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Original Research

Examination of Genetic Background of Intrahepatic Cholangiocarcinoma by Bioinformatics Applications

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Abstract

Objectives: Intrahepatic cholangiocarcinoma (ICC), the second most common primary liver cancer, is associated with a poor prognosis with a very low survival rate. Therefore, a comprehensive understanding of the molecular pathways involved in the disease is of great importance for the development of targeted therapies and personalised treatment strategies. The aim of this study was to identify possible biomarkers associated with ICC by analysing gene expression in ICC tumor and non-tumour liver tissues.

Methods: The dataset included in the study comprises gene expression data from ICC and non tumor liver 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. 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: ICC tumor tissues and non tumor liver 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 and down regulated in ICC tissues compared to non tumor liver tissues.

Conclusion: Genetic research has a pivotal role in enhancing investigate molecular pathways and treatment of ICC. Genes that have been identified can function as biomarkers, which can assist in the creation of medication therapies that are specifically targeted and enhance the quality of patient care and the efficiency of healthcare. As genetic research advances, the utilization of these biomarkers is anticipated to improve personalization.

Keywords: Intrahepatic cholangiocarcinoma, Tumor free liver tissue, Gene expression, Biological behavior

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Hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC) account for approximately 85% and 15% of primary liver cancer, respectively.[1, 2] ICC show a rapidly increasing incidence, especially in western countries. The two types are quite different in terms of their morphology, metastatic capacity and response to cancer therapies.[1, 3] ICCs are composed of ductular, papillary, or solid tumor formations that are surrounded by a dense tumor stroma.[2] While HCC is invasive, the occurrence of metastatic tumours is more common in ICC. Interestingly,

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HCCs show a positive response to therapy with multikinase inhibitors, whereas ICCs are resistant to these medications. In contrast, ICCs exhibit positive therapeutic responses to traditional cytotoxic therapy, while HCC is characterised by its resistance to chemotherapy.[1, 3, 4]

ICC has a lower occurrence rate in comparison to other types of liver cancer, such as hepatocellular carcinoma. However, its prevalence is increasing worldwide, especially in areas with high rates of chronic liver illnesses, such as hepatitis C and cirrhosis.[5-7] ICC is also associated with a poor prognosis with a five-year survival rate of only 22-24% after curative resection.[8] The rising prevalence of ICC can be due to a combination of various risk factors, such as nonalcoholic fatty liver disease, chronic viral hepatitis, and primary sclerosing cholangitis.[6, 9, 10]

The clinical manifestation of ICC is frequently subtle, with a significant number of patients being discovered coincidentally during imaging examinations for other ailments. Common symptoms of this condition may encompass unintentional weight loss, yellowing of the skin and eyes (jaundice), discomfort in the stomach region, and elevated body temperature. However, it is important to note that these symptoms are sometimes vague and can result in delays in the identification of the underlying condition.[11] Imaging investigations, such as computed tomography (CT) and magnetic resonance imaging (MRI), usually show a mass with consistent low-attenuation and uneven enhancement at the edges. This may be accompanied by the shrinking of the outer layer and the widening of the ducts within the liver.[12, 13] The diagnosis is typically verified through histological examination after a biopsy or surgical removal of tissue.

The molecular composition of ICC is intricate, with multiple genetic changes and modifications involved in its development. Significantly, mutations in the KRAS, IDH1, and IDH2 genes have been found to be prevalent in ICC, especially in patients with pre-existing liver illness.[14, 15] The involvement of epithelial-mesenchymal transition (EMT) in the advancement of ICC has also attracted interest, as it is thought to contribute to the invasive properties of the tumor.[16, 17] Gaining a comprehensive understanding of these molecular pathways is of utmost importance for the advancement of targeted treatments and tailored treatment strategies. Although there have been improvements in diagnostic and treatment approaches, the outlook for individuals with ICC remains quite poor, especially for those who are discovered at a late stage. The significant frequency of reappearance after curative removal emphasizes the necessity for continuous monitoring and the investigation of additional treatments to enhance long-term results.[8, 18] Research into novel biomarkers and therapeutic targets is ongoing, with the aim of identifying patients who may benefit from specific treatment modalities based on their tumor's molecular profile.[19]

