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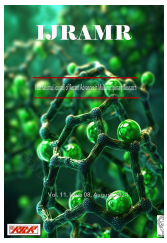

RESEARCH ARTICLE

THE IMPORTANCE OF GENETIC BACKGROUND IN RECURRENT HCC AND THE BIOINFORMATICS ANALYSIS OF GENE DATA ASSOCIATED WITH RECURRENT HCC

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ABSTRACT

Aim: Known for its high recurrence rates and potential to metastasize and recur after liver transplantation or hepatectomy, HCC requires effective management, comprehensive surveillance and specialized therapies. Bioinformatics and machine learning play a key role in analyzing large datasets to reveal genetic and molecular insights into HCC metastasis and help identify potential biomarkers and therapeutic targets. This study aims to identify biomarkers associated with HCC recurrence by analyzing gene expression in primary and recurrent tumor tissues. **Material and methods:** The dataset included in the study comprises gene expression data from both recurrent and primary HCC tissue. The gene expression analysis of this data set was conducted using the capabilities provided by the limma package. The distribution of each tissue in the dataset is shown by the distribution graph and the expression density graph. The UMAP graph represents the association of tissue types. The genes exhibiting different regulation are represented in the volcano plot. **Results:** The UMAP analysis revealed a perfect separation of the tissues in the dataset into two distinct groups: recurrent tumor tissues and primer tissues. The analysis showed that many genes differed in both groups under log2FC>1 p<0.05 and log2FC<-1 and p<0.05 conditions. The resultsshow that there are genes that are upregulated in recurrent tissues compared to primary tissues and no downregulated genes. **Conclusion:** Genetic research is crucial for advancing the treatment of recurrent hepatocellular carcinoma (HCC). Identified genes may serve as biomarkers, aiding in the development of targeted drug therapies and improving patient care and healthcare efficiency. As genetic research progresses, the use of these biomarkers is expected to enhance personalized medicine. Understanding the genetic basis of recurrent HCC is essential for prevention and treatment, leading to more effective strategies and early detection for high-risk individuals. Future advancements in genetic research are anticipated to yield innovative methods for preventing and treating recurrent HCC.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is a type of liver cancer that originates in the liver and is caused by a combination of genetic and non-genetic factors. These factors include persistent alcohol misuse, hepatitis B and C infections, and liver cirrhosis(1, 2).The development of HCC is intricate, encompassing the interaction of several genetic changes, non-genetic variables, and the existence of liver cancer stem cells(3).It is important to mention that the occurrence of HCC can be preceded by a condition called non-alcoholic steatohepatitis (NASH), which is defined by the accumulation of fat in the liver, inflammation, and scarring(4).

Extensive research has been conducted on diagnostic and therapeutic approaches for HCC. Alpha-fetoprotein (AFP), glypican-3, and vascular endothelial growth factor (VEGF) are biomarkers that are essential for the diagnosis and prognosis of HCC (5-7).Furthermore, the discovery of genes that contribute to chemoresistance and the investigation of innovative therapeutic approaches, such as the combination of lenvatinib with transarterial chemoembolization, demonstrate the continuous endeavors to enhance the management of HCC (7, 8).Research has focused on understanding the molecular mechanisms that drive the advancement of hepatocellular carcinoma (HCC). Studies have shown that signaling pathways such as JAK/STAT, PI3K/AKT, and Notch1-Stat3

are involved in the growth and spread of HCC(2, 9). Moreover, the disruption of cancer-causing signaling pathways and the presence of certain proteins such as LECT2 have been linked to the development of HCC. The presence of metastasis in HCC is an essential component of the disease, which has a considerable influence on the prognosis of patients and the therapeutic techniques that are utilized. The capacity of HCC to metastasis to atypical places is a clear indication of the complexity of the disease, which highlights the importance of employing methodological techniques that are thorough(10).Recurrent liver metastases in hepatocellular carcinoma (HCC) present a substantial difficulty in clinical management, affecting patient outcomes and therapeutic approaches. Research has indicated that tumor recurrence following liver transplantation for hepatocellular carcinoma (HCC) is a frequent event, particularly in individuals with advanced illness(11-13).The rates of recurrence following hepatectomy for hepatocellular carcinoma (HCC) are very high, with data suggesting that recurrence surpasses 60% over a span of 5 years. This emphasizes the importance of implementing efficient surveillance and treatment approaches for managing recurrent disease(14).Overall, the occurrence of liver metastases in HCC is a complicated clinical situation that necessitates a thorough approach. This approach should take into account the many locations of metastasis, the frequent recurrence rates, and the importance of tailoring treatment techniques to individual patients in order to enhance their results.

