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INVESTIGATION OF THE RELATIONSHIP BETWEEN HEPATITIS C AND LIVER CANCER DATABASES

HEPATİT C İLE KARACİĞER KANSERİ ARASINDAKİ İLİŞKİNİN VERİ TABANLARI ÜZERİNDEN ARAŞTIRILMASI

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ABSTRACT

Objective: The global prevalence of hepatitis C virus (HCV) infections has recently reached epidemic levels. Liver cancer, known as hepatocellular carcinoma (HCC), is extremely lethal once detected and is common in those with persistent HCV infection without access to appropriate therapy. Recent studies have shown that HCV-encoded proteins contribute to cancer development in infected hepatocytes. To develop treatments for HCC and liver cancer, it is essential to understand how viral proteins interact with host cell proteins.

Material and Methods: We used the Reactive, WikiPathways, KEGG, and Biocarta databases to identify common DEG pathways associated with HCC and HCV.

Results: Through bioinformatics approaches, this study identified common differential genes and related pathways to determine the molecular mechanisms underlying the pathogenesis of hepatitis C-related liver cancer. Investigating gene-gene interactions may lead to more effective treatment approaches. The liver cancer transcriptome identified 708 genes associated with HCC. Data on hepatitis C infection revealed 1768 genes linked to HCV. The Venn diagram then identified 152 DEGs common to HCV and HCC.

Conclusion: We believe that hepatitis C-related liver cancer can be predicted using the first 20 target genes identified through PPI analysis.

Keywords: Hepatocellular carcinoma, hepatitis C virus, databases, genes, bioinformatics, pathways

ÖZ

Amaç: Hepatit C virüsü (HCV) enfeksiyonlarının küresel prevalansı son zamanlarda epidemik seviyelere ulaşmıştır. Hepatoselüler karsinom (HCC) olarak bilinen karaciğer kanseri, tespit edildiğinde çok öldürücüdür ve tedaviye erişimi olmayan, kalıcı HCV enfeksiyonu olan kişilerde yaygındır. Son çalışmalar, HCV tarafından kodlanan proteinlerin, enfekte hepatositlerde kanser gelişimine katkıda bulunduğunu göstermiştir. HCC ve karaciğer kanserine yönelik tedaviler geliştirmek için viral proteinlerin konak hücre proteinleriyle nasıl etkileşime girdiğini anlamak önemlidir.

Gereç ve Yöntemler: HCC ve HCV ile ilişkili yaygın DEG'lerin yolaklarını tanımlamak için Reactome, WikiPathways, KEGG ve Biocarta veritabanlarını kullandık.

Bulgular: Biyoenformatik yaklaşımlar aracılığıyla bu çalışmada, hepatit C ile ilişkili karaciğer kanserinin patogenezinin altında yatan moleküler mekanizmaları belirlemek için ortak diferansiyel genler ve ilgili yolaklar keşfedildi. Gen-gen etkileşimlerinin araştırılması daha etkili tedavi yaklaşımlarına yol açabilir. Karaciğer kanseri transkriptom verileri, 708 genin HCC ile ilişkili olduğunu tanımladı. Hepatit C enfeksiyonuna ilişkin veriler, HCV ile bağlantılı 1768 gen ortaya çıkardı. Daha sonra Venn diyagramı HCV ve HCC'de ortak olan 152 DEG'i tanımladı.

Sonuç: Hepatit C ile ilişkili karaciğer kanserinde, PPI analizi yoluyla belirlediğimiz ilk 20 hedef genin biyobelirteç olarak kullanabileceğine inanıyoruz.

Anahtar Kelimeler: Hepatoselüler karsinom, hepatit C virüsü, veritabanları, genler, biyoinformatik, yolaklar

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INTRODUCTION

Globally, liver cancer (LC) is the second-leading cause of cancer-related mortality. Hepatocellular carcinoma (HCC) originates in liver cells and is the most common histological subtype (1). HCC is the second most common type of cancer worldwide, and it is frequently discovered late in development. As a result, early detection of the disease is critical for improving the prognosis of patients with HCC. Blood alpha-fetoprotein detection and liver ultrasonography are the current early clinical screening modalities for HCC. Hence, it is imperative that we promptly develop a more efficient and precise technique for detecting liver cancer. With advancements in our understanding of cancer biology, liquid biopsy will increasingly emerge as a valuable method for early detection of HCC. HCC is associated with many risk factors, including cirrhosis, aflatoxin B ingestion, alcohol use, infection with the hepatitis B virus (HBV), and infection with the hepatitis C virus (HCV) (2).

HCC is a type of cancer that is associated with ongoing inflammation, even in the absence of infectious agents or exposure to harmful substances. Abnormal microenvironment is a significant factor in HCC progression. Endothelial cells, pericytes, dendritic cells, stem/progenitor cells, extracellular matrix components, growth factors, cytokines, and inflammatory cells constitute the HCC microenvironment (3). It is interesting that these factors that cause cancer and HCC are linked to problems in the MDM2-p53 axis, which show up as p53 being turned off and *MDM2* (a transcriptional target and negative regulator of p53) being turned on too much. Mechanistically, dysregulation of the MDM2-p53 feedback loop in HCC tissues controls the initiation and progression of HCC (1).

HCV is a leading cause of liver cancer. As an RNA virus, HCV cannot incorporate itself into the host genome, in contrast to the hepatitis B virus. HCV infection may trigger the progression of HCC, with the intricate interplay between viral and host proteins causing the body to react, leading to inflammation, fibrosis, and eventually cirrhosis. HCV facilitates the oncogenic process by activating cellular oncogenes, inactivating tumour suppressor genes, and deregulating several signal transduction pathways. Epigenetic modifications and alterations are also involved in this process. Recent developments in genetics and gene expression profiling have enhanced our knowledge of the mechanisms underlying the progression of HCV-associated liver cancer (4).

The *TP53* tumour suppressor gene is one of the most frequently identified genetic abnormalities in a wide variety of human cancers, including liver cancer. Both HBV- and HCV-associated HCCs localised more than 60% of the nucleotide changes to the untranscribed strand, according to analysis of mutated TP53 nucleotides. G to A nucleotide changes and C to T mirror transitions were more abundant in the HCV-associated group (36%), compared to HBV-associated HCC (25%). Various forms of cancer, including HCC, have been shown to exhibit abnormal Wnt signalling activity and nuclear β-catenin accumulation. Most of the extra β-catenin in HCC arises from changes in the *CTNNB1* gene, which is found in 20%–40% of liver tumours, and in genes that encode the AXIN and AXIN2 proteins, which are part of the β-catenin degradation complex (5).

Growth hormone (GH) activates signal transducer and activator of transcription 5b (STAT5b), a transcription factor that regulates the expression of genes associated with sexual differences in the liver. If the GH hypothalamo-pituitary-liver axis does not turn on STAT5b, metabolic problems, steatosis, and liver cancer (6). Regardless of liver function, decreased expression of the GHR/STAT5/IGF-1 signalling pathway may influence the development, aggressiveness, and prognosis of HCV-associated HCC (7). Phosphatase and tensin homologue (*PTEN*) deletion on chromosome 10, a tumour suppressor, is frequently mutated or deleted in HCC tumours. *PTEN* has been shown to inhibit HCV secretion (8). They found that lower *PTEN* expression was associated with disease stage, tumour grade, and higher expression of alpha-fetoprotein, a tumour marker for HCC (9).

Bioinformatics methods are rapidly developing to identify genes that enable the detection of the relationship between disease and disease through genomic databases. These bioinformatics methods can identify candidate disease genes. In our study, the relationship between hepatitis C virus and liver cancer was integrated with different bioinformatic strategies by performing gene ontology (GO) and pathway enrichment analyses using various databases. Thus, this study aimed to identify candidate disease genes that will reveal the relationship between these two diseases and use them as possible biomarkers.

MATERIAL AND METHODS

Working area

The study was conducted between February and March 2024 using data analysis techniques.

TCGA and DisGeNET databases

Liver cancer transcriptome data were obtained from the Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov/) (10). The study identified 708 genes associated with liver cancer.

Hepatitis C infection data were obtained through the DisGeNET database. A total of 1,768 genes were found to be associated with Hepatitis C. Then, 152 differentially expressed genes (DEGs) common to Hepatitis C and LC were identified by Venn diagram using the Bioinformatics & Evolutionary Genomics database (https://bioinformatics.psb.ugent.be/webtools/Venn/) (11).