The latest developments in the field of bioinformatics have made it easier to identify precise genetic changes and biomarkers that play a critical role in the diagnosis, prognosis, and treatment approaches.[20, 21] Bioinformatics is crucial in the molecular profiling of ICC, enabling researchers to examine extensive databases of genomic and transcriptome information. For example, thorough analysis of molecules has shown that mutations in genes like IDH1, FGFR2, and BAP1 are common in ICC, and these changes can affect therapy choices and patient results.[20, 22, 23] In addition, the application of bioinformatics in examining gene expression patterns has resulted in the identification of prognostic biomarkers, such as CXCL12, that are linked to the spread of cancer to other parts of the body and unfavorable survival rates.[18, 21]

Ultimately, the incorporation of bioinformatics into the investigation of ICC is revolutionizing our comprehension of this cancerous condition. It aids in the detection of genetic changes, predictive markers, and immunological landscape features, all of which are crucial for enhancing diagnosis, therapy, and patient results. As research progresses, the utilization of bioinformatics is expected to have a growing significance in the management of ICC facilitating the development of more efficient and tailored therapeutic approaches.

Therefore, in this study, in order to understand the genetic background of ICC and to identify the associated biomarker genes by bioinformatic analyses, the data obtained from ICC tumour tissue and non-tumour liver tissue samples from 15 patients were examined by expression analysis. Genes showing differential regulation in ICC tumour tissues were identified.

Material And Methods

Dataset

The study utilised a dataset comprising of data collected from ICC tumour tissues and non-tumour liver tissues of 15 individuals. The purpose was to examine the alterations in RNA editing in ICC. The dataset was acquired through the utilisation of RNA-seq, employing Illumina HiSeq2000, on 15 pairs of ICC tumours and corresponding non-tumour liver tissues. The study utilised a data collection acquired from the National Centre for Biotechnology Information (NCBI). The current code assigned to the data collection at the NCBI is GSE119336.

RNA-Sequence Analysis (RNA-Seq)

RNA sequence analysis technologies, a widely recognized and efficient technology, have shown remarkable outcomes in characterizing all RNA transcripts produced by cells. RNA-seq is an innovative tool in the field of transcriptomics that allows for a thorough and precise study of complete transcriptomes in a quantitative manner. This strategy is the first of its kind to use sequencing technology to reveal the entire collection of RNA transcripts in an organism. In contrast to hybridization-based approaches, the RNA-seq method not only aims to identify known transcripts, but also seeks to discover and link new transcripts while examining and revealing the known ones. An important advantage of the RNA-seq technique is its capacity to evaluate expression levels throughout a wide and variable spectrum, rather than solely evaluating relative values. RNA-seq technologies provide substantial advantages, including little background contamination, resulting in very precise and dependable results. Moreover, these techniques enable accurate identification of exon and intron borders, as well as the detection of SNPs and other genetic variations within transcripts. RNA-seq offers multiple advantages, making it an ideal tool for most research projects aimed at discovery.[24]

Transcriptomics

The transcriptome encompasses all RNA molecules, such as mRNA, tRNA, rRNA, and non-coding RNAs, that are produced by the genome of a cell, tissue, or organism during a certain period. Transcriptomes have a constantly changing structure and experience ongoing modifications as a result of fluctuations in gene expression. Contrary to the largely unchanging genetic makeup of a cell, the transcriptome is vulnerable to changes produced by several environmental factors, such as changes in pH, differences in nutrient supply, fluctuations in temperature, and interactions with signals from nearby cells. Gene transcription related to these processes may differ in response to various cellular activities, resulting in alterations in the transcriptome, which includes all the messenger RNAs (mRNAs) found in the cell. Hence, the transcriptome offers a momentary and contextual representation of the genes that are now working. The detection of changes in gene expression induced by environmental factors has emphasized the importance of interactions between the environment and biological systems.[24, 25]