Bioinformatics analyses handle high-volume gene expression data and other omics data, allowing for a comprehensive genetic and molecular profile linked to HCC(15, 16). Large datasets and complicated computer approaches are frequently used to examine this data, allowing for detailed understanding of the biological processes of HCC. Machine learning algorithms are highly successful at uncovering concealed links and patterns within extensive data sets. These algorithms facilitate the comprehension of the genetic and biochemical underpinnings of the disease by employing data mining and predictive modeling techniques to pinpoint molecular pathways linked to the advancement and spread of HCC. Specifically, comprehending the molecular pathways that cause HCC metastasis is an essential stride in the advancement of accurate therapeutic therapies. The integration of bioinformatics and machine learning methodologies provides a potent approach to uncover the relationships and functions of genes, proteins, and other biomolecules involved in this process. By doing an analysis of different gene expression profiling and protein interaction networks, it is possible to identify the crucial molecules and signaling pathways that enhance the metastatic characteristics of hepatocellular carcinoma (HCC). This information lays the foundation for the advancement of specific therapy methods and individualized treatment plans. Ultimately, the incorporation of bioinformatics and machine learning methodologies in HCC research has the potential to profoundly transform our comprehension of the molecular underpinnings of the illness and pinpoint viable therapy targets. Therefore, in this study, tissue samples from primary hcc tumors and recurrent tumors were examined by expression analysis to identify possible biomarkers for the recurrent state. For this purpose, regulated genes in recurrent tissues were identified and analyzed.

MATERIAL AND METHODS

Dataset: The dataset used in the study included patients who underwent liver transplantation for HCC. To create the dataset, paired FFPE tumor samples were collected from seven patients at both initial resection and repeat. Total RNA was extracted from the collected tumors for gene expression analysis using the Nano String nCounter Tumor Signaling 360 Panel. The data set used in the study was obtained from the National Center for Biotechnology Information (NCBI). The current code of the data set in NCBI is GSE102340.

RNA-Sequence Analysis (RNA-Seq): RNA sequence analysis technologies, a renowned and effective method, have demonstrated exceptional results in describing all RNA transcripts generated by cells. RNA-seq is a groundbreaking technology in transcriptomics that enables the comprehensive and quantitative investigation of entire transcriptomes. It is the first sequencing-based approach that allows researchers to uncover an organism's complete set of RNA transcripts. Unlike methods based on hybridization, the RNA-seq method not only focuses on identifying existing transcripts, but also strives to find and associate new transcripts while investigating and disclosing known ones. One significant benefit of the RNA-seq method is its ability to assess expression levels across a broad and fluctuating range, as opposed to measuring only relative values. RNA-seq methods offer significant benefits, such as minimal background contamination, leading to highly accurate and reliable data. Additionally, these methods allow for precise determination of exon and intron boundaries, as well as the identification of SNPs and other variants within transcripts. RNA-seq possesses numerous benefits, rendering it a highly suitable technique for most research endeavors focused on discovery**(17).**

Transcriptomics: The transcriptome refers to the complete set of RNA molecules, including mRNA, tRNA, rRNA, and non-coding RNAs, that are generated by the genome of a cell, tissue, or organism within a specific timeframe. Transcriptomes exhibit a highly dynamic structure and undergo ongoing changes due to variations in gene expression. Unlike the relatively stable genome of a cell, the transcriptome is susceptible to alterations caused by several environmental conditions, including fluctuations in pH, variations in nutrition availability, shifts in temperature, and interactions with signals from neighboring cells. Transcription of genes associated with these functions might vary in response to diverse cellular activities, leading to changes in the transcriptome, which encompasses all the mRNAs present in the cell. Consequently, the transcriptome provides a snapshot of the genes that are actively functioning in a specific moment and context. The identification of alterations in gene expression caused by environmental variables has highlighted the significance of interactions between the environment and biological systems (17, 18).

Transcriptomics is a scientific discipline that investigates the complete set of mRNA transcripts produced by the transcription process in a cell's genome, and provides insights on their patterns of expression. Microarray and next-generation sequencing are advanced technologies commonly employed in transcriptomics applications. These technologies

enable the examination of particular changes in the transcriptome that occur at specific periods and for specific reasons (18). In recent times, transcriptomics studies have gained significant importance and their prevalence has notably risen. These research endeavors have particularly focused on elucidating the role of genetic variant expression alterations, both in terms of increase and decrease, in the development of complex diseases such as cancer. Furthermore, these studies aim to uncover the interrelationships and manifestations of these effects. Moreover, as a result of these investigations, scientists and researchers are able to obtain additional data regarding the biochemical pathways and molecular mechanisms that govern and guide the life cycles of cells, and consequently, the progression of diseases (18, 19).

