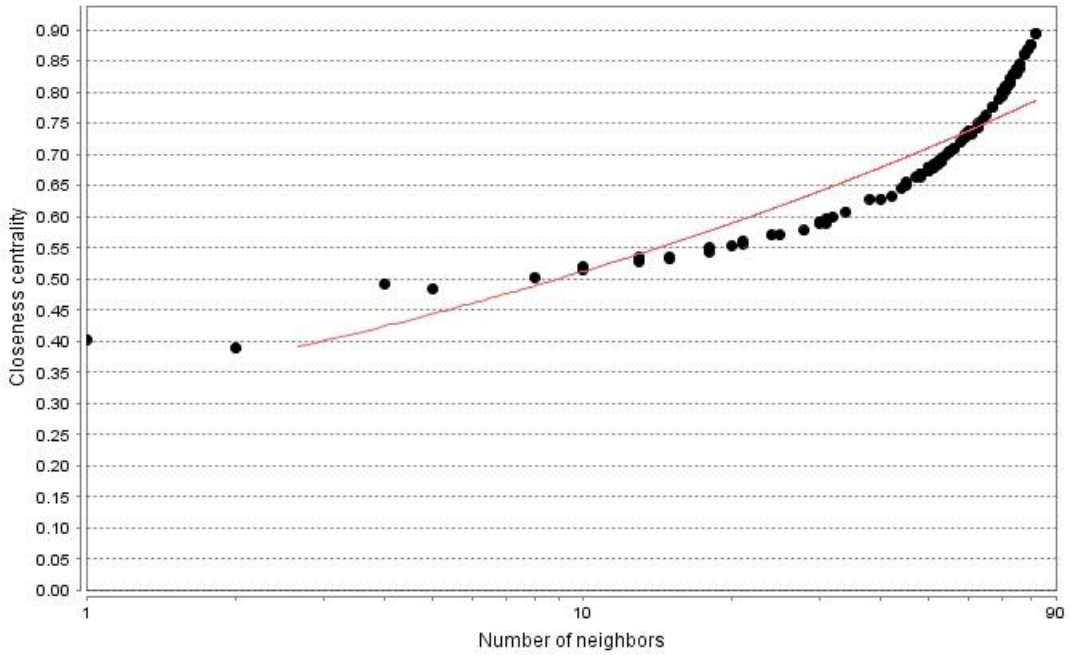

Figure 4. Closeness centrality distribution of nodes of liver cancer network. The fitted line is presented in red. Centrality amounts are reported as unnormalized data.

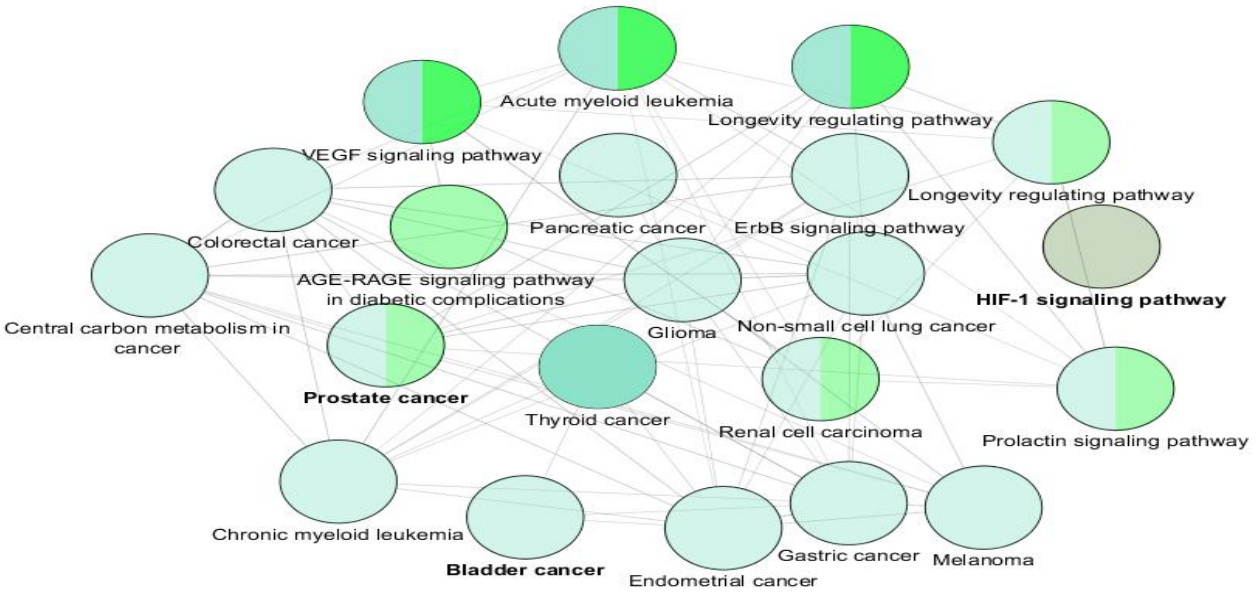

Figure 5. Biochemical pathways are related to the central nodes of liver cancer network. The names of groups are bolded. HIF-1 signaling pathway, Bladder cancer, and Prostate cancer are the three pathways classes.

