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Original Research

Selective Gene Up- and Down-Regulation as Potential Predictors of the Behavior of HBV-Associated Hepatocellular Carcinoma?

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Abstract

Objectives: The current study aimed to identify genes that show differential expression in tumor tissue by performing bioinformatic analysis from matched tumor and non-tumoral liver tissue samples obtained from HBV- HCC patients.

Methods: mRNA data from 21 HBV-HCC patients were used in this open-access database-based study. The mRNA sequence data were obtained from 21 pairs of tumors and non-tumoral liver tissue samples. Gene expression analysis was used in bioinformatics analyses and log2FC value was used to identify genes showing up- and down-regulation. To illustrate differentially expressed genes, the volcano plot was utilized.

Results: Our analysis showed that many genes showed quite different expression levels in tumor tissues. Among these genes, the genes that showed very high fold upregulation were GNG4, IGF2BP1, GPC3, PEG10, AFP, SPINK1, EPS8L3, MYCN, DUSP9, and DKK1 genes, respectively. The down-regulated genes were CNDP1, WAKMAR1, LINC01818, TH, LINC01093, MARCO, LOC101927078, LOC105372263, FCN2, and CLEC4M.

Conclusion: Our study defined various genes that might be utilized as potential biomarkers for HBV-related HCC. Targeted treatment for these genes can be developed and verified for efficacy in treatment.

Keywords: Biological behavior, Down-regulated genes, Hepatocellular carcinoma, Hepatitis B infection, Up-regulated genes

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Nearly two billion individuals worldwide are infected with the hepatitis B virus (HBV). At this point, there are around 350 million carriers, HBV is one of the most common public health problems, especially in the Middle East and Eastern Asia.[1] Even though the HBV vaccine is efficient and safe, and it has decreased the incidence of HCC to a certain extent, HBV infection remains the main etiologic factor for hepatocellular carcinoma (HCC) globally. More than half of all HCC cases are caused by HBV-related chronic liver disease.[2, 3] HBV surface antigen (HBsAg) carriers had a 25-37-fold higher lifetime risk of developing HCC than non-infected persons, according to recent research in Asia and North America.[4, 5] HCC risk is known to be increased by viral load;[6-8] however, other variables may be involved. Several studies in extremely prevalent areas, notably Mainland China and South Africa, have discovered a synergistic link between HBV infection and aflatoxin B1.[9]

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HCC is the seventh most common cancer disease and the third main cause of cancer deaths globally. According to a recent epidemiological analysis, the incidence of liver cancer is rising and will continue to rise, globally. This is attributed to the rising incidence of fatty liver disease and Non-Alcoholic Steatohepatitis (NASH).[10] HCC has non-specific symptoms and the clinical diagnosis of the patients is difficult. The most important therapeutic approach is screening and treatment at an early stage.[11] However, there is no cost-effective screening protocol. For these reasons, the majority of the patients are diagnosed at a later stage, and these important results in poor prognosis for the patients. Advances in the molecular basis of HCC are of vital importance in these circumstances because it enables scientists to develop biomarkers for early diagnosis of the tumors and novel targeted therapeutics with higher potential for treatment.[12,13] The genetic and molecular characteristics of HCC are complex. There is a vast amount of intricate genetic changes that play a role in the pathogenesis of HCC. During hepatocarcinogenesis, point mutations, chromosomal amplification/deletions, gene methylations, and histone deacetylations contribute to hepatocarcinogenesis by disrupting the regulation of signal transduction pathways. Molecular studies on HCC have revealed that activation of cellular oncogenes, inactivation of tumor suppressor genes, and disruptions in signaling pathways such as PI3K/AKT, Wnt/-catenin, Ras, p53, pRB, , MAPK, STAT, JAK, and HGF/cMET all play important roles in the development, progression, and metastasis of HCC.

With the widespread use of genome-wide sequencing techniques, significant progress has been made in examining the genetic factors that play critical functions in diseases. Studies have focused on revealing the genetic mechanisms of diseases that have high mortality, are important public health problems, and have led to the determination of more efficient diagnostic biomarkers. Determining the prognostic importance of a new biomarker that plays a major role in carcinogenesis might inspire translational research to create targeted treatments against these new therapeutic indicators and their corresponding signaling pathways. In addition, such a biomarker can also help clinicians predict the success of transplantation and determine the nature of the immune response.