Bioinformatics and gene expression analysis: Bioinformatics involves the methodical gathering, retention, arrangement, examination, and demonstration of information acquired via the utilization of theoretical and practical principles in fields such as biology, medicine, behavioral sciences, and health sciences. The primary goal of this project is to examine and improve computational tools and techniques, with the aim of expanding the utilization and modification of data derived from research endeavors or the application of established protocols. Acquired by meticulous intellectual investigation or by adhering to established protocols. Bioinformatic analyses are conducted by choosing a suitable database and employing a technology that allows for the execution of bioinformatic analysis, based on the specific biological query, molecule, or structure under investigation. The data gathered and the insights derived from the investigations are merged, and the resulting assessments are thoroughly reviewed in comparison to the existing body of literature(20).

Any alterations in the physiological state of an organism or cell will inevitably lead to corresponding modifications in the pattern of gene expression. Hence, the evaluation of gene expression holds great importance in every field of biological research. The DNA microarray technology, which is now in the developmental stage, is utilized for the investigation of gene expression. This is achieved by the process of hybridization, when mRNA molecules are bound to a densely packed array of immobilized target sequences. Each of these target sequences corresponds to a distinct gene. The impact of chemical substances on the regulation of gene expression can provide useful insights into both functional and toxicological properties. Performing analyses on clinical samples, encompassing both those in good health and those afflicted with diseases, holds the potential to unveil previously undiscovered biomarkers(21).

Bioinformatics analysis phase: This study conducted gene expression analyses at the transcriptome level to investigate HCC recurrence following liver transplantation. The study's dataset comprised samples of both initial and recurring tumors from the patients. The experiment utilized the limma package, a software tool available in the R programming language that aids expression analysis (22). Limma is a software suite designed primarily for the analysis of gene expression microarray data using linear models. The primary objective is to employ linear models to analyze specific experiments and identify differential expression. The packet's capabilities can be employed in many gene expression methodologies, including microarrays, RNA-seq, and quantitative PCR. The Limma software employs Empirical Bayes methods to get reliable findings, even in scenarios with a limited number of sequences. The bioinformatic investigation yielded Lof2FC, a metric that quantifies the fold change in gene expression discrepancies. This measure organizes the genes in a descending sequence according to their degree of significance. Genes with higher expression levels are identified by applying a threshold of log2 fold change (log2FC) larger than 1, while genes with lower expression levels are identified by employing a threshold of log2FC less than -1. The data distribution in the study was shown using box plots and density plots. The graphs depict examples with similar attributes, indicated by the use of uniform colors. The study opted to utilize the Uniform Manifold Approximation and Projection (UMAP) graph to visually represent the connections between the samples under investigation. The volcano plot was selected as the optimum technique for showing genes that have differential expression, encompassing both upregulation and downregulation. The volcano plot illustrates the logarithmic correlation between significance and fold-change. The y-axis represents significance, while the x-axis represents fold-change on a logarithmic scale with a base of 2. This visual representation allows for the rapid identification of genes that exhibit differential expression. The graph illustrates the levels of gene expression, with red indicating up-regulated genes, blue indicating down-regulated genes, and black indicating genes with no significant difference in expression. In addition to the Volcano plot, we utilized the Mean Difference (MD) plot, which provides a clear visual representation of genes that show differential expression between groups. The MD plot graphically represents the log2 fold change of genes that are expressed differently, relative to the average log2 expression levels. The volcano plot utilizes color coding to differentiate between genes that are up-regulated and those that are down-regulated. The coloration in the volcano graph bears a similarity to that of this graph.

RESULTS

Figure 1 and Figure 2 display the scatter plots of the 7 matched main and recurrent tumor samples utilized in the investigation, respectively. The graphs use the symbol K to represent recurrent tissues and the symbol L to represent primary tissues. Both graphs were utilized to illustrate the distribution of values within the chosen samples. The graphs depict samples that have been color-coded based on their respective groupings. These plots are utilized to assess data normalization before to doing differential expression analysis, and the expression intensity plot is frequently favored as a complementary tool to the boxplot.

Figure 3 shows the UMAP graph, which clearly illustrates the connections between the samples. The graph shows that samples with similar characteristics cluster together. In the graph, green dots indicate recurrent tissues while purple dots indicate primary tissue samples. In the graph, K indicates recurrent tissues and L indicates primary tissues. Based on the results obtained by analyzing the data in the data set, the

results of the first 10 genes showing regulation between the two groups are given in table 1.