Gene ontology (GO) and pathway enrichment analyses

An important goal of gene set enrichment analysis is to sort basic biological data into groups, like the molecular pathways of chromosomal areas linked to a group of diseases. We performed gene ontology and functional enrichment analyses using the Enrichr tool (https://maayanlab.cloud/Enrichr/) (12), specifically focusing on cellular components (CC), biological processes (BP), molecular functions (MF), and pathway en-

richment. Enrichr, a web-based programme, enhances gene sets to evaluate biological processes and signalling pathways associated with common genes. We searched four repositories for this research: WikiPathways, BioCarta, Reactive, and the Kyoto Encyclopaedia of Genes and Genomes (KEGG). We used these data repositories to identify the pathways that LC and hepatitis C share. The KEGG pathway identified overlapping genes associated with HCV-related LC.

PPI network analysis and hub protein detection

Subsequently, the study investigated the interaction associations among proteins using STRING (Search Tool for Retrieval of Interaction Genes and Proteins), a web-based application available at https://string-db.org/ (13). Using STRING to analyse the PPI DEG network, you can learn more about the connections among genes. We constructed the PPI protein network from common DEGs using the STRING repository to identify the physical and functional relationships between liver cancer and hepatitis C. Edges, nodes, and the relationships that connect them comprise the PPI network. In this context, the most common nodes are called hub genes. The STRING database presents the interactions between genes in tabular form, starting with the highest scores.

Evaluation of data using statistical methods

Bioinformatics analysis was performed using the R/Bioconductor package, which allows querying, downloading, and performing integrative analyses of TCGA data through the Cancer Genomic Atlas (TCGA) portal. P<0.05 values were used as the standard threshold to evaluate the pathways mentioned in gene ontology (GO) and pathway enrichment analyses. The STRING method was used to examine DEG interactions. Interaction scores >0.7 were considered to indicate high confidence significance and were used to prevent unclear PPIs.

RESULTS

Identification of hepatitis C-associated liver cancer target genes

By comparing genes associated with hepatitis C and liver cancer, we identified 152 DEGs as common therapeutic targets (Figure 1 and Table 1).

GO and KEGG enrichment analyses

Enrichr was used to perform GO and KEGG enrichment analyses to assess the biological values of common genes and their enriched pathways. Three categories emerged from the GO analysis: biological processes (BS), molecular function (MF), and cellular component (HB). We used the GO database as a source to annotate the data. Table 2 provides a concise overview of the 10 most significant terms in the BS, MF, and HB categories. For each of the distinct categories, the bar chart in Figure 2 presents a comprehensive ontological study in a linear manner. The bar chart in Figure 3 presents the top ten terms across the WikiPathways, BioCarta, Reactive, and KEGG pathways.

The Reactome, WikiPathways, KEGG, and Biocarta databases were used to identify pathways of common DEGs associated with liver cancer and hepatitis C. Table 3 summarises the most important pathways identified via analysis of the Reactive, WikiPathways, KEGG, and Biocarta datasets. Figure 3 presents the pathway enrichment analysis results as a bar graph. Hepatitis C and Hepatocellular Carcinoma WP3646 (p=1.014e-20) are included in term 38 via WikiPathways (Figure 3a). Although there are cancer-related pathways in BioCarta (Figure 3a) and Reactome (Figure 3b), none are directly related to hepatitis or HCC. In the KEGG pathway, hepatocellular carcinoma (p=2.101e-34) was found in the 11th term, whereas hepatitis C (p=3.949e-26) was found in the 31st term. The following list includes other cancers linked to HCC associated with HCC (Figure 3d).

Identification of Hub proteins using STRING analysis

We used STRING with Cytoscape to construct a differential gene interaction network through protein-protein interaction (PPI) analysis. A total of 152 nodes and 3077 edges make up the network's final topology (Figure 4). Using the STRING v12.0 analysis (https://string-db.org/), we could construct gene-gene and network relationships.

Our evaluation of hepatitis C-associated liver cancer identified 152 genes with altered expression levels. Using STRING, a web-based tool for network analysis, the possible connections between these 152 genes showed that there were more physiologically important links in the final network (PPI enrichment p value: < 1.0e-16).

We selected the 20 hub genes (Tumour Protein P53 *(TP53)*, Murine double minute 2 *(MDM2)*, E1A Binding Protein P300 *(EP300)*, Heat Shock Protein 90 Alpha Family Class A Member 1 *(HSP90AA1)*, ATM serine/threonine kinase *(ATM)*, Murine double minute 4 *(MDM4)*, B Cell Leukaemia /Lymphoma 2 *(BCL2)*, Breast cancer 1 *(BRCA1)*, Cyclin-dependent kinase inhibitor 1

Table 1: Comparison of two gene expression datasets for identifying co-expressed genes

Features	Total	Target genes
Coexpressed genes of X and Y	152	*CNBP CXCR4 EP300 MET BRCA1 ACSL3 FEV ALDH2 NFKB2 BCL2 PIK3CB NPM1 HLF APOBEC3B FUBP1 NFE2L2 BRD4 VTI1A SET KIT FHIT B2M CTNNB1 CCND1 NOTCH2 ESR1 PRF1 CDK4 ARHGAP26 HRAS BCL6 MYC ARID2 FOXO1 CCR7 PTPRC EXT1 SMAD2 MAPK1 MDM2 AKT2 EGFR NDRG1 ERBB2 CREB1 CD28 STAT5B MYCN TERT SOCS1 RB1 PTPN6 SRC FBXW7 CD209 TP53 CASP9 CDKN1A FAT1 PIK3R1 GPC3 CDKN2C PER1 POU5F1 PDCD1LG2 LMO1 MLH1 APC TPR PAX5 ERC1 SOX2 ROS1 BCL2L12 PTEN FCGR2B PTPN11 SMAD3 FAS CDKN1B MUC1 BCL9 SND1 CASP8 KRAS PPARG NRAS KLF6 MTOR CBLB LCK RAF1 SDC4 HLA-A STAT3 TRIM27 WAS ATM SPECC1 SMAD4 STAT6 TFRC CLTC HSP90AA1 CASP3 HNF1A EPHA3 RARA TNC MYD88 PML PIK3CA IL2 SYK DDIT3 CDH1 DDX6 IL6ST FCRL4 WRN BAP1 MSH2 NCKIPSD CEBPA SALL4 IL7R SDHC NFKBIE TCF7L2 JAK1 CCND2 AKT1 EIF4A2 CUX1 RAD21 MAP2K1 CLIP1 DCC FOXO3 CDKN2A JUN NF2 NRG1 DDX3X FLNA NOTCH1 CD274 TP63 MDM4 ERCC2 PMS2 TOP*1