Global gene expression study provides quantitative information on the RNA types and quantity in the cells and tissues. It is a very effective research technique for investigating the fundamental biology of diseases, developing diagnostic markers, facilitating drug discovery and surveillance of response to therapy, and creating databases with information on biological processes. Consequently, expression analysis has become one of the most widely used methods in modern biology.[14] This study aims to determine the genes that may play a role in the genomic mechanism and are expressed differently in tumor tissues by mRNA sequence analysis of tumor and distant non-neoplastic liver tissues from HBV-related HCC patients.

Methods

Dataset

In the current study, we used an open-access dataset obtained by sequencing the mRNAs of 21 pairs of tumor and non-tumoral liver tissues obtained from patients with HBV-related HCC (HBV- HCC). These 21 pairs of tumors and non-tumoral liver tissues that were employed for RNA sequencing were obtained from HBV-HCC patients who underwent primary surgical resection between 2008 and 2013 at Mount Sinai Medical Center in New York, USA.[14] RNAs obtained from tissues were utilized for cDNA library preparation after quality control. The resulting library was subjected to purification. Before loading onto the sequencer, the size and concentration of the RNAseq collection were evaluated using a Bioanalyzer and Qubit fluorometry (Life Technologies, NY, USA) and compared to human transcript reference sequences from the ENSEMBLE database.[15]

Bioinformatics and Gene Expression Analyses

Bioinformatics generally includes collecting, storing, organizing, archiving, analyzing, and presenting the results by visualizing the data obtained based on both theory and practice in a field in biology, medicine, behavioral or health sciences. In addition, it is concerned with the research and development of computational tools and approaches to expand the use and processing of data obtained as a result of studies, or the application of known methods. In bioinformatics, analyses are made by selecting a database and a program that allows the performance of bioinformatic analysis following the biological question, molecule, or structure to be examined. The data and results obtained at the end of the analyses are blended in the light of the previously defined information about the subject in the literature and interpreted analytically.[16]

Changes in the physiology of an organism or cell will cause changes in the pattern of gene expression, making gene expression analysis significant in many disciplines of biological inquiry. By hybridizing mRNA to a high-density array of immobilized target sequences, each of which corresponds to a different gene, DNA microarray technology is used to examine gene expression. The impact of substances on gene expression, for example, can provide information on their functional and toxicological properties. Expression investigations on clinical samples, both normal and sick, may lead to the discovery of new biomarkers.[17]

Bioinformatics Analysis

In this study, gene expression analyses were performed on mRNA data obtained from 21 pairs of samples. In the investigation, the Limma package, which is accessible in the R coding language and enables expression analysis, was employed (18Limma (Linear Models for Microarray Analysis) is a library for analyzing gene expression microarray data, with a focus on using linear models to evaluate specific experiments and determine differential expression. The packet's functionalities apply to all gene expression methods, such as microarrays, RNA-seq, and quantitative PCR. The obtained results are displayed in the form of a table of genes ranked in order of importance and a graph depicting differentially expressed genes. Genes with the lowest p values are the most trustworthy, and the results table contains corrected P and log2-fold change (log2FC) values. Up-regulated genes were identified using log2FC >1 and a p-value of 0.05, whereas down-regulated genes were found using log2FC -1 and a p-value of 0.05. The volcano graph and Mean difference (MD) graph were used to show differentially expressed genes in the study. Uniform Manifold Approximation and Projection (UMAP) graphs were preferred to visualize the relations of the samples in the study with each other. In addition, a boxplot was used to show the distribution of each sample.

Study Protocol and Ethics Approval

The National Center for Biotechnology Information (NCBI) Gene Expression Omnibus open-access dataset was used in this work, which involved human participants and was established in compliance with institutional and national research committee ethical requirements. Since the open-access dataset was utilized, no approval from the local ethics commission was required.