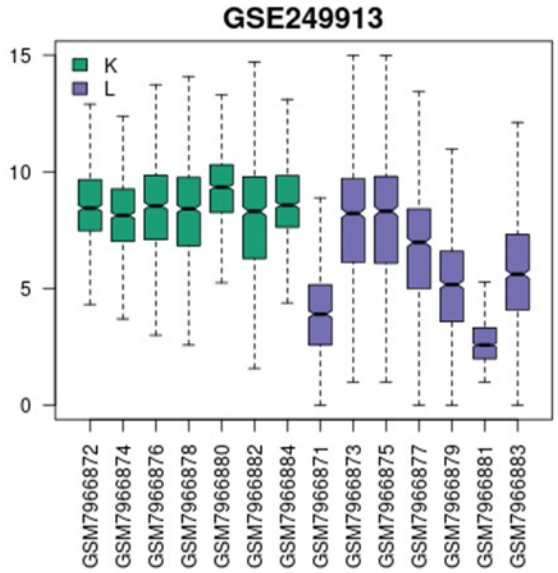

Figure 1. Distribution plot of the samples

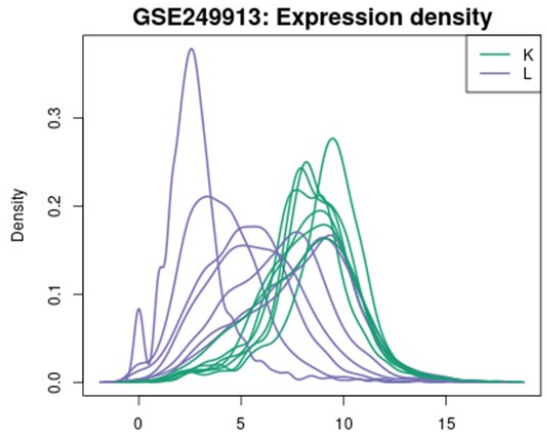

Figure 2: The expression density graph of the samples

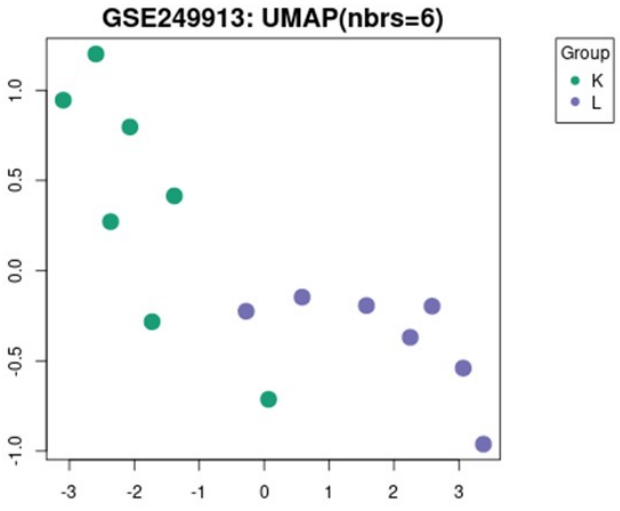

Figure 3. UMAP plot of the samples

The conditions |log2FC| > 1.0, p 0.05 were considered when determining the regulation of gene expression. As a result of the analysis in Table 1, all genes were found to be up-regulated. There are no down-regulated genes.

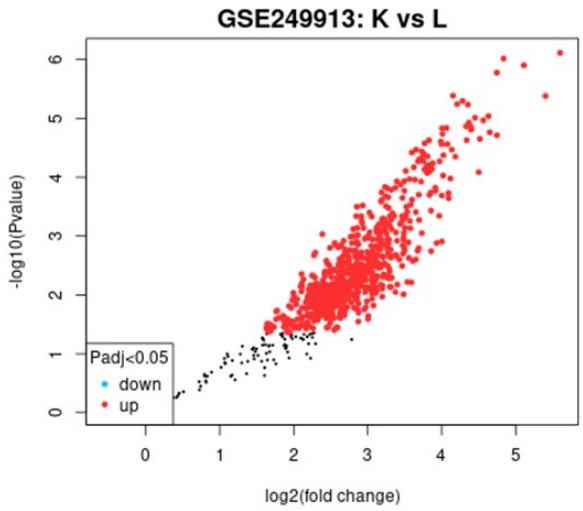

Figure 4 illustrates the volcano plot, which visually portrays the genes that exhibit differential expression among the several groups. Figure 4: Volcano plot of transcripts in primer tumor and recurrent tumor tissues. (Red dots represent transcripts that increased and black dots represent transcripts whose expression level remained unchanged.) Figure 4 shows all genes and when the graph is analyzed, it is seen that there is no down-regulated gene. If there was down-regulation, there should be extra blue dots on the graph. Figure 5 depicts the Mean Difference (MD) plot, which clearly illustrates genes exhibiting differential expression among the four groups. The MD plot visually represents genes that exhibit differential expression by displaying the log2 fold change relative to the mean log2 expression levels. Just with the volcano graphic, you may hover over data points to see detailed gene annotations. The highlighted genes exhibit a significant disparity in their levels of expression. The color red signifies an increase in activity, whereas blue signifies a decrease in activity. This differentiation is established by employing a predetermined P-value threshold of 0.05.