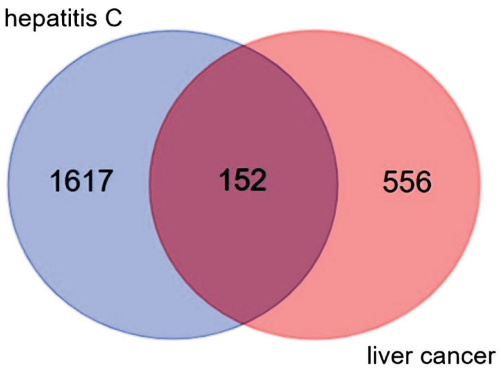

Figure 1: 152 DEGs identified in liver cancer and hepatitis C target genes

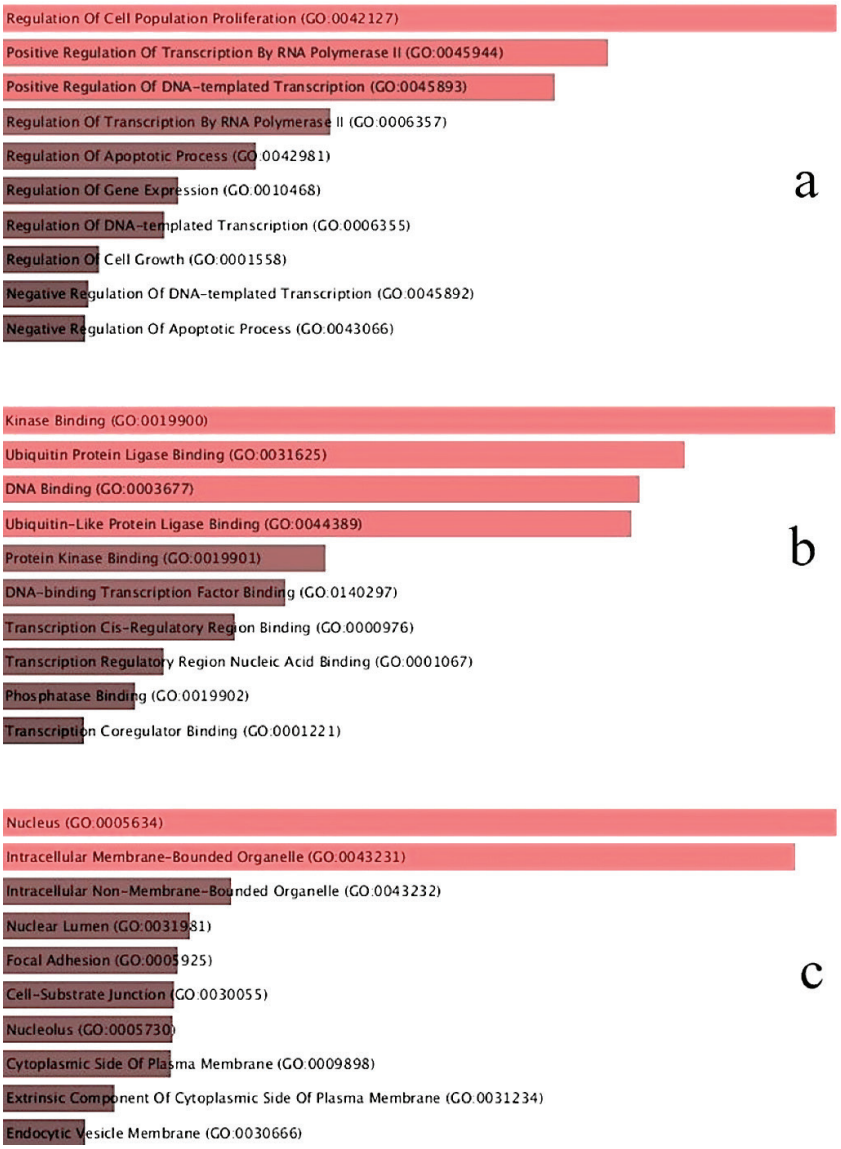

Figure 2: The Enrichr online programme was used to create bar graphs showing the ontological evaluation of shared DEGs between hepatitis C and liver cancer for (a) biological processes, (b) molecular function, and (c) cellular components

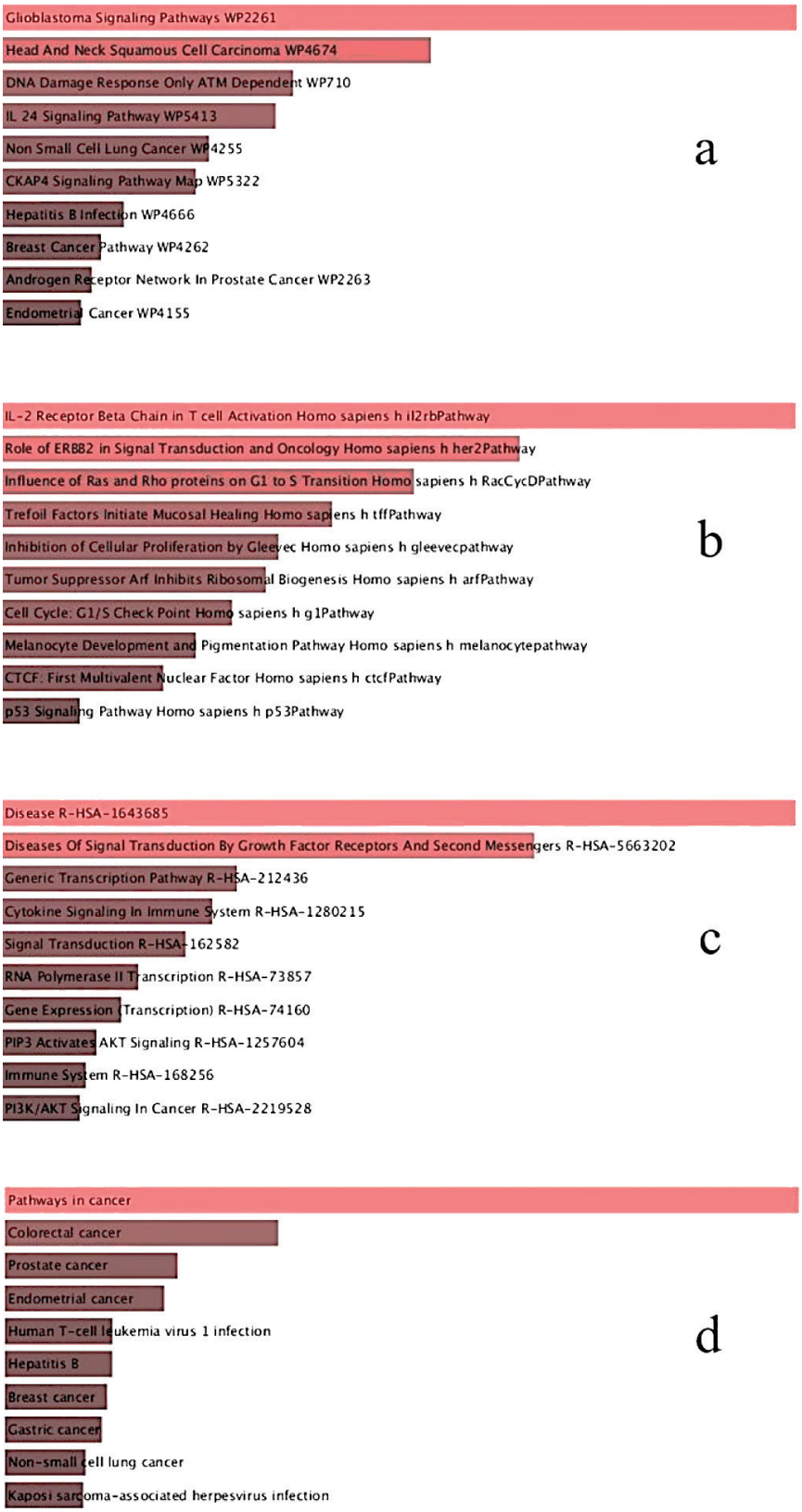

Figure 3: Bar graphs showing the pathway enrichment findings for shared differentially expressed genes (DEGs) between HCV and liver cancer. The data were gathered from four databases: (a) WikiPathways, (b) BioCarta, (c) Reactive, and (d) Enrichr database for KEGG