Results

The mRNA sequence data used in the current study contains 26760 expressions. There were 21 pairs of samples which make up 42 in total, and the graph of the distribution of these samples is given in Figure 1. The UMAP graph that shows how the samples are related to each other is given in Figure 2. According to the gene expression analyses performed, statistically important differences were determined between both groups in the level of gene expression ($|log2FC| > 1.0$, $p<0.05$). According to the results of the bioinformatics analysis, the GNG4 gene has 165.42-fold higher gene expression in tumor tissues than in non-tumoral liver tissues. Likewise, IGF2BP1, GPC3, PEG10, AFP, SPINK1, EPS8L3, MYCN, DUSP9, and DKK1 genes had higher gene expression in the tumors of 143.01, 83.86, 62.24, 60.54, 53.81, 52.70, 42.25, 43.41, 40.22-fold, respectively. The CNDP1 gene, on the other hand, has 49.86-fold lower gene expression in tumor tissues than in non-tumoral liver tissues. Similarly, WAKMAR1, LINC01818, TH, LINC01093, MARCO, LOC101927078, LOC105372263, FCN2, and CLEC4M genes have 43.11, 40.22, 39.67, 38.85, 36.75, 36.50, 36.25, 35.01, 33.59-fold lower gene expression, respectively.

Information on the first 20 genes showing an increase and decrease in expression between the two groups is given in Tables 1 and Table 2. Figure 3 depicts the volcano plot utilized to display the differentially expressed genes between groups.

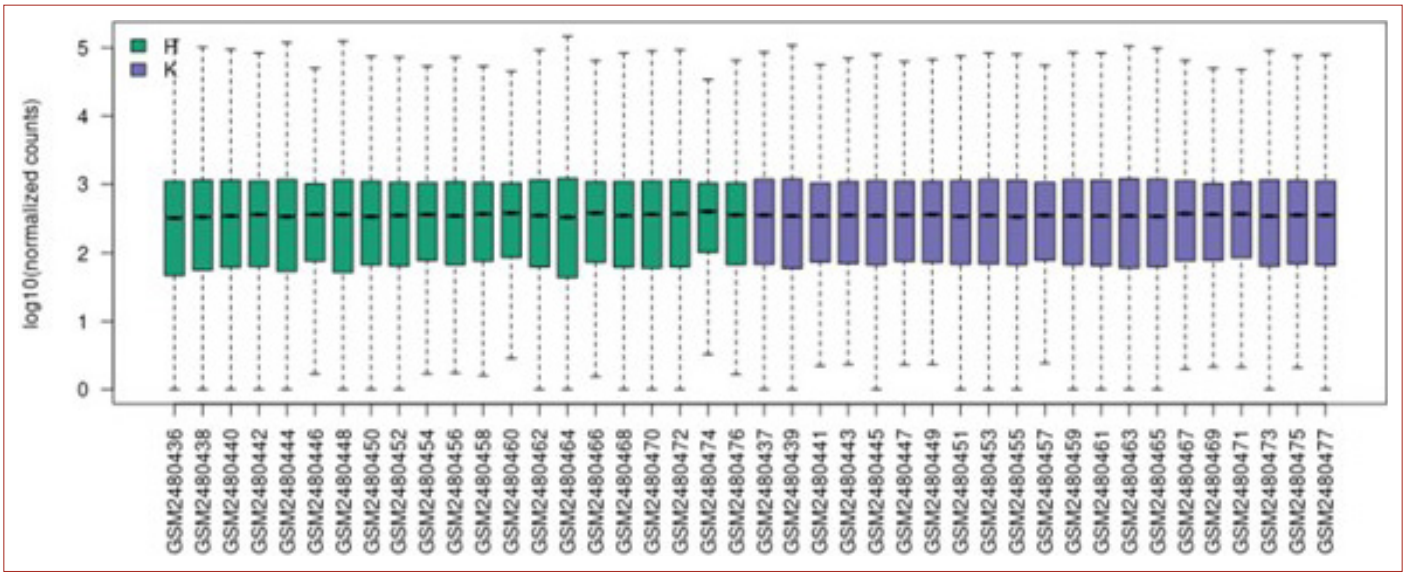

Figure 1. The distribution of the values of the selected Samples.