Figure5: MD plot of transcripts in primer tumor and recurrent tumor tissues (Red dots represent transcripts that increased, and black dots represent transcripts whose expression level remained unchanged). When figure 5 is analyzed as in figure 4, it is observed that there is no down-regulated gene.

DİSCUSSİON

Aggressive in nature, HCC has distinct epidemiological characteristics. Due to the substantial financial and illness burden it creates, HCC is still a major global public health concern (23-26).HCC has substantial variation in its occurrence and mortality rates throughout different regions of the globe. Disparities in the timing and amount of exposure to environmental and infectious risk factors, the accessibility to healthcare resources, and the ability to detect HCC at an earlier stage and provide potentially curative treatment are all factors that contribute to these variations.(27, 28).HBV, HCV, alcoholism, NASH, NAFLD, and exposure to food toxins such aflatoxins and aristolochic acid are common risk factors for

Table 1. Transcripts found to be regulated in recurrent tissue samples relative to primer tissue

NAME	adj,P,Val	P,Value	t	B	logFC
KIR3DL1	0,000332	7,72E-07	6,749	5,79499	5,6001
KIR2DL3	0,000516	4,17E-06	6,023	4,29084	5,4026
IFNA1	0,000332	1,25E-06	6,537	5,36439	5,1108
ESR2	0,000332	9,69E-07	6,649	5,59354	4,8363
POU5F1	0,000734	1,94E-05	5,381	2,90323	4,747
FAM30A	0,000332	1,67E-06	6,412	5,10796	4,7457
KIR2DS4	0,000724	1,73E-05	5,428	3,00606	4,6483
LTA	0,000684	9,18E-06	5,691	3,57985	4,6329
HDC	0,000684	1,08E-05	5,623	3,43147	4,5623
ALK	0,000778	2,23E-05	5,323	2,77575	4,5164
RAD51AP1	0,001284	8,24E-05	4,79	1,58832	4,5001
FGFBP1	0,000684	9,78E-06	5,665	3,52301	4,4507
EME1	0,000684	1,55E-05	5,473	3,10572	4,3971
TERT	0,000684	1,40E-05	5,517	3,20162	4,3948
FCRL2	0,000684	1,18E-05	5,586	3,35124	4,3667
TNF	0,000516	5,84E-06	5,88	3,987	4,3541
TNFSF13B	0,000778	2,35E-05	5,302	2,7295	4,3379
MLANA	0,000684	1,37E-05	5,524	3,21713	4,3299
GRHL2	0,000516	5,06E-06	5,941	4,11731	4,2812
KIR3DL1	0,000332	7,72E-07	6,749	5,79499	5,6001

HCC, particularly in countries with ample resources. In these circumstances, HCC is primarily caused by long-term chronic hepatitis. In this scenario, patients have acquired liver cirrhosis as a result of infection with either HBV or HCV. The annual occurrence of hepatocellular carcinoma (HCC) in individuals with cirrhosis caused by chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infection varies between 2 and 5 percent (29).

The biggest problem with HCC is its recurrence and metastasis in various locations. These conditions have an impact on the treatment procedures of the disease and also affect survival rates.Recurrent HCC continues to be a major obstacle, even after successful therapies like hepatectomy. Research has indicated that a significant percentage of individuals have recurrence, with the majority of recurring lesions being of tiny dimensions(30). The recurrence of HCC can be classified into two categories: early recurrence and late recurrence. Each category presents unique problems in terms of treatment and prognosis(31).The possibility of performing a salvage hepatectomy for locally recurrent HCC after locoregional therapy has been investigated. It has been highlighted that it is crucial to take into account the use of adjuvant medicines, such as immune checkpoint inhibitors, for cases with recurrence risk factors (32). HCC, there is a significant likelihood of the cancer recurring within the liver, even after aggressive treatment.

This recurrence has a substantial risk of mortality(33). Patients who have been free of recurrence for five years after receiving initial therapy for early-stage hepatocellular carcinoma (HCC) nevertheless have a significant likelihood of experiencing a recurrence within the following five years(34). Researchers have identified factors that can predict the likelihood of recurrence in early-stage HCC patients who have surgical resection. They have found that patients with more severe recurrence patterns tend to have poorer survival outcomes(35). The study has examined the factors that affect the recurrence of HCC in liver transplant patients. It has emphasized the significance of pre-transplant factors in determining the rates of recurrence after transplantation(36).The recurrence of HCC can occur in multiple locations within the liver, either as new tumors or as the spread of cancer cells inside the liver itself.