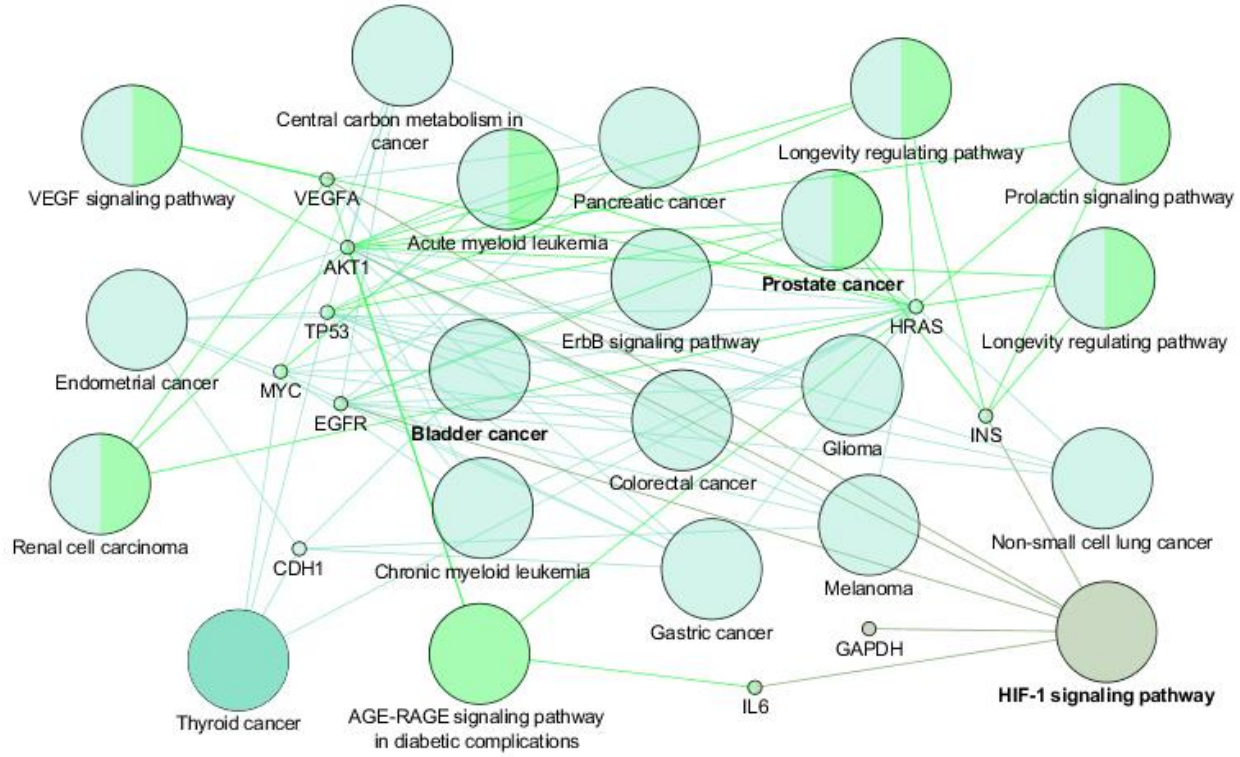

Figure 6. Biochemical pathways are related to the central nodes of liver cancer network in connection with the related proteins. The names of groups are bolded.

Discussion

There are many documents about the molecular mechanism of liver cancer and the dysregulated genes related to this type of cancer (19, 20). As a suitable method, bioinformatic analysis can be used to explore molecular aspects of different diseases. As reported, TOP2A, RRM2, NEK2, CDK1, and CCNB1 are introduced as prognostic biomarkers of liver cancer via bioinformatic analysis (14). In the present study,

network analysis is applied to discover possible biomarker candidates related to liver cancer. As depicted in Figure 1, the dysregulated proteins related to liver cancer participate in a network via different strengths (different numbers of connections). The nodes of the illustrated network have appeared as elements with different centrality properties. The distribution of central parameters of the analyzed nodes of the network that are presented in Figures 2-4 indicates that a few central nodes are highlighted from the other nodes by higher values of centrality amounts. These highlighted nodes are introduced as the central nodes in Table 1. As it is shown in Table 1, GAPDH, TP53, EGFR, MYC, INS, ALB, IL6, AKT1, VEGFA, CDH1, and HRAS are the 11 central nodes of the liver cancer network.