Transcriptomics is a scientific field that examines the entire array of mRNA transcripts generated by transcription within a cell's genome, offering insights on their expression patterns. Microarray and next-generation sequencing areadvanced technologies frequently utilized in transcriptomics applications. These technologies provide the analysis of precise alterations in the transcriptome that transpire at designated times and for certain purposes.[25]

Transcriptomics studies have become increasingly important and their prevalence has significantly increased in recent decades. These research efforts have specifically concentrated on clarifying the impact of changes in the expression of genetic variants, whether they increase or decrease, in the development of complex diseases like cancer. Moreover, these investigations seek to reveal the connections and expressions of these impacts. Furthermore, through these investigations, scientists and researchers can get supplementary data about the biochemical pathways and molecular mechanisms that regulate the life cycles of cells, and thus, the development of diseases.[25, 26]

Bioinformatics and Gene Expression Analysis

Bioinformatics encompasses the systematic collection, storage, organization, analysis, and presentation of data obtained via the application of theoretical and practical concepts in disciplines such as biology, medicine, behavioral sciences, and health sciences. The main objective of this project is to analyze and enhance computational tools and techniques in order to broaden the use and customization of data obtained from research efforts or the implementation of existing procedures. Obtained through careful and thorough intellectual inquiry or by following established procedures. Bioinformatic analyses are performed by selecting an appropriate database and utilizing a technology that enables the execution of bioinformatic analysis, depending on the particular biological inquiry, molecule, or structure being investigated. The collected data and the obtained insights from the studies are combined, and the ensuing assessments are carefully evaluated in relation to the available literature.[27]

Any alterations in the physiological state of an organism or cell will inevitably lead to corresponding modifications in the pattern of gene expression.Therefore, the assessment of gene expression is highly significant in all areas of biological investigation. The DNA microarray technology, now in the developmental stage, is employed for the examination of gene expression. This is accomplished by the process of hybridization, when mRNA molecules are attached to a tightly packed array of immobilized target sequences. Each of these target sequences corresponds to a unique gene. Studying the influence of chemical substances on the control of gene expression can offer valuable knowledge on both functional and toxicological characteristics. Conducting investigations on clinical samples, including both individuals who are in excellent health and those who are affected by diseases, has the potential to reveal previously unknown biomarkers.[28]

Bioinformatics Analysis Phase

In this study, gene expression analyses were performed at the transcriptome level to investigate the role of RNA editing on ICC. The data set of the study consisted of ICC tumour tissues and non-tumour liver tissues samples from paired 15 patients. The current study utilized the limma package, a software tool available in the R programming language that aids expression analysis.[29]

Limma is a software suite specifically developed for analyzing gene expression microarray data using linear models. The main goal is to utilize linear models to examine certain experiments and detect differential expression. The packet's functionalities can be utilized in many gene expression techniques, such as microarrays, RNA-seq, and quantitative PCR. The Limma software utilizes Empirical Bayes algorithms to obtain trustworthy results, especially in situations when there is a small number of sequences. The bioinformatic inquiry resulted in the identification of Lof2FC, a metric that measures the magnitude of differences in gene expression fold change. This metric arranges the genes in a descending order based on their level of importance. Genes with greater expression levels are determined by using a threshold of log2 fold change (log2FC) more than 1, whereas genes with lower expression levels are determined by using a threshold of log2FC less than -1.

The study utilized box plots to display the distribution of data. The graphs illustrate instances with comparable characteristics, denoted by the utilization of consistent colors. The study chose to employ the Uniform Manifold Approximation and Projection (UMAP) graph to visually depict the relationships between the samples being examined. The volcano plot was chosen as the optimal method for visualizing genes with differential expression, including both upregulation and downregulation. The volcano plot depicts the logarithmic relationship between the level of significance and the magnitude of fold-change. The y-axis denotes the level of significance, while the x-axis indicates the fold-change on a logarithmic scale with a base of 2. This graphical depiction enables the quick identification of genes that display differential expression. The graph depicts the levels of gene expression, where red represents genes that are up-regulated, blue represents genes that are down-regulated, and black represents genes that show no significant difference in expression. Furthermore, we employed the Mean Difference (MD) plot alongside the Volcano plot to visually depict genes that exhibit distinct expression patterns between various groups. The MD plot visually illustrates the log2 fold change of genes that exhibit differential expression, in comparison to the average log2 expression levels. The volcano plot use color coding to distinguish between up-regulated and down-regulated genes. The coloration in the volcanic graph exhibits a resemblance to that of this graph.