Each of these situations presents distinct difficulties in terms of managing and treating the disease(37).Biomarkers such as AFP, Ezrin, and Ki67 have been linked to the recurrence of hepatocellular carcinoma (HCC) after liver transplantation. These biomarkers help in predicting the likelihood of recurrence and the overall survival in cases of HCC recurrence (35). Reoccurrence of autoimmune liver disorders Post-liver transplantation can create complications during the recovery process, requiring diligent monitoring and the implementation of effective management techniques(38).Overall, the recurrence of HCC presents a substantial clinical obstacle, with multiple factors impacting the likelihood and characteristics of recurrence. Gaining knowledge of these aspects and employing predictive biomarkers can assist in promptly recognizing, tailoring treatment approaches, and enhancing results for patients with recurring HCC. Investigation of various genomic factors plays an important role in the prediction of recurrent HCC.Research has investigated the genetic characteristics of HCC recurrence, identifying possible indicators and pathways linked to the reappearance of the illness.Genetic changes, including as mutations and increases in the number of copies of genes like BCL9, have been linked to the activation of the WNT/β-catenin signaling pathway and the formation of a tumor microenvironment in recurrent HCC where the immune system is unable to penetrate(39).Moreover, the detection of Aurora kinase B, a protein kinase associated with chromosomes, has been recognized as a major indicator of aggressive recurrence of HCC after hepatectomy(40).HCC) after liver transplantation has been studied using deep learning methods. Genes such as IL6 have been found to be strongly linked to HCC recurrence, highlighting the importance of certain genes in the progression of the illness(41).Genomic studies have yielded useful insights into the molecular mechanisms that cause recurrent HCC. These studies have also identified possible biomarkers, pathways, and gene signatures that could aid in predicting, understanding, and managing recurrent illness.The objective of this study was to identify genes that are controlled differently by analyzing the expression of tissue samples from recurrent and main cases. The purpose was to find prospective gene candidates that could serve as biomarkers for recurrent hepatocellular carcinoma (HCC).

We examined an open-access dataset with the expectation that genetic investigations will shed light on the underlying process. The dataset consisted of both recurrent and primary tumor tissues. Bioinformatics analyses revealed genes that were differently regulated in recurring tumor tissues versus initial tumor tissues. These genes were also shown using volcano and MD plots. Furthermore, the distributions of the samples in the dataset were evaluated and shown using expression density plots and scatter plots. The association of the samples in the data was shown using the UMAP plot, which revealed that the two groups differed. When the results of bioinformatic analyses were examined, it was determined that a lot of genes showed different regulation (up) in recurrent tumor compared to primer tumor tissues.No down-regulated genes were found.According to the results, KIR3DL1 gene was up-regulated 48.6-fold in recurrent tumor tissues. Similarly, KIR2DL3, IFNA1, ESR2, POU5F1, FAM30A, KIR2DS4, LTA, HDC, ALK genes had 42.22, 34.53, 28.44, 26.72, 24.93, 24.76, 23.58, 22.78 fold up-regulated gene expression, respectively.

Further investigation and studies on the identified genes may reveal that they are biomarkers that can aid in the effective treatment of recurrent HCC. Drug therapy can be devised and implemented using these biomarkers. The accurate and effective use of genetic biomarkers can improve patient care while also increasing healthcare system efficiency. As genetic research advances, more genes are predicted to be employed as biomarkers, resulting in the further expansion of personalized techniques in the medical sector. In conclusion, HCC is one of the most frequent tumors worldwide, and genetic research is critical to understanding the illness. Treatment techniques are heavily influenced by the disease's extremely metastatic and recurring nature. Understanding the genetic basis of recurrent HCC is crucial for disease prevention and treatment. In this context, genetic studies provide knowledge that allows for the creation of focused preventative tactics and tailored treatment approaches. Genetic testing and screening programs for high-risk individuals provide early detection and prevention.Finally, in the future, with the further advancement of genetic research, more innovative and effective methods for the prevention and treatment of recurrent HCC are expected to be developed.