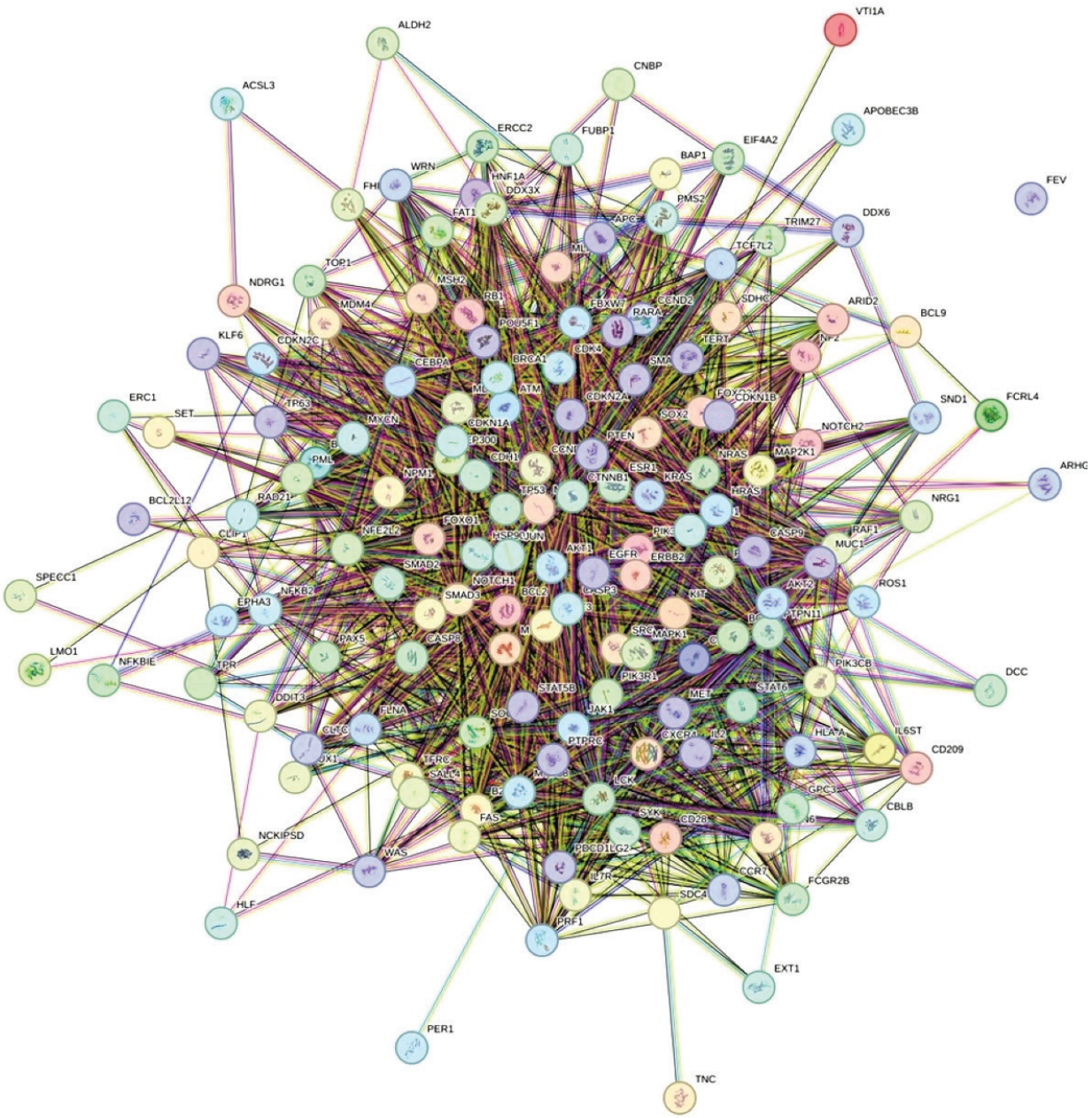

Figure 4: Interaction diagram showing 152 co-expressed genes associated with liver cancer and hepatitis C using the String v12.0 database

(CDKN1A), Cyclin-dependent kinase inhibitor 2A *(CDKN2A)*, Phosphatase and tensin homologue *(PTEN)*, Transcription factor 7-like 2 *(TCF7L2)*, Katenin beta 1 *(CTNNB1)*, Spleen Associated Tyrosine Kinase *(SYK)*, SRC Proto-Oncogene, Non-Receptor Tyrosine Kinase *(SRC)*, Signal transducer and activator of transcription 5B *(STAT5B)*, Janus kinase 1 *(JAK1)*, Signal transducer and activator of transcription 3 *(STAT3)*, Erb-B2 Receptor Tyrosine Kinase 2 *(ERBB2)*, Epidermal growth factor receptor *(EGFR)*) with the highest score from the network using the genomic association method via the Cytoscape plugin (Table 4). New therapeutic approaches may be possible because of the discovery of hub genes, which may also serve as biomarkers for diseases currently under investigation.

DISCUSSION

In this study, common differentially expressed genes for liver cancer and HCV infection were identified using bioinformatics techniques. Furthermore, PPI analysis of common differential genes identified the top 20 target genes. These target genes can guide potential treatment options and serve as biomarkers for liver cancer and hepatitis C infection.

Table 2: Ontology assessment of shared DEGs in liver cancer and hepatitis C

Category	GO ID	Term	P value	Genes
GO biological process	GO:0042127	Regulation of cell population proliferation	1.030e-26	*CEBPA;CDKN1A;NOTCH1;CDKN1B;TFRC;PTEN;EGFR;CCND2;MYC;AKT2;ERBB2;AKT1;STAT6;ARID2;HRAS;STAT5B;TCF7L2;MAP2K1;JUN;NPM1;SMAD3;CDKN2C;CDKN2A;CNBP;STAT3;NRG1;IL2;PML;BCL6;APC;CDK4;KIT;BCL2;MDM2;RARA;CTNNB1;MDM4;PTPN6;KRAS;NF2;RAF1;IL6ST;IL7R;TP53*
	GO:0045944	Positive regulation of transcription by RNA polymerase II	4.227e-24	*CEBPA;NOTCH1;DDX3X;WAS;PIK3R1;BRCA1;FOXO3;FOXO1;EGFR;SOX2;BCL2L12;MUC1;MYC;EP300;STAT6;HRAS;TP63;BRD4;LMO1;SMAD2;STAT5B;TCF7L2;SMAD4;JUN;NPM1;SMAD3;CNBP;STAT3;HNF1A;ESR1;POU5F1;NFKB2;BCL9;PER1;KLF6;MYCN;CREB1;DDIT3;RARA;CTNNB1;ATM;PPARG;TP53;MET;NFE2L2*
	GO:0045893	Positive regulation of DNA-templated transcription	1.724e-23	*DDX3X;BRCA1;SOX2;CDH1;MYC;EP300;AKT1;HRAS;TP63;LMO1;MAP2K1;HNF1A;POU5F1;MYCN;CREB1;DDIT3;RARA;PPARG;TP53;MET;CEBPA;NOTCH1;WAS;PIK3R1;FOXO3;FOXO1;EGFR;BCL2L12;TERT;ERBB2;STAT6;BRD4;SMAD2;STAT5B;TCF7L2;SMAD4;JUN;NPM1;SMAD3;CNBP;STAT3;ESR1;MTOR;NFKB2;BCL9;PER1;KLF6;CTNNB1;ATM;NFE2L2*
	GO:0006357	Regulation of transcription by RNA polymerase II	6.412e-21	*RB1;DDX3X;BRCA1;SOX2;CCND1;MYC;SALL4;EP300;TRIM27;ARID2;HRAS;TP63;LMO1;HNF1A;PAX5;POU5F1;MYCN;CREB1;DDIT3;RARA;PPARG;TP53;MET;CEBPA;NOTCH1;WAS;PIK3R1;FOXO3;FOXO1;EGFR;BCL2L12;MUC1;CUX1;RAD21;TPR;STAT6;BRD4;SMAD2;STAT5B;TCF7L2;SMAD4;JUN;NPM1;SMAD3;CNBP;STAT3;ESR1;NFKB2;BCL9;PER1;KLF6;BCL6;CDK4;FEV;MDM2;CTNNB1;ATM;MDM4;NFE2L2*
	GO:0042981	Regulation of the apoptotic process	4.578e-20	*NOTCH2;DDX3X;TFRC;SRC;PIK3R1;FOXO3;FOXO1;EGFR;CASP9;CCND2;CASP8;MYC;AKT2;CASP3;ERBB2;AKT1;FLNA;JUN;HSP90AA1;NPM1;NRG1;IL2;MTOR;BCL6;APC;BCL2;MDM2;CD28;CTNNB1;FAS;ATM;PTPN6;NF2;RAF1;IL6ST;TP53*
	GO:0010468	Regulation of gene expression	3.531e-19	*RB1;NOTCH2;CEBPA;NOTCH1;DDX3X;TFRC;TNC;BRCA1;PIK3CB;SOX2;CASP8;TERT;CDH1;MYC;AKT1;ERC1;HRAS;MAP2K1;SMAD3;FBXW7;CNBP;STAT3;NRG1;ESR1;POU5F1;MTOR;PML;NFKB2;PER1;MYCN;PTPRC;BCL6;CDK4;DDIT3;FUBP1;MDM2;CD28;ATM;PPARG;KRAS;TP53;MYD88;NFE2L2*
	GO:0006355	Regulation of DNA-templated transcription	5.111e-19	*RB1;CDKN1B;BRCA1;SOX2;CDH1;MYC;SALL4;EP300;AKT1;ERC1;ARID2;HRAS;TP63;MAP2K1;NRG1;HNF1A;PAX5;POU5F1;MYCN;CREB1;DDIT3;RARA;PPARG;TP53;CEBPA;SET;NOTCH1;FOXO3;FOXO1;EGFR;CUX1;RAD21;STAT6;BRD4;SMAD2;STAT5B;TCF7L2;SMAD4;JUN;NPM1;SMAD3;CDKN2A;CNBP;STAT3;ESR1;PML;NFKB2;PER1;KLF6;BCL6;FEV;MDM2;CTNNB1;MDM4;NFE2L2*
	GO:0001558	Regulation of cell growth	2.860e-18	*RB1;SMAD4;CDKN1A;SMAD3;CDKN1B;DDX3X;CDKN2C;TFRC;CDKN2A;TNC;NRG1;BRCA1;IL2;EGFR;MTOR;PML;BCL6;ERBB2;BCL2;AKT1;ROS1;TP53*
	GO:0045892	Negative regulation of DNA-templated transcription	3.784e-18	*RB1;CEBPA;SET;NOTCH1;CDKN1B;BRCA1;FOXO3;FOXO1;SOX2;MUC1;CCND1;CUX1;MYC;TPR;EP300;FLNA;TRIM27;TP63;SMAD2;TCF7L2;SMAD4;JUN;SMAD3;CDKN2A;CNBP;STAT3;NRG1;ESR1;POU5F1;PML;PER1;CREB1;BCL6;DDIT3;MDM2;RARA;CTNNB1;MDM4;PPARG;TP53*
	GO:0043066	Negative regulation of the apoptotic process	4.117e-18	*RB1;NOTCH2;SET;NOTCH1;DDX3X;TFRC;SRC;PIK3R1;FOXO1;EGFR;CCND2;MYC;AKT2;ERBB2;AKT1;FLNA;NPM1;IL2;MTOR;MSH2;PIK3CA;BCL2;MDM2;CD28;CTNNB1;FAS;RAF1;IL6ST;TP53*