Results

Figure 1 displays the distribution plots of the samples from 15 pairs of ICC tumors and corresponding non-tumour liver tissues that were utilised in the investigation. The term 'cancer' is employed to symbolise the ICC tumour tissues, whereas 'non-tumour' is used to symbolise the corresponding non-tumour liver tissues. The graph depicted the distribution of values within the chosen samples. The graph utilizes colour coding to distinguish between different samples. The green colour indicates the presence of ICC tumour tissues, while the purple colour indicates the presence of matched non-tumour liver tissues. This graph is utilised to evaluate data normalisation prior to doing differential expression analysis.

Figure 2 displays the UMAP graph, which effectively depicts the relationships among the samples. The graph demonstrates that samples with comparable traits form

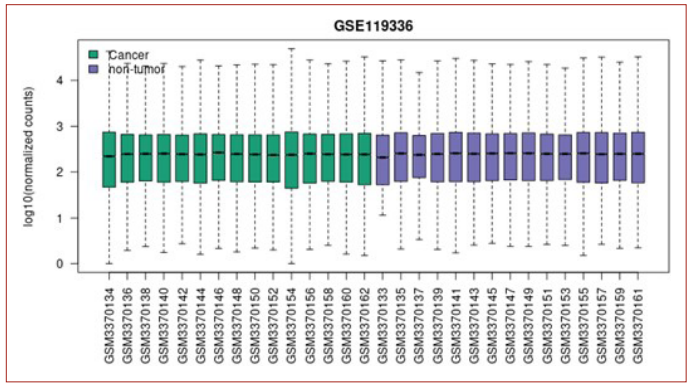

Figure 1. Distribution plot of the samples.

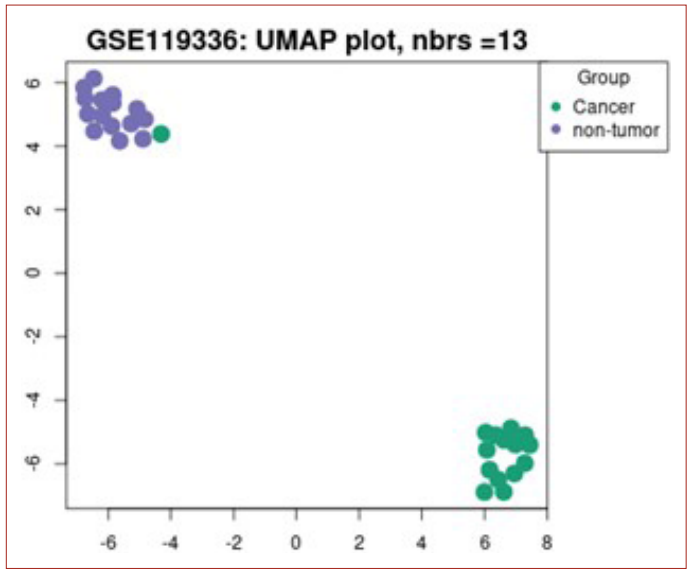

Figure 2. UMAP plot of the samples.

distinct clusters. The graph displays ICC tumor tissues as green dots and matched non-tumour liver tissue samples as purple dots. In the graph, "cancer" indicates ICC tumor tissues and "non-tumor" indicates non-tumour liver tissues.

The gene expression of 17327 genes in the dataset was analyzed, and the results of the top 10 genes exhibiting up- and down-regulation between the two groups are shown in Table 1 and Table 2. When evaluating the control of gene expression, conditions with an |log2FC| > 1.0 and a p-value less than 0.05 were considered.