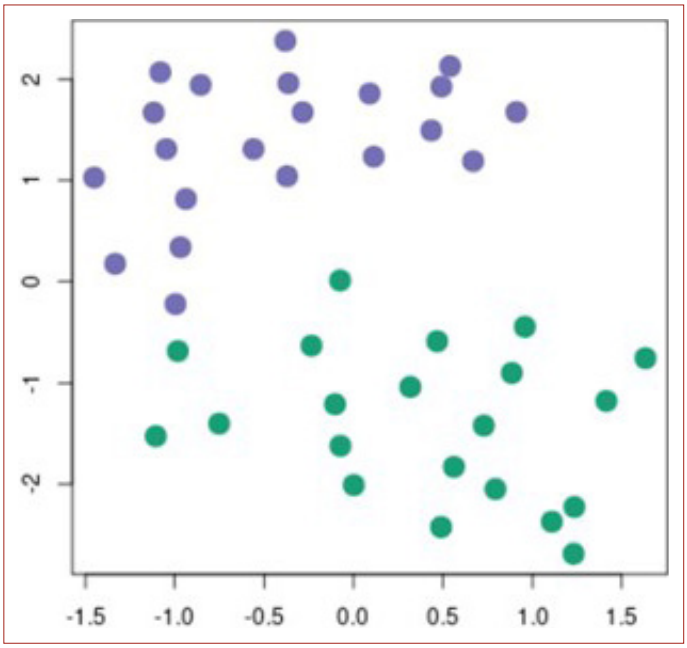

Figure 2. UMAP plot of the samples (Green Dots: Tumor specimens, Purple dots: non-neoplastic liver tissues samples).

Discussion

HCC is a frequent cause of cancer-related mortality which accounts for the fourth most prevalent cause of cancer-associated mortality.[19,20] The incidence and mortality of HCC vary significantly across the world. This can be attributed to regional variations in the duration and intensity of exposure to environmental and infectious factors. Also, accessibility to medical resources and the ability to identify HCC in its earlier stages. All these enable patients to receive potentially curative treatment. In Eastern Asia and Sub-Saharan Africa, low- and middle-income countries account for nearly 80% of all HCC cases.[21,22] Alcoholism, HBV, non-alcoholic fatty liver disease, HCV, and dietary toxins are all potential risk factors for HCC development.[20] HBV and HCV infection are the most common causes of HCC, accounting for 4 in 5 of all HCC cases globally. With the exception of Northern Africa, where HCV prevalence is highest, HBV infection is the leading cause of this cancer across Eastern Asia and the majority of African countries.[21,22] It is predicted that 257 million people globally are infected with chronic HBV. As a result of this condition, chronic viral liver disease and HCC are extremely common. Between 2015 and 2030, HBV is predicted to cause 20 million deaths due to cirrhosis, acute or chronic hepatitis, and HCC, with HCC alone accounting for 5 million deaths.[20]

The annual incidence of HCC in cirrhotic people due to chronic HBV or HCV infection is between 2 and 5%. HBV-related HCC can occur even in the absence of liver cirrhosis and accounts for 30 to 50% of HCC cases in HBV-endemic

Table 1. Up-regulated genes in tumor tissues relative to non-tumoral liver tissue samples