REFERENCES

1. Q L, Ling Y, Zhou C, Chen J, Li X, Huang M. Circular RNA circCDK13 Suppresses Cell Proliferation, Migration and Invasion by Modulating the JAK/STAT and PI3K/AKT Pathways in Liver Cancer. International Journal of Oncology. 2018.
2. Ho DWH, Lo RCL, Chan LK, Ng IOL. Molecular Pathogenesis of Hepatocellular Carcinoma. Liver Cancer. 2016;5(4):290-302.
3. Tu C, Xiong H, Hu Y, Wang W, Mei G, Wang H, et al. Cardiolipin Synthase 1 Ameliorates NASH Through Activating Transcription Factor 3 Transcriptional Inactivation. Hepatology. 2020;72(6):1949-67.
4. Xie Q, Huang L, Gao F-W, Liu D-Q, Wang X, Jiang K, et al. Efficacy of Lenvatinib Combined With Sequential Transarterial Chemoembolization for Primary Hepatocellular Carcinoma and the Effects on Serum Basic Fibroblast Growth Factor and Vascular Endothelial Growth Factor. Frontiers in Pharmacology. 2022;13.
5. Yamauchi N, Watanabe A, Hishinuma M, Ohashi K, Midorikawa Y, Morishita Y, et al. The Glypican 3 Oncofetal Protein Is a Promising Diagnostic Marker for Hepatocellular Carcinoma. Modern Pathology. 2005;18(12):1591-8.
6. Ng KTP, Lo CM, Guo D, Xiang Q, Li CX, Wang G, et al. Identification of Transmembrane Protein 98 as a Novel Chemoresistance-Conferring Gene in Hepatocellular Carcinoma. Molecular Cancer Therapeutics. 2014;13(5):1285-97.
7. Wu C, Xu A, Zhang CC, Olson P, Cc L, Lee T, et al. Notch Inhibitor PF-03084014 Inhibits Hepatocellular Carcinoma Growth and Metastasis via Suppression of Cancer Stemness Due to Reduced Activation of Notch1–Stat3. Molecular Cancer Therapeutics. 2017;16(8):1531-43.
8. Yao M, Fang R-F, Xie Q, Xu M, Sai W-L, Yao D-F. Early Monitoring Values of Oncogenic Signalling Molecules for Hepatocellular Carcinoma. World Journal of Gastrointestinal Oncology. 2024;16(6):2350-61.
9. Xie Y, Fan KW, Guan S-X, Hu Y, Gao Y, Zhou W. <scp>LECT2</Scp>: A Pleiotropic and Promising Hepatokine, From Bench to Bedside. Journal of Cellular and Molecular Medicine. 2022;26(13):3598-607.
10. Romanas MM, Cherian R, McGregor DH, Wu Y, May CL, Baranda J. Hepatocellular Carcinoma Diagnosed by Fine-needle Aspiration of the Parotid Gland. Diagnostic Cytopathology. 2004;30(6):401-5.
11. Yu S, Guo H, Li Z, Yu J, Yan S, Zhang M, et al. A Case Report of De Novo Hepatocellular Carcinoma After Living Donor Liver Transplantation. World Journal of Surgical Oncology. 2013;11(1).
12. Natsuizaka M, Omura T, Akaike T, Kuwata Y, Yamazaki K, Sato T, et al. Clinical Features of Hepatocellular Carcinoma With Extrahepatic Metastases. Journal of Gastroenterology and Hepatology. 2005;20(11):1781-7.
13. Pusceddu C, Mascia L, Ninniri C, Ballicu N, Zedda S, Melis L, et al. The Increasing Role of CT-Guided Cryoablation for the Treatment of Liver Cancer: A Single-Center Report. Cancers. 2022;14(12):3018.
14. Franssen B, Jibara G, Tabrizian P, Schwartz M, Roayaie S. Actual 10-Year Survival Following Hepatectomy for Hepatocellular Carcinoma. HPB. 2014;16(9):830-5.
15. Xie Y, Huang X, Zhang L, Shu K. Screening of Hub Genes in Hepatocellular Carcinoma Based on Network Analysis and Machine Learning. Computational and Mathematical Methods in Medicine. 2022;2022:1-32.
16. Yang T, Chen Y, Xu J, Jin-yuan LI, Liu H, Liu N. Bioinformatics Screening the Novel and Promising Targets of Curcumin in Hepatocellular Carcinoma Chemotherapy and Prognosis. BMC Complementary Medicine and Therapies. 2022;22(1).
17. Pertea M. The human transcriptome: an unfinished story. Genes. 2012;3(3):344-60.
18. Lee KJ, Yin W, Arafat D, Tang Y, Uppal K, Tran V, et al. Comparative transcriptomics and metabolomics in a rhesus macaque drug administration study. Frontiers in cell and developmental biology. 2014;2:54.
19. Mortazavi A, Williams BA, McCue K, Schaeffer L, Wold B. Mapping and quantifying mammalian transcriptomes by RNA-Seq. Nature methods. 2008;5(7):621-8.