Figure 3: Volcano plot of genes in ICC tumor and non-tumor liver tissues. (Red dots represent transcripts that increased, blue dots represent transcripts that dropped, and black dots represent transcripts whose expression level remained unchanged.)

Figure 4 displays the MD plot, which effectively demonstrates genes that show differential expression across the various groups. The MD plot graphically illustrates genes with differential expression by showing the log2 fold change in relation to the average log2 expression levels. The highlighted genes display a notable discrepancy in their levels of expression. The color red represents heightened levels of activity, whereas blue represents diminished levels of activity. This distinction is determined by utilizing a pre-established P-value threshold of 0.05.

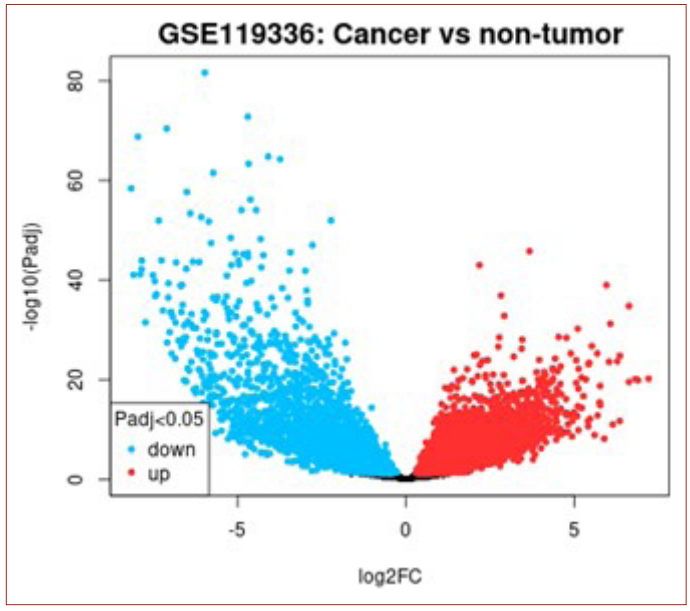

Figure 3. Illustrates the volcano plot, which visually portrays the genes that exhibit differential expression among the several groups.

Table 1. Transcripts found to be up-regulated in ICC tumor tissues samples relative to non-tumour liver tissues.

GeneID	padj	p	log2FoldChange	Symbol	Description
1311	6,54E-21	1,21E-22	7,201741	COMP	cartilage oligomeric matrix protein
5349	1,47E-20	2,87E-22	6,887498	FXYD3	FXYD domain containing ion transport regulator 3
5744	7,88E-21	1,48E-22	6,811435	PTHLH	parathyroid hormone like hormone
79574	2,99E-20	6,13E-22	6,618167	EPS8L3	EPS8 like 3
2810	1,73E-35	7,30E-38	6,617754	SFN	stratifin
4582	1,70E-25	2,01E-27	6,348982	MUC1	mucin 1, cell surface associated
131368	2,16E-12	1,27E-13	6,345546	ZPLD1	zona pellucida like domain containing 1
221416	2,20E-24	2,89E-26	6,272728	LINC03040	long intergenic non-protein coding RNA 3040
5266	1,11E-11	7,28E-13	6,136162	PI3	peptidase inhibitor 3
105371453	6,55E-32	3,82E-34	6,068467	BCAN-AS1	BCAN antisense RNA 1

Table 2. Genes found to be down-regulated in ICC tumor tissues samples relative to non-tumour liver tissues.