GeneID	PADJ	p	Log2FoldChange	Symbol	Description
2786	2,41E-30	1,29E-32	7,3706792	GNG4	G protein subunit gamma 4
10642	7,36E-32	3,58E-34	7,1650115	IGF2BP1	Insulin like growth factor 2 mRNA binding protein 1
2719	1,08E-38	3,12E-41	6,3956802	GPC3	Glypican 3
23089	1,56E-21	1,89E-23	5,9686012	PEG10	Paternally expressed 10
174	1,05E-15	2,74E-17	5,9220562	AFP	Alpha- fetoprotein
6690	1,76E-15	4,82E-17	5,753456	SPINK1	Serine peptidase inhibitor Kazal type 1
79574	2,47E-19	3,95E-21	5,7214354	EPS8L3	EPS8 like 3
4613	3,53E-21	4,48E-23	5,5005538	MYCN	MYCN proto-oncogene, bHLH transcription factor
1852	4,62E-22	5,39E-24	5,4477672	DUSP9	Dual specificity phosphatase 9
22943	4,78E-12	2,39E-13	5,3304587	DKK1	dickkopf WNT signaling pathway inhibitor 1
5865	8,46E-19	1,44E-20	5,2286698	RAB3B	RAB3B, member RAS oncogene family
4751	6,43E-46	6,05E-49	5,1911583	NEK2	NIMA related kinase 2
29944	3,08E-19	4,97E-21	5,0813647	PNMA3	PNMA family member 3
256714	9,87E-16	2,56E-17	4,952008	MAP7D2	MAP7 domain containing 2
2563	2,86E-30	1,56E-32	4,9170355	GABRD	gamma-aminobutyric acid type A receptor subunit delta
1063	6,85E-55	7,17E-59	4,8550639	CENPF	Centromere protein F
11082	1,11E-28	6,96E-31	4,8386647	ESM1	Endothelial cell specific molecule 1
114794	1,53E-14	4,84E-16	4,8192059	ELFN2	Extracellular leucine rich repeat and fibronectin type III domain containing 2
220134	2,74E-41	6,17E-44	4,8055116	SKA1	Spindle and kinetochore associated complex subunit 1
55872	1,04E-33	4,29E-36	4,7363249	PBK	PDZ binding kinase

Table 2. Down- regulated genes in tumor tissues relative to non-tumoral liver tissue samples

GeneID	PADJ	p	Log2FoldChange	Symbol	Description
84735	1,58E-26	1,23E-28	-5,64658	CNDP1	Carnosine Dipeptidase 1
105372576	4,04E-19	6,60E-21	-5,43271	WAKMAR1	wound and keratinocyte migration associated lncRNA 1
105373684	5,16E-36	1,73E-38	-5,33103	LINC01818	long intergenic non-protein coding RNA 1818
7054	5,59E-14	1,96E-15	-5,31748	TH	Tyrosine hydroxylase
100506229	9,32E-27	7,17E-29	-5,28676	LINC01093	long intergenic non-protein coding RNA 1093
8685	8,90E-20	1,32E-21	-5,2043	MARCO	macrophage receptor with collagenous structure
101927078	9,88E-38	2,95E-40	-5,19588	LOC101927078	uncharacterized LOC101927078
105372263	5,50E-16	1,37E-17	-5,18188	LOC105372263	uncharacterized LOC105372263
2220	2,38E-19	3,78E-21	-5,13387	FCN2	Ficolin 2
10332	5,38E-16	1,33E-17	-5,07029	CLEC4M	C-type lectin domain family 4 member M
3781	9,27E-42	1,99E-44	-5,01768	KCNN2	potassium calcium-activated channel subfamily N member 2
339390	2,76E-16	6,50E-18	-4,89947	CLEC4G	C-type lectin domain family 4 member G
5816	3,19E-40	8,01E-43	-4,80921	PVALB	Parvalbumin
51266	7,60E-20	1,11E-21	-4,72287	CLEC1B	C-type lectin domain family 1 member B
107985462	2,43E-14	8,00E-16	-4,68485	LOC107985462	uncharacterized LOC107985462
8547	1,61E-19	2,49E-21	-4,58575	FCN3	Ficolin 3
101928384	3,80E-19	6,18E-21	-4,58488	LOC101928384	uncharacterized LOC101928384
102724019	1,94E-12	8,99E-14	-4,55773	LOC102724019	uncharacterized LOC102724019
143941	6,83E-25	6,25E-27	-4,42997	TTC36	tetratricopeptide repeat domain 36
27302	2,80E-13	1,11E-14	-4,4283	BMP10	bone morphogenetic protein 10

areas.[23] HBV infection is responsible for 30% and 45% of people with liver cirrhosis and HCC, respectively, worldwide.[24,25]

The total survival of HCC patients is low, and prevention of exposure to the risk factors is important to reduce the global burden of the disease. There is a rising interest in identifying diagnostic and prognostic markers, and new and effective targeted treatments. Furthermore, research aimed at defining the mechanisms of hepatocyte carcinogenesis and means of increasing the clinical care of patients with HCC is also a very popular subject.[20,26]