Table 2: Ontology assessment of shared DEGs in liver cancer and hepatitis C (Continue)

Category	GO ID	Term	P value	Genes
	GO:0005634	Nucleus	6.773e-28	*RB1;SOX2;CCND2;CCND1;MYC;AKT2;SALL4;EP300;AKT1;TRIM27;TP63;LMO1;MAP2K1;FBXW7;HNF1A;SND1;CLIP1;MYCN;MSH2;DDIT3;TP53;NOTCH2;DDX6;SET;NOTCH1;PIK3R1;FOXO3;FHIT;NDRG1;FOXO1;BCL2L12;TERT;RAD21;TPR;SMAD2;STAT5B;SMAD4;JUN;SMAD3;ESR1;NFKB2;BCL6;CDK4;FEV;FUBP1;BCL2;MDM2;FAT1;ATM;MDM4;NF2;MYD88;APOBEC3B;NFE2L2;CDKN1A;CDKN1B;DDX3X;PTEN;BRCA1;PIK3CB;CASP3;JAK1;HSP90AA1;SYK;POU5F1;CREB1;RARA;PPARG;TOP1;CEBPA;WAS;CXCR4;EGFR;WRN;CUX1;ERBB2;MAPK1;FLNA;PMS2;STAT6;BRD4;TCF7L2;NPM1;CDKN2C;CDKN2A;CNBP;STAT3;PTPN11;MLH1;MTOR;PML;PER1;KLF6;APC;ERCC2;CTNNB1;PTPN6*
	GO:0043231	Intracellular membrane-bound organelles	1.320e-26	*RB1;TFRC;SOX2;CCND2;CCND1;MYC;AKT2;SALL4;EP300;AKT1;TRIM27;TP63;LMO1;MAP2K1;FBXW7;HNF1A;SND1;CLIP1;MYCN;MSH2;DDIT3;TP53;NOTCH2;DDX6;SET;NOTCH1;PIK3R1;FOXO3;FHIT;NDRG1;FOXO1;BCL2L12;TERT;RAD21;TPR;VTI1A;SMAD2;SPECC1;STAT5B;SMAD4;JUN;SMAD3;ESR1;NFKB2;BCL9;BCL6;CDK4;FEV;FUBP1;BCL2;MDM2;FAT1;ATM;MDM4;NF2;MYD88;APOBEC3B;NFE2L2;CDKN1A;CDKN1B;DDX3X;PTEN;BRCA1;PIK3CB;CASP3;JAK1;HSP90AA1;SYK;POU5F1;CREB1;RARA;PPARG;TOP1;CEBPA;WAS;CXCR4;EGFR;MUC1;WRN;CUX1;ERBB2;MAPK1;FLNA;PMS2;STAT6;BRD4;TCF7L2;NPM1;CDKN2C;CDKN2A;CNBP;STAT3;PTPN11;MLH1;MTOR;PML;PER1;KLF6;APC;ERCC2;CTNNB1;PTPN6*
GO Cellular Component	GO:0043232	Intracellular Non-membrane-bound organelles	5.826e-9	*CDKN1A;SET;CLTC;BRCA1;PIK3CB;WRN;CCND2;CASP8;TERT;MYC;RAD21;MAPK1;FLNA;TRIM27;NPM1;ACSL3;MLH1;PML;KLF6;MYCN;BCL6;CDK4;ERCC2;MDM2;RARA;ATM;PTPN6;NF2;TOP1;TP53*
	GO:0031981	Nuclear lumen	1.128e-7	*JUN;CDKN1A;NPM1;PIK3CB;PML;WRN;KLF6;CCND2;MYCN;TERT;BCL6;CDK4;MYC;MDM2;RARA;FLNA;ATM;PTPN6;TRIM27;NF2;TOP1;TP53*
	GO:0005925	Focal adhesion	2.747e-7	*MAP2K1;NPM1;SRC;CLTC;TNC;CXCR4;EGFR;PTPRC;FAT1;MAPK1;FLNA;CTNNB1;KRAS;B2M;JAK1*
	GO:0030055	Cell-substrate junction	3.565e-7	*MAP2K1;NPM1;SRC;CLTC;TNC;CXCR4;EGFR;PTPRC;FAT1;MAPK1;FLNA;CTNNB1;KRAS;B2M;JAK1*
	GO:0005730	Nucleolus	4.006e-7	*CDKN1A;NPM1;PIK3CB;PML;WRN;KLF6;CCND2;MYCN;TERT;BCL6;CDK4;MYC;MDM2;RARA;FLNA;ATM;PTPN6;TRIM27;NF2;TOP1;TP53*
	GO:0009898	Cytoplasmic side of the plasma membrane	4.498e-7	*PTPRC;SYK;CDH1;LCK;SRC;PTEN;KRAS;MYD88;JAK1*
	GO:0031234	Extrinsic component of the cytoplasmic side of a plasma membrane	0.00002653	*SYK;LCK;SRC;KRAS;MYD88;JAK1*
	GO:0030666	Endocytic vesicle membrane	0.0002169	*TFRC;CLTC;MDM2;HLA-A;IL7R;B2M;EGFR*

Table 2: Ontology assessment of shared DEGs in liver cancer and hepatitis C (Continue)

Category	GO ID	Term	P value	Genes
	GO:0019900	Kinase binding	9.156e-21	*RB1;CEBPA;CDKN1A;CDKN1B;TFRC;CLTC;WAS;FOXO3;EGFR;CASP9;CCND2;SOCS1;CCND1;FLNA;TCF7L2;NPM1;SMAD3;CDKN2C;CDKN2A;STAT3;PTPN11;ACSL3;ESR1;PER1;MYCN;PTPRC;APC;LCK;CTNNB1;FAS;PTPN6*
	GO:0031625	Binding of ubiquitin protein ligase	6.171e-19	*RB1;SMAD2;JUN;CDKN1A;HSP90AA1;SMAD3;CDKN1B;FBXW7;CXCR4;BRCA1;FHIT;FOXO1;POU5F1;EGFR;PML;PER1;CASP8;APC;BCL2;MDM2;CTNNB1;TP53;NFE2L2;JAK1*
	GO:0003677	DNA binding	2.197e-18	*CEBPA;DDX3X;BRCA1;FOXO3;FOXO1;EGFR;SOX2;WRN;TERT;CUX1;MYC;EP300;PMS2;TP63;SMAD2;TCF7L2;SMAD4;JUN;SMAD3;CNBP;STAT3;HNF1A;MLH1;POU5F1;PER1;MYCN;MSH2;BCL6;DDIT3;FUBP1;ERCC2;BCL2;RARA;PPARG;TOP1;TP53;NFE2L2*
	GO:0044389	Ubiquitin-like protein ligase binding	2.761e-18	*RB1;SMAD2;JUN;CDKN1A;HSP90AA1;SMAD3;CDKN1B;FBXW7;CXCR4;BRCA1;FHIT;FOXO1;POU5F1;EGFR;PML;PER1;CASP8;APC;BCL2;MDM2;CTNNB1;TP53;NFE2L2;JAK1*
GO-Molecular Function	GO:0019901	Protein kinase binding	1.443e-14	*CDKN1A;CDKN1B;TFRC;CLTC;WAS;FOXO3;CASP9;CCND2;SOCS1;CCND1;TPR;TCF7L2;HSP90AA1;NPM1;SMAD3;CDKN2C;CDKN2A;STAT3;PTPN11;ACSL3;ESR1;PTPRC;APC;LCK;RARA;PTPN6*
	GO:0140297	DNA-binding transcription factor binding	4.472e-14	*RB1;LMO1;SMAD2;CEBPA;TCF7L2;JUN;NPM1;SMAD3;STAT3;CREB1;BCL6;MYC;DDIT3;BCL2;EP300;FLNA;CTNNB1;PPARG;TP53;NFE2L2*
	GO:0000976	Transcription of Cis regulatory region binding	1.842e-13	*RB1;SMAD2;CEBPA;TCF7L2;SMAD4;JUN;NPM1;SMAD3;STAT3;HNF1A;BRCA1;FOXO3;POU5F1;SOX2;PER1;CUX1;MYC;DDIT3;RARA;PPARG;STAT6;TP53;NFE2L2;BRD4*
	GO:0001067	Transcription regulatory region nucleic acid binding	1.346e-12	*CEBPA;TCF7L2;SMAD4;JUN;SMAD3;STAT3;HNF1A;BRCA1;FOXO3;POU5F1;SOX2;PER1;DDIT3;PPARG;TP53;NFE2L2;BRD4*
	GO:0019902	Phosphatase binding	2.988e-12	*SMAD2;SMAD3;CDKN1B;STAT3;PIK3R1;EGFR;LCK;MAPK1;CTNNB1;STAT6;ROS1;MET;JAK1*
	GO:0001221	Transcription coregulator binding	1.254e-11	*PER1;SMAD4;SMAD3;TERT;BCL6;MYC;RARA;EP300;CTNNB1;PPARG;STAT6;ESR1*