GeneID	padj	p	log2FoldChange	Symbol	Description
1549	4,31E-59	2,24E-62	-8,16581	CYP2A7	cytochrome P450 family 2 subfamily A member 7
641654	9,55E-42	2,48E-44	-8,09746	HEPN1	hepatocellular carcinoma, down-regulated 1
131669	1,71E-69	3,95E-73	-7,96448	UROC1	urocanate hydratase 1
1548	7,34E-42	1,86E-44	-7,894	CYP2A6	cytochrome P450 family 2 subfamily A member 6
10332	7,06E-43	1,67E-45	-7,85558	CLEC4M	C-type lectin domain family 4 member M
220296	1,43E-44	2,65E-47	-7,84927	HEPACAM	hepatic and glial cell adhesion molecule
1544	3,25E-32	1,86E-34	-7,74055	CYP1A2	cytochrome P450 family 1 subfamily A member 2
101928384	1,09E-41	2,91E-44	-7,51731	LOC101928384	uncharacterized LOC101928384
1543	1,73E-40	4,90E-43	-7,46271	CYP1A1	cytochrome P450 family 1 subfamily A member 1
319	1,76E-37	5,98E-40	-7,44875	APOF	apolipoprotein F

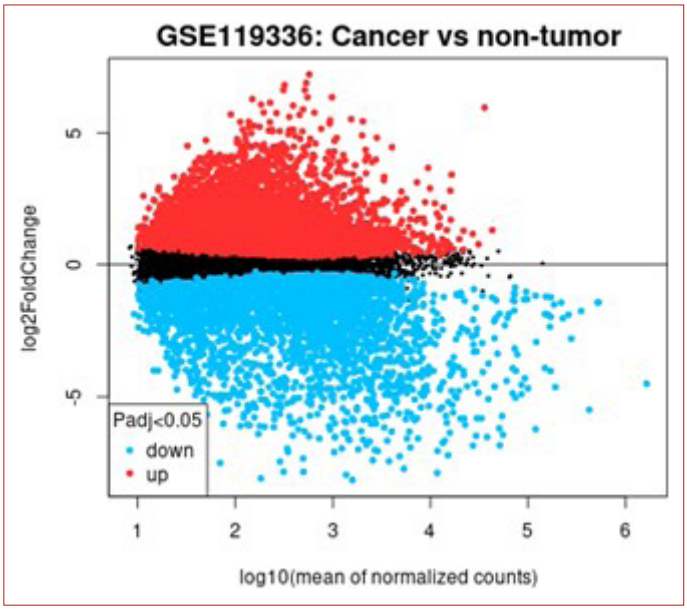

Figure 4. MD plot of genes in ICC tumor tissues and non-tumor liver tissues (Red dots represent transcripts that increased, blue dots represent transcripts that decreased and black dots represent transcripts whose expression level remained unchanged).

Discussion

ICC is a cancerous tumor that develops from the cells lining the bile ducts within the liver. It is the second most prevalent form of primary liver cancer, occurring after hepatocellular carcinoma, and makes up around 5-10% of all cases of primary liver malignancies.[30, 31] The global prevalence of ICC has been on the rise, especially in Western nations, and is linked to several risk factors such as chronic liver disorders, viral hepatitis, and bile duct stones.[31-33] The primary treatment for ICC is surgical excision, which aims to cure the disease, particularly in its early stages.[30, 31] Nevertheless, because ICC frequently lacks noticeable symptoms during its initial phases, a large number of patients are diagnosed with advanced disease. This greatly complicates the available treatment options and has a negative impact on the prognosis.[32, 34] When surgical removal is not possible, systemic chemotherapy, specifically with gemcitabine and cisplatin, has become a recognized treatment plan that has shown better results in terms of survival rates.[35, 36] Furthermore, ongoing research is investigating the effectiveness of targeted treatments and immunotherapy in treating ICC, but more examination is needed to determine their efficacy.[30, 37]

Genomic research has made great progress in improving our knowledge of the molecular basis of this disease. It has uncovered specific genetic changes that can affect the outlook and treatment approaches. Genomic research has discovered numerous crucial mutations linked to ICC, including changes in the KRAS, IDH1, and FGFR2 genes. The prevalence of KRAS mutations in ICC has been documented to vary from 8.6% to 25%, highlighting a significant frequency of this mutation among patients.[38] Furthermore, there is evidence that IDH1 mutations are associated with better survival rates, indicating that this genetic change could be used as a possible indicator for predicting prognosis.[39] Moreover, the existence of FGFR2 fusions has been identified as a crucial focus for therapeutic treatment. Studies have shown that these changes do not occur together with other important mutations, thus indicating the presence of discrete molecular subtypes of ICC.[22, 40] Recent study has emphasized the connection between changes in the genetic makeup and clinical characteristics, such as perineural invasion. This suggests that the specific genetic subtype of a tumor may provide more accurate information about prognosis compared to only considering tumor size.[41] This finding emphasizes the importance of comprehensive genomic profiling in guiding treatment decisions and improving patient outcomes.