Gene ontology revealed that three classes of biological terms, including 21 biochemical pathways, are related to the central nodes. As it is presented in Figure 6, no biological terms are related to albumin. Two potent central nodes are seen in Figure 6, which are characterized by large connections with the introduced biological terms. These central nodes are AKT1 and HRAS. AKT1 and HRAS are connected to most of the identified biochemical pathways.

Some documents refer to the significant role of Akt1 in regulating the development of inflammation and fibrosis accompanied by alcoholic liver disease (21). Xu Z et al. concluded that AKT1 upregulation is accompanied by poor survival in patients with hepatocellular carcinoma (22). JX Zhao et al. reported that the progress of hepatocellular carcinoma is associated with the up-regulation of aldose reductase. They showed that a higher value of aldolase reductase leads to dysregulation of AKT1, which increases AKT/mTOR signaling (23).

Researchers report the crucial role of HRAS in energy homeostasis. It is shown that the gonadal fat pad weight of the adipose-specific HRAS transgenic mice is reduced, and the adipocyte size is condensed (24). V Pecenka et al. published data about the activation of HRAS in liver tumors (25).

B Xin et al. published a document about the role of AKT and HRAS in inducing hepatocarcinogenesis via the activation of endogenous Myc. In this report, it is discussed that MYC, as an enhancing factor, facilitates the hepatocarcinogenesis process via alterations in the metabolism of the cells (26).

The HIF-1 signaling pathway appears in Figures 5 and 6 as a class of related biochemical pathways. J Han et al. assessed the role of the hypoxia-inducible factor 1 (HIF-1) signaling pathway in promoting liver fibrosis (27). DKC Chiu et al. showed that HIF/HEY1/PINK1 pathway regulates mitochondrial activity in hepatocellular carcinoma cells (28). The key elements in liver cancer progression are the two highlighted proteins AKT1 and HRAS and the related biochemical pathways.

The other two biochemical pathways related to the central nodes of the liver cancer network are prostate and bladder cancers (see Figures 5 and 6). The investigation by Bubendorf et al. indicates that the liver and two other organs (lung and bone) are the most frequent locations of distant prostate cancer metastases (29). This finding corresponds to the results of gene ontology in the present assessment.

Conclusion

Our assessment revealed that AKT1 and HRAS are suitable pair of biomarker candidates for liver cancer. The three biochemical pathways ("HIF-1 signaling pathway", "prostate cancer", and "bladder cancer") are the three common pathways in liver cancer, and therefore "HIF-1 signaling pathway" can be a possible drug target in the treatment of liver cancer.

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Conflict of interests

There is no conflict of interest.

References

1. Fu J, Wang H. Precision diagnosis and treatment of liver cancer in China. Cancer Lett 2018;412:283-288.

2. Billington S, Ray AS, Salphati L, Xiao G, Chu X, Humphreys WG, et al. Transporter expression in noncancerous and cancerous liver tissue from donors with hepatocellular carcinoma and chronic hepatitis C infection quantified by LC-MS/MS proteomics. Drug Metab Dispos 2018;46:189-196.

3. Jiang Y, Sun A, Zhao Y, Ying W, Sun H, Yang X, et al. Proteomics identifies new therapeutic targets of early-stage hepatocellular carcinoma. Nature 2019;567:257-261.

4. Liu J, Dang H, Wang XW. The significance of intertumor and intratumor heterogeneity in liver cancer. Exp Mol Med 2018;50:416.

5. Carulli JP, Artinger M, Swain PM, Root CD, Chee L, Tulig C, et al. High throughput analysis of differential gene expression. J Cell Biochem 1998;72:286-296.

6. Büssow K, Nordhoff E, Lübbert C, Lehrach H, Walter G. A human cDNA library for high-throughput protein expression screening. Genomics 2000;65:1-8.

7. Wu J, Vallenius T, Ovaska K, Westermarck J, Mäkelä TP, Hautaniemi S. Integrated network analysis platform for protein-protein interactions. Nat Methods 2009;6:75-77.

8. Ideker T, Sharan R. Protein networks in disease. Genome Res 2008;18:644-652.

9. Zhang Q, Ma C, Gearing M, Wang PG, Chin L-S, Li L. Integrated proteomics and network analysis identifies protein hubs and network alterations in Alzheimer's disease. Acta Neuropathol Commun 2018;6:1-19.

10. Zotenko E, Mestre J, O'Leary DP, Przytycka TM. Why do hubs in the yeast protein interaction network tend to be essential: reexamining the connection between the network topology and essentiality. PLoS Comput Biol 2008;4:1000140.