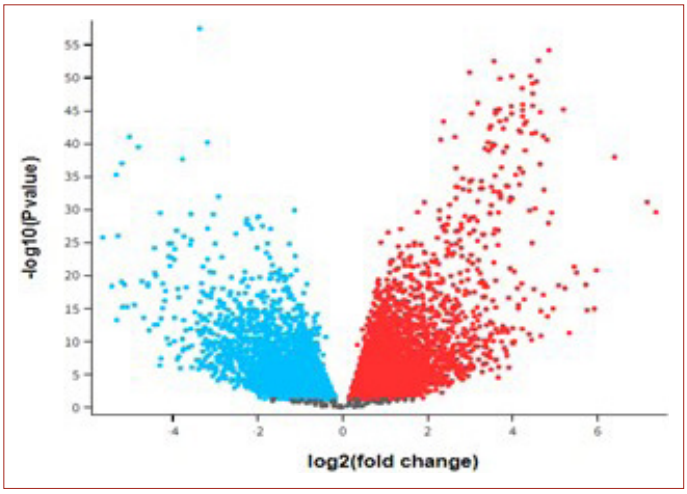

Figure 3. Volcano plot of genes with increased (red dots) and decreased (blue dots) gene expression among the group of tumorous and non-neoplastic liver tissues.

The current study examined liver tissues from HBV- HCC patients and utilized mRNA sequence data to identify genes with differences in gene expression between tumor tissues and non-neoplastic liver tissues. For this purpose, gene expression analysis was applied to the data set as a bioinformatic analysis, and the genes that differed between the two groups were determined. According to the results of the bioinformatics analysis, the GNG4 gene, IGF2BP1, GPC3, PEG10, AFP, SPINK1, EPS8L3, MYCN, DUSP9, and DKK1 genes had higher gene expression in the tumors. The CNDP1 gene had lower expression in tumor tissues than in non-tumoral liver tissues. Similarly, WAKMAR1, LINC01818, TH, LINC01093, MARCO, LOC101927078, LOC105372263, FCN2, and CLEC4M genes had lower expression in the tumors.

It has been shown that the abnormal expression of the GNG4 gene plays a significant role in the development of HCC.[27] GNG4 is upregulated in the HCC cells.[28] Further-

more, the GNG4 gene was shown to be among the differentially expressed genes for HBV-related HCC.[29] In a clinical study, IGF2BP1 was up-regulated in tumor tissues at a very high fold in the case of HCC.[30] Several studies have also been undertaken to examine the link between the IGF2BP1 gene and HCC.[31,32] Many studies on the GPC3 gene have also supported the idea that this gene can be a reliable biomarker for HCC, and this gene has shown upregulation in HCC.[33-35] Likewise, the PEG10 gene has been defined as the gene that plays a role in the development of HCC and is one of the genes that is upregulated in the case of HCC.[36,37] The CNDP1 gene is significantly downregulated in HCC.[38] It has been shown that downregulation of MORCO was associated with tumor progression in HCC.[39]

HCC, like all other cancers, has gene mutations and mutation-related mRNA expression alterations. As demonstrated by the findings of this study, the potential alterations in the expression of genes are highly related to HCC, and patients who have these genetic mutations are susceptible to HCC. Therefore, a surveillance program is required as well as preventive measures should be taken.

Conclusion

This study identified differentially expressed genes for tumor and non-tumoral liver tissue using genomic data from HBV- HCC patients. With further and comprehensive analysis of the target genes that we have defined, significant biomarkers for HCC can be developed. In addition, with the use of these biomarkers in clinical practice, the patient treatment approaches might be determined and personalized treatment strategies may be developed.

Disclosures

Ethics Committee Approval: This article was produced from the NCBI open-access dataset. Therefore, it has been reported by the institute that ethics committee approval is not required.

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship Contributions: Concept – Z.K., S.A.; Design – Z.K., S.A., C.C.; Supervision – C.C.; Materials – Z.K., S.A.; Data collection &/or processing – Z.K., T.T.S.; Analysis and/or interpretation – Z.K., S.A; Literature search – Z.K., T.T.S., S.A; Writing – Z.K., S.A; Critical review – S.A., C.C.

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