The attention on ICC has extended beyond mutations to include the tumor microenvironment and immune response. Research has shown that natural immune mechanisms can affect the growth and spread of tumors, making the management of the condition more complex.[20, 42] Understanding these interactions may provide insights into novel therapeutic strategies aimed at enhancing the efficacy of existing treatments. To summarize, the genetic makeup of ICC is marked by a wide range of mutations and changes that have a substantial effect on prognosis and treatment approaches. Continued investigation into the molecular pathways that cause ICC is essential for the development of precise treatments and enhancing patient results. Therefore, in order to determine the pathophysiology of the disease and to personalise treatment modalities, it is very important to increase advanced bioinformatics and genetic-based studies and to approach the disease in the light of the results that can be obtained from these studies. In this study, it was aimed to determine the biomarkers that may play a role in the course of the disease by examining RNA seq data, which is very important in genomic studies, with bioinformatic analyses.

This study utilized an open access ICC dataset to conduct bioinformatic analysis. Through this analysis, genes that exhibited distinct regulation in ICC tumor tissues, as opposed to non-tumor liver tissue, were found. The volcano plot and MD plot were used to visualize these genes. Furthermore, the distribution of the samples in the data set was analyzed and represented using a scatter plot. The data samples were visualized using the UMAP graph, revealing a noticeable distinction between the two groups.

When the results of bioinformatic analyses were examined, it was determined that a lot of genes showed different regulation (up or down) in ICC tumor tissues compared to non tumor liver tissues. COMP gene showed 147,03 fold up-regulation in ICC tumor tissues compared to non tumor liver tissues. Likewise, FXYD3, PTHLH, EPS8L3, SFN, MUC1, ZPLD1, LINC03040, PI3,and BCAN-AS1 genes had up-regulated gene expression of 117.78, 112.20, 98.22, 98.15, 81.00, 79.98, 77.17, 70.03, and 66.71 fold, respectively. CYP2A7 gene showed 286.02 fold down-regulation in CRC tumor tissues compared to CRC normal tissues. Likewise, HEPN1, UROC1, CYP2A6, CLEC4M, HEPACAM, CYP1A2, LOC101928384, CYP1A1, and APOF genes had down-regulated gene expression of 272.47, 248.99, 237.20, 230.72, 229.12, 213.78, 182.27, 176.06, and 173.64 fold, respectively.

Additional analysis and research on the identified genes may reveal that these genes could serve as biomarkers that contribute to the development of successful treatment strategies for ICC. Biomarkers can be utilized to develop and implement drug therapy. The precise and efficient utilization of genetic biomarkers can enhance patient care and optimize the performance of healthcare systems. As genetic research progresses, an increasing number of genes are anticipated to serve as biomarkers, hence facilitating the wider adoption of personalized approaches in the field of medicine.

Genetic research into ICC, the second most prevalent primary liver cancer, is crucial for comprehending the illness. The unfavorable prognosis of the disease significantly impacts the choice of treatment techniques. Hence, comprehending the genetic foundation of ICC is vital for the prevention and management of the ailment. Genetic investigations in this context yield valuable information that enables the development of targeted prevention strategies and individualized treatment approaches. Genetic testing and screening programs for persons at high risk offer timely identification and prevention. In the future, as genetic research continues to progress, it is anticipated that more advanced and efficient approaches for preventing and treating ICCC will be created.

Disclosures

Ethics Committee Approval: This article was produced from 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.

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