11. Kar G, Gursoy A, Keskin O. Human cancer protein-protein interaction network: a structural perspective. PLoS Comput Biol 2009;5:1000601.

12. Zamanian-Azodi M, Rezaei-Tavirani M, Rahmati-Rad S, Hasanzadeh H, Tavirani MR, Seyyedi SS. Protein-Protein Interaction Network could reveal the relationship between the breast and colon cancer. Gastroenterol Hepatol Bed Bench 2015;8:215.

13. Rezaei-Tavirani M, Mansouri V, Tavirani MR, Rostami-Nejad M, Bashash D, Azodi MZ. Gene and biochemical pathway evaluation of burns injury via protein-protein interaction network analysis. Galen Med J 2019;8:1257.

14. Nguyen TB, Do DN, Nguyen-Thanh T, Tatipamula VB, Nguyen HT. Identification of five hub genes as key prognostic biomarkers in liver cancer via integrated bioinformatics analysis. Biology 2021;10:957.

15. Azodi MZ, khatoon Hajisayyah S, Razzaghi Z, Tavirani MR. Introducing physical exercise as a potential strategy in liver cancer prevention and development. Gastroenterol Hepatol Bed Bench 2021.

16. Szklarczyk D, Gable AL, Nastou KC, Lyon D, Kirsch R, Pyysalo S, et al. The STRING database in 2021: customizable protein–protein networks, and functional characterization of user-uploaded gene/measurement sets. Nucleic Acids Res 2021;49:605-612.

17. Asadzadeh-Aghdaei H, Okhovatian F, Razzaghi Z, Heidari M, Vafaee R, Nikzamir A. Radiation therapy in patients with brain cancer: post-proteomics interpretation. J Lasers Med Sci 2019;10:59.

18. Azodi MZ, Arjmand B, Zali A, Razzaghi M. Introducing APOA1 as a key protein in COVID-19 infection: a bioinformatics approach. Gastroenterol Hepatol Bed Bench 2020;13:367.

19. Lu X, Paliogiannis P, Calvisi DF, Chen X. Role of the mammalian target of rapamycin pathway in liver cancer: from molecular genetics to targeted therapies. Hepatology 2021;73:49-61.

20. Lu SC. Dysregulation of glutathione synthesis in liver disease. Liv Res 2020;4:64-73.

21. Reyes-Gordillo K, Shah R, Arellanes-Robledo J, Cheng Y, Ibrahim J, Tuma PL. Akt1 and Akt2 isoforms play distinct roles in regulating the development of inflammation and fibrosis associated with alcoholic liver disease. Cells 2019;8:1337.

22. Xu Z, Xu M, Liu P, Zhang S, Shang R, Qiao Y, et al. The mTORC2-Akt1 cascade is crucial for c-Myc to promote hepatocarcinogenesis in mice and humans. Hepatology 2019;70:1600-1613.

23. Zhao J-X, Yuan Y-W, Cai C-F, Shen D-Y, Chen M-L, Ye F, et al. Aldose reductase interacts with AKT1 to augment hepatic AKT/mTOR signaling and promote hepatocarcinogenesis. Oncotarget 2017;8:66987.

24. Park J-C, Jeong W-J, Seo SH, Choi K-Y. WDR76 mediates obesity and hepatic steatosis via HRas destabilization. Sci Rep 2019;9:1-12.

25. Pecenka V, Pajer P, Karafiat V, Kasparova P, Dudlova J, Dvorak M. HRAS, EGFR, MET, and RON genes are recurrently activated by provirus insertion in liver tumors induced by the retrovirus myeloblastosis-associated virus 2. J Virol 2017;91:00467-17.

26. Xin B, Yamamoto M, Fujii K, Ooshio T, Chen X, Okada Y, et al. Critical role of Myc activation in mouse hepatocarcinogenesis induced by the activation of AKT and RAS pathways. Oncogene 2017;36:5087-5097.

27. Han J, He Y, Zhao H, Xu X. Hypoxia inducible factor-1 promotes liver fibrosis in nonalcoholic fatty liver disease by activating PTEN/p65 signaling pathway. J Cell Biochem 2019;120:14735-14744.

28. Chiu DK-C, Tse AP-W, Law C-T, Xu IM-J, Lee D, Chen M, et al. Hypoxia regulates the mitochondrial activity of hepatocellular carcinoma cells through HIF/HEY1/PINK1 pathway. Cell Death Dis 2019;10:1-16.

29. Bubendorf L, Schöpfer A, Wagner U, Sauter G, Moch H, Willi N, et al. Metastatic patterns of prostate cancer: an autopsy study of 1,589 patients. Hum Pathol 2000;31:578-583.