GO: gene ontology, p value: probability value

Table 3: Evaluation of common differentially expressed genes between liver cancer and hepatitis C according to pathway enrichment analysis

Category	Term	P value	Genes
WikiPathways	Glioblastoma signalling pathway WP2261	2.063e-47	*RB1;CDKN1A;CDKN1B;SRC;PTEN;PIK3CB;BRCA1;PIK3R1;FOXO3;FOXO1;EGFR;NRAS;CCND2;CCND1;AKT2;ERBB2;AKT1;EP300;MAPK1;HRAS;MAP2K1;CDKN2C;CDKN2A;PIK3CA;CDK4;MDM2;KRAS;MDM4;ATM;RAF1;MET;TP53*
	Head and neck squamous cell carcinoma (WP4674)	3.077e-41	*RB1;NOTCH2;CDKN1A;NOTCH1;PTEN;PIK3CB;PIK3R1;EGFR;NRAS;CASP8;CCND1;TERT;AKT2;ERBB2;AKT1;HRAS;TP63;SMAD4;CDKN2A;MTOR;NFKB2;PIK3CA;CDK4;FAT1;CTNNB1;KRAS;TP53;NFE2L2*
	DNA damage response of ATM-dependent WP710	6.551e-39	*CDKN1A;CDKN1B;PTEN;PIK3R1;PIK3CB;FOXO3;NRAS;CCND2;CCND1;MYC;AKT2;ERBB2;AKT1;MAPK1;HRAS;TCF7L2;JUN;SMAD4;SMAD3;CDKN2A;NFKB2;BCL6;PIK3CA;APC;BCL2;MDM2;CTNNB1;ATM;KRAS;TP53*
	IL-24 signalling pathway WP5413	1.301e-38	*RB1;CDKN1A;CDKN1B;SRC;PRF1;PTEN;CXCR4;PIK3R1;NDRG1;EGFR;SOX2;CASP9;CASP8;CCND1;CDH1;MYC;CASP3;AKT1;MAPK1;JAK1;JUN;HSP90AA1;CDKN2A;STAT3;IL2;MTOR;PTPRC;BCL6;PIK3CA;APC;DDIT3;BCL2;CTNNB1;FAS;ATM;TP53;NFE2L2*
	Nonsmall cell lung cancer WP4255	1.739e-37	*RB1;CDKN1A;PIK3CB;PIK3R1;FOXO3;FHIT;EGFR;CASP9;NRAS;CASP8;CCND1;AKT2;CASP3;ERBB2;AKT1;MAPK1;HRAS;STAT5B;MAP2K1;CDKN2A;STAT3;PIK3CA;CDK4;KRAS;RAF1;TP53*
	CKAP4 signalling pathway map of WP5322	2.892e-37	*CDKN1B;SRC;PTEN;BRCA1;EGFR;SOX2;CASP9;CASP8;CCND1;CDH1;MYC;CASP3;AKT1;MAPK1;MAP2K1;JUN;SMAD4;SMAD3;FBXW7;ESR1;POU5F1;MYCN;PIK3CA;CDK4;MDM2;FAS;CTNNB1;ATM;TP53*
	Hepatitis B infection with WP4666	4.753e-36	*CDKN1A;DDX3X;SRC;PIK3R1;PIK3CB;CASP9;NRAS;CASP8;MYC;AKT2;CASP3;EP300;AKT1;MAPK1;STAT6;HRAS;JAK1;SMAD2;STAT5B;MAP2K1;JUN;SMAD4;SMAD3;STAT3;CREB1;PIK3CA;BCL2;FAS;KRAS;RAF1;MYD88*
	Breast Cancer pathway WP4262	1.152e-35	*RB1;NOTCH2;CDKN1A;NOTCH1;PTEN;PIK3R1;BRCA1;EGFR;NRAS;CCND1;MYC;AKT2;ERBB2;AKT1;MAPK1;HRAS;TCF7L2;MAP2K1;JUN;ESR1;MTOR;NFKB2;PIK3CA;APC;CDK4;KIT;CTNNB1;ATM;KRAS;RAF1;TP53*
	Androgen receptor network in prostate Cancer WP2263	1.653e-35	*RB1;PTEN;BRCA1;NDRG1;CASP9;CASP8;CCND1;MYC;CASP3;AKT1;MAPK1;HRAS;JAK1;SMAD2;MAP2K1;JUN;SMAD3;STAT3;PTPN11;MTOR;MSH2;PIK3CA;CDK4;BCL2;MDM2;ATM;RAF1;TP53*
	Endometrial cancer WP4155	2.523e-35	*TCF7L2;CDKN1A;MAP2K1;PTEN;PIK3CB;PIK3R1;FOXO3;EGFR;CASP9;NRAS;CCND1;PIK3CA;APC;CDH1;AKT2;MYC;ERBB2;AKT1;MAPK1;CTNNB1;KRAS;RAF1;HRAS;TP53*

Table 3: Evaluation of common differentially expressed genes between liver cancer and hepatitis C according to pathway enrichment analysis (Continue)

Category	Term	P value	Genes
BioCarta	IL-2 receptor beta chain in t-cell activation homo sapiens h ıl2rb pathway	2.394e-26	*RB1;STAT5B;MAP2K1;PIK3R1;IL2;CCND2;SOCS1;CCND1;PIK3CA;MYC;BCL2;AKT1;MAPK1;FAS;PTPN6;RAF1;HRAS;JAK1*
	Role of ERBB2 in signal transduction and oncology of homo sapiens h her2 pathway	5.672e-21	*MAP2K1;STAT3;PIK3R1;ESR1;EGFR;PIK3CA;ERBB2;EP300;AKT1;MAPK1;RAF1;HRAS;JAK1*
	Influence of ras and rho proteins on g1-to-s transition in homo sapiens h raccycdpathway	6.538e-19	*RB1;CDKN1A;MAP2K1;CDKN1B;CCND1;PIK3CA;CDK4;AKT1;MAPK1;PIK3R1;RAF1;HRAS*
	Trefoil factors ınitiate mucosal healing by homo sapiens h off pathway	2.554e-17	*CASP9;MAP2K1;PIK3CA;CASP3;ERBB2;AKT1;MAPK1;CTNNB1;PIK3R1;RAF1;HRAS;EGFR*
	Inhibition of cellular proliferation by the Gleevec Homo sapiens h Gleevec pathway	2.847e-16	*STAT5B;JUN;MAP2K1;PIK3CA;MYC;BCL2;AKT1;PIK3R1;RAF1;HRAS*
	Tumour suppressor arf ınhibits ribosomal biogenesis homo sapiens h arf pathways	5.005e-16	*RB1;HSP90AA1;PIK3CA;CDKN2A;MYC;MDM2;AKT1;ATM;PIK3R1;TP53*
	Cell Cycle: G1/S checkpoint pathway of Homo sapiens h g1	2.279e-15	*RB1;CDKN1A;SMAD4;CDKN1B;SMAD3;CCND1;CDKN2A;CDK4;ATM;TP53*
	Melanocyte development and pigmentation pathway of homo sapiens	1.151e-14	*MAP2K1;CREB1;KIT;BCL2;EP300;MAPK1;RAF1;HRAS*
	CTCF: First multivalent nuclear factor of homo sapiens h ctcf pathway	4.967e-14	*SMAD4;CDKN1B;PIK3CA;CDKN2A;MYC;PTEN;MDM2;PIK3R1;MTOR*
	p53 signalling pathway of homo sapiens h p53	2.104e-12	*RB1;CDKN1A;CCND1;CDK4;BCL2;MDM2;TP53*