20. Akalın PK. Introduction to bioinformatics. Molecular nutrition & food research. 2006;50(7):610-9.
21. van Hal NL, Vorst O, van Houwelingen AM, Kok EJ, Peijnenburg A, Aharoni A, et al. The application of DNA microarrays in gene expression analysis. Journal of biotechnology. 2000;78(3):271-80.
22. Smyth GK. Limma: linear models for microarray data. Bioinformatics and computational biology solutions using R and Bioconductor: Springer; 2005. p. 397-420.
23. Fitzmaurice C, Allen C, Barber RM, Barregard L, Bhutta ZA, Brenner H, et al. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 32 cancer groups, 1990 to 2015: a systematic analysis for the global burden of disease study. JAMA Oncol. 2017;3(4):524-48.
24. Yang JD, Hainaut P, Gores GJ, Amadou A, Plymoth A, Roberts LR. A global view of hepatocellular carcinoma: trends, risk, prevention and management. Nat Rev Gastroenterol Hepatol. 2019;16(10):589-604.
25. Sayiner M, Golabi P, Younossi ZM. Disease Burden of Hepatocellular Carcinoma: A Global Perspective. Dig Dis Sci. 2019;64(4):910-7.
26. Aly A, Ronnebaum S, Patel D, Doleh Y, Benavente F. Epidemiologic, humanistic and economic burden of hepatocellular carcinoma in the USA: a systematic literature review. Hepat Oncol. 2020;7(3):Hep27.
27. El-Serag HB. Epidemiology of viral hepatitis and hepatocellular carcinoma. Gastroenterology. 2012;142(6):1264-73.
28. Tang A, Hallouch O, Chernyak V, Kamaya A, Sirlin CB. Epidemiology of hepatocellular carcinoma: target population for surveillance and diagnosis. Abdom Radiol (NY). 2018;43(1):13-25.
29. Zhang M, Wang B, Liu Y, Sun X, Luo X, Wang C, et al. Cumulative increased risk of incident type 2 diabetes mellitus with increasing triglyceride glucose index in normal-weight people: the Rural Chinese Cohort Study. Cardiovasc Diabetol. 2017;16(1):30.
30. Saito R, Amemiya H, Hosomura N, Kawaida H, Maruyama S, Shimizu H, et al. Prognostic Factors for Post-Recurrent Survival in Hepatocellular Carcinoma After Curative Resection. Anticancer Research. 2019;39(6):3033-8.
31. Yang Y, Mao S, Fang J, Wang G, Shan Y, Yu X, et al. Macrotrabecular-Massive Subtype-Based Nomogram to Predict Early Recurrence of Hepatocellular Carcinoma After Surgery. European Journal of Gastroenterology & Hepatology. 2023;35(4):505-11.
32. Minagawa T, Itano O, Kitago M, Abe Y, Yagi H, Hibi T, et al. Surgical and Oncological Outcomes of Salvage Hepatectomy for Locally Recurrent Hepatocellular Carcinoma After Locoregional Therapy: A Single-Institution Experience. Cancers. 2023;15(8):2320.
33. Yamasaki T, Sakaida I. Hepatic Arterial Infusion Chemotherapy for Advanced Hepatocellular Carcinoma and Future Treatments for the Poor Responders. Hepatology Research. 2011;42(4):340-8.
34. Kim J, Kang W, Sinn DH, Gwak GY, Paik YH, Choi MS, et al. Substantial Risk of Recurrence Even After 5 Recurrence-Free Years in Early-Stage Hepatocellular Carcinoma Patients. Clinical and Molecular Hepatology. 2020;26(4):516-28.
35. Cheng T-H, Cheng YM, Wu CC, Tzeng IS, Wang CC. Predictors of Recurrence in Early Hepatocellular Carcinoma Patients Treated With Surgical Resection. Advances in Digestive Medicine. 2021;10(1):21-7.
36. Zucker K, Gómez P, Kezirian O, Mehta S. Pre-Transplant Factors Influencing Rates of Hepatocellular Carcinoma Recurrence in Liver Transplant Recipients. Gastroenterology Research. 2021;14(3):190-3.
37. Takamura M, Sakamoto M, Genda T, Ichida T, Asakura H, Hirohashi S. Inhibition of Intrahepatic Metastasis of Human Hepatocellular Carcinoma by Rho-Associated Protein Kinase Inhibitor Y-27632. Hepatology. 2001;33(3):577-81.
38. Wang S, Dong V, Montaño-Loza AJ, Mason AL. Autoimmune Liver Diseases, Hepatocellular Carcinoma, and Recurrence of Autoimmunity Post-Liver Transplantation. 2021.
39. Zhou S-L, Zhou ZJ, Song C, Xin H-Y, Hu Z, Luo C-B, et al. Whole-Genome Sequencing Reveals the Evolutionary Trajectory of HBV-related Hepatocellular Carcinoma Early Recurrence. Signal Transduction and Targeted Therapy. 2022;7(1).
40. Tanaka S, Arii S, Yasen M, Mogushi K, Su N, Zhao C, et al. Aurora Kinase B Is a Predictive Factor for the Aggressive Recurrence of Hepatocellular Carcinoma After Curative Hepatectomy. British Journal of Surgery. 2008;95(5):611-9.
41. To J, Ghosh S, Zhao X, Pasini E, Fischer S, Sapisochin G, et al. Deep Learning-Based Pathway-Centric Approach to Characterize Recurrent Hepatocellular Carcinoma After Liver Transplantation. Human Genomics. 2024;18(1).

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