Table 3: Evaluation of common differentially expressed genes between liver cancer and hepatitis C according to pathway enrichment analysis (Continue)

Category	Term	P value	Genes
Reactome	Disease R-HSA-1643685	1.661e-43	*RB1;CDKN1A;CDKN1B;CLTC;PTEN;BRCA1;PIK3CB;CASP9;CCND2;CASP8;CCND1;MYC;AKT2;EP300;AKT1;TRIM27;B2M;HRAS;JAK1;MAP2K1;HSP90AA1;SYK;FBXW7;NRG1;SND1;HLA-A;CREB1;MSH2;PIK3CA; LCK;KIT;TP53;NOTCH2;NOTCH1;SDC4;SRC;WAS;CXCR4;PIK3R1;FOXO3;FOXO1;EGFR;NCKIPSD;MUC1;NRAS;WRN;CUX1;TPR;ERBB2;GPC3;MAPK1;PMS2;BRD4;SMAD2;STAT5B;TCF7L2;SMAD4;JUN;NPM1;SMAD3;CDKN2A;CNBP;STAT3;PTPN11;MLH1;ESR1;MTOR;PML;NFKB2;EXT1;CDK4;ERCC2;MDM2;CD28;CTNNB1;ATM;PTPN6;MYD88;NFE2L2*
	Diseases of signal transduction via growth factor receptors and second messengers R-HSA-5663202	1.152e-37	*CDKN1A;NOTCH1;CDKN1B;SRC;CLTC;PTEN;PIK3R1;PIK3CB;FOXO3;FOXO1;EGFR;CASP9;NRAS;CUX1;MYC;AKT2;TPR;ERBB2;EP300;AKT1;MAPK1;HRAS;SMAD2;STAT5B;TCF7L2;MAP2K1;SMAD4;HSP90AA1;NPM1;SMAD3;FBXW7;STAT3;NRG1;PTPN11;ESR1;SND1;MTOR;CREB1;PIK3CA;LCK;KIT;MDM2;CD28;CTNNB1*
	Generic transcription pathway R-HSA-212436	5.144e-31	*RB1;CDKN1A;CDKN1B;PTEN;BRCA1;SOX2;CCND2;CCND1;MYC;AKT2;EP300;AKT1;ARID2;TP63;LMO1;FBXW7;HNF1A;PAX5;CREB1;MSH2;DDIT3;KIT;RARA;PPARG;TP53;NOTCH2;NOTCH1;SRC;FOXO3;NDRG1;FOXO1;EGFR;NRAS;WRN;ERBB2;MAPK1;PMS2;SMAD2;TCF7L2;SMAD4;JUN;NPM1;SMAD3;CDKN2A;PTPN11;MLH1;ESR1;IL2;MTOR;PML;BCL6;CDK4;ERCC2;MDM2;CTNNB1;ATM;MDM4*
	Cytokine signalling in immune system R-HSA-1280215	1.822e-30	*EIF4A2;CDKN1A;CDKN1B;PIK3R1;PIK3CB;FOXO3;FOXO1;SOX2;MUC1;NRAS;SOCS1;CASP8;CCND1;MYC;AKT2;CASP3;TPR;AKT1;MAPK1;FLNA;STAT6;B2M;HRAS;JAK1;STAT5B;MAP2K1;JUN;HSP90AA1;SMAD3;SYK;STAT3;PTPN11;IL2;PML;HLA-A;NFKB2;CREB1;BCL6;PIK3CA;LCK;BCL2;PTPN6;IL6ST;IL7R;TP53;MYD88*
	Signal transduction R-HSA-162582	7.225e-30	*CDKN1A;CDKN1B;TFRC;CLTC;PTEN;PIK3CB;SOX2;CASP9;CASP8;CCND1;MYC;AKT2;SALL4;CASP3;EP300;AKT1;CCR7;TRIM27;HRAS;JAK1;MAP2K1;HSP90AA1;SYK;FBXW7;NRG1;CLIP1;-MYCN;CREB1;PIK3CA;LCK;KIT;RARA;PPARG;IL6ST;TP53;NOTCH2;CD274;NOTCH1;SRC;WAS;CXCR4;PIK3R1;FOXO3;FOXO1;EGFR;NCKIPSD;NRAS;SOCS1;TERT;RAD21;ERBB2;MAPK1;FLNA;STAT6;SMAD2;STAT5B;TCF7L2;SMAD4;JUN;SMAD3;STAT3;PTPN11;ARHGAP26;ESR1;IL2;MTOR;PML;BCL9;CDK4;BCL2;MDM2;CD28;CTNNB1;PTPN6;NF2;MYD88*
	RNA Polymerase II Transcription R-HSA-73857	8.311e-29	*RB1;CDKN1A;CDKN1B;PTEN;BRCA1;SOX2;CCND2;CCND1;MYC;AKT2;EP300;AKT1;ARID2;TP63;LMO1;FBXW7;HNF1A;PAX5;CREB1;MSH2;DDIT3;KIT;RARA;PPARG;TP53;NOTCH2;NOTCH1;SRC;FOXO3;NDRG1;FOXO1;EGFR;NRAS;WRN;ERBB2;MAPK1;PMS2;SMAD2;TCF7L2;SMAD4;JUN;NPM1;SMAD3;CDKN2A;PTPN11;MLH1;ESR1;IL2;MTOR;PML;BCL6;CDK4;ERCC2;MDM2;CTNNB1;ATM;MDM4*
	Gene expression (Transcription) R-HSA-74160	1.993e-28	*RB1;CDKN1A;CDKN1B;PTEN;BRCA1;SOX2;CCND2;CCND1;MYC;AKT2;EP300;AKT1;ARID2;TP63;LMO1;HSP90AA1;FBXW7;HNF1A;PAX5;CREB1;MSH2;DDIT3;KIT;RARA;PPARG;TP53;NOTCH2;NOTCH1;SRC;FOXO3;NDRG1;FOXO1;EGFR;NRAS;WRN;TPR;ERBB2;MAPK1;PMS2;SMAD2;TCF7L2;SMAD4;JUN;NPM1;SMAD3;CDKN2A;PTPN11;MLH1;ESR1;IL2;MTOR;PML;BCL6;CDK4;ERCC2;MDM2;CTNNB1;ATM;MDM4*
	PIP3 activates AKT signalling R-HSA1257604	7.098e-28	*CDKN1A;CDKN1B;SRC;PTEN;PIK3R1;PIK3CB;FOXO3;FOXO1;EGFR;CASP9;AKT2;SALL4;ERBB2;AKT1;MAPK1;TRIM27;JUN;NRG1;PTPN11;ESR1;MTOR;PML;CREB1;PIK3CA;LCK;KIT;MDM2-;CD28;PPARG;TP53;MYD88*
	Immune System R-HSA-168256	1.230e-27	*EIF4A2;CDKN1A;CDKN1B;DDX3X;CLTC;PTEN;CBLB;PIK3CB;SOX2;CASP9;CASP8;CCND1;MYC;AKT2;CASP3;EP300;AKT1;B2M;HRAS;JAK1;MAP2K1;HSP90AA1;SYK;FBXW7;HLA-A;PDCD1LG2;CREB1;PIK3CA;LCK;IL6ST;TP53;CD274;SRC;WAS;PIK3R1;FOXO3;FOXO1;NCKIPSD;MUC1;NRAS;SOCS1;TPR;MAPK1;FLNA;STAT6;STAT5B;JUN;SMAD3;STAT3;PTPN11;IL2;MTOR;PML;NFKB2;PTPRC;BCL6;CD209;BCL2;CD28;CTNNB1;PTPN6;NF2;IL7R;NFKBIE;FCGR2B;MYD88*
	PI3K/AKT signalling in cancer R-HSA-2219528 Cells	1.679e-27	*CDKN1A;CDKN1B;SRC;PTEN;NRG1;PTPN11;PIK3R1;PIK3CB;FOXO3;ESR1;FOXO1;EGFR;MTOR;CASP9;CREB1;PIK3CA;LCK;AKT2;KIT;ERBB2;CD28;MDM2;AKT1*

Table 3: Evaluation of common differentially expressed genes between liver cancer and hepatitis C according to pathway enrichment analysis (Continue)

Category	Term	P value	Genes
KEGG	Pathways in cancer	6.708e-62	*RB1;CDKN1A;CDKN1B;PTEN;PIK3CB;CASP9;CCND2;CASP8;CCND1;CDH1;MYC;AKT2;CASP3;EP300;AKT1;HRAS;JAK1;MAP2K1;HSP90AA1;DCC;MSH2;PIK3CA;KIT;RARA;PPARG;RAF1;IL6ST;TP53;MET;NOTCH2;CEBPA;NOTCH1;CXCR4;PIK3R1;FOXO1;EGFR;NRAS;TERT;TPR;ERBB2;MAPK1;STAT6;SMAD2;STAT5B;TCF7L2;SMAD4;JUN;SMAD3;CDKN2A;STAT3;MLH1;ESR1;IL2;MTOR;PML;NFKB2;APC;CDK4;BCL2;MDM2;CTNNB1;FAS;KRAS;IL7R;NFE2L2*
	Colorectal cancer	1.159e-42	*CDKN1A;PIK3CB;PIK3R1;EGFR;CASP9;NRAS;CCND1;AKT2;CASP3;MYC;AKT1;MAPK1;HRAS;SMAD2;TCF7L2;MAP2K1;JUN;SMAD4;SMAD3;DCC;MLH1;MTOR;MSH2;PIK3CA;APC;BCL2;CTNNB1;KRAS;RAF1;TP53*
	Prostate cancer	5.973e-39	*RB1;CDKN1A;CDKN1B;PTEN;PIK3R1;PIK3CB;FOXO1;EGFR;CASP9;NRAS;CCND1;AKT2;ERBB2;AKT1;EP300;MAPK1;HRAS;TCF7L2;MAP2K1;HSP90AA1;MTOR;CREB1;PIK3CA;BCL2;MDM2;CTNNB1;KRAS;RAF1;TP53*
	Endometrial cancer	1.870e-38	*CDKN1A;PTEN;PIK3CB;PIK3R1;FOXO3;EGFR;CASP9;NRAS;CCND1;CDH1;AKT2;MYC;ERBB2;AKT1;MAPK1;HRAS;TCF7L2;MAP2K1;MLH1;PIK3CA;APC;CTNNB1;KRAS;RAF1;TP53*
	Human T-cell leukaemia virus 1 infection	1.549e-36	*RB1;CDKN1A;PTEN;PIK3R1;PIK3CB;NRAS;CCND2;CCND1;TERT;MYC;AKT2;EP300;AKT1;MAPK1;B2M;HRAS;JAK1;SMAD2;STAT5B;MAP2K1;SMAD4;JUN;SMAD3;CDKN2C;CDKN2A;IL2;HLA-A;NFKB2;CREB1;PIK3CA; CDK4; LCK;ATM;KRAS;TP53*
	Hepatitis B	1.573e-36	*RB1;CDKN1A;DDX3X;SRC;PIK3R1;PIK3CB;CASP9;NRAS;CASP8;MYC;AKT2;CASP3;EP300;AKT1;MAPK1;STAT6;HRAS;JAK1;STAT5B;MAP2K1;SMAD4;JUN;SMAD3;STAT3;CREB1;PIK3CA;BCL2;FAS;KRAS;RAF1;TP53;MYD88*
	Breast cancer	2.403e-36	*RB1;NOTCH2;CDKN1A;NOTCH1;PTEN;PIK3R1;BRCA1;PIK3CB;EGFR;NRAS;CCND1;AKT2;MYC;ERBB2;AKT1;MAPK1;HRAS;TCF7L2;MAP2K1;JUN;ESR1;MTOR;NFKB2;PIK3CA;APC;CDK4;KIT;CTNNB1;KRAS;RAF1;TP53*
	Gastric cancer	3.793e-36	*RB1;CDKN1A;CDKN1B;PIK3R1;PIK3CB;EGFR;NRAS;CCND1;TERT;CDH1;AKT2;MYC;ERBB2;AKT1;MAPK1;HRAS;SMAD2;TCF7L2;MAP2K1;SMAD4;SMAD3;MLH1;MTOR;PIK3CA;APC;BCL2;CTNNB1;KRAS;RAF1;TP53;MET*
	Nonsmall cell lung cancer	1.502e-35	*RB1;CDKN1A;PIK3CB;PIK3R1;FOXO3;FHIT;EGFR;CASP9;NRAS;CCND1;AKT2;ERBB2;AKT1;MAPK1;HRAS;STAT5B;MAP2K1;CDKN2A;STAT3;PIK3CA;CDK4;KRAS;RAF1;MET;TP53*
	Kaposi sarcoma-associated herpesvirus infection	1.848e-35	*RB1;CDKN1A;SRC;PIK3R1;PIK3CB;CASP9;NRAS;CASP8;CCND1;MYC;AKT2;CASP3;EP300;AKT1;MAPK1;HRAS;JAK1;TCF7L2;MAP2K1;JUN;SYK;STAT3;MTOR;HLA-A;CREB1;PIK3CA;CDK4-;CTNNB1;FAS;KRAS;RAF1; IL6ST;TP53*

WP: WikiPathways, R-HSA: Reactome Homo sapiens, KEGG: Kyoto Encyclopaedia of Genes and Genomes

Table 4: Identification of 20 highly linked genes based on Cytoscape score values

Node 1	Node 2	Node 1 access	Node 2 access	Score
TP53	*MDM2*	ENSP00000269305	ENSP00000258149	0.999
TP53	*EP300*	ENSP00000269305	ENSP00000263253	0.999
TP53	*HSP90AA1*	ENSP00000269305	ENSP00000335153	0.999
TP53	*ATM*	ENSP00000269305	ENSP00000278616	0.999
TP53	*MDM4*	ENSP00000269305	ENSP00000356150	0.999
TP53	*BCL2*	ENSP00000269305	ENSP00000381185	0.999
TP53	*BRCA1*	ENSP00000269305	ENSP00000418960	0.999
TP53	*CDKN1A*	ENSP00000269305	ENSP00000384849	0.999
TP53	*CDKN2A*	ENSP00000269305	ENSP00000418915	0.999
TP53	*PTEN*	ENSP00000269305	ENSP00000361021	0.999
TCF7L2	*CTNNB1*	ENSP00000486891	ENSP00000495360	0.999
SYK	*SRC*	ENSP00000364898	ENSP00000362680	0.999
STAT5B	*JAK1*	ENSP00000293328	ENSP00000499900	0.999
STAT3	*EP300*	ENSP00000264657	ENSP00000263253	0.999
STAT3	*JAK1*	ENSP00000264657	ENSP00000499900	0.999
STAT3	*SRC*	ENSP00000264657	ENSP00000362680	0.999
SRC	*STAT3*	ENSP00000362680	ENSP00000264657	0.999
SRC	*ERBB2*	ENSP00000362680	ENSP00000269571	0.999
SRC	*EGFR*	ENSP00000362680	ENSP00000275493	0.999
SRC	*HSP90AA1*	ENSP00000362680	ENSP00000335153	0.999

In the ontological evaluation between liver cancer and hepatitis C, *TP53, MDM2, MDM4, BCL2, CDKN1A, CDKN2A, PTEN, TCF7L2, CTNNB1, STAT5B, STAT3, ERBB2,* and *EGFR* genes are involved in the GO biological process and the regulation of cell population proliferation. *TP53, EP300, ATM, BRCA1, TCF7L2, CTNNB1, STAT5B, STAT3,* and *EGFR* genes play a role in the positive regulation of transcription by RNA polymerase II. While *TP53* and *EGFR* genes function in the positive regulation of DNA template transcription, *TP53, MDM2, EP300, ATM, MDM4, BRCA1, TCF7L2, CTNNB1, STAT5B, STAT3*, and *EGFR* genes are important in the regulation of transcription by RNA polymerase II. *MDM2, HSP90AA1, ATM, BCL2, CTNNB1, ERBB2,* and *EGFR* genes function in regulating the apoptotic process, and *MDM2, ATM, BRCA1,* and *STAT3* genes function in regulating gene expression. *MDM2, EP300, MDM4, BRCA1, CDKN2A, TCF7L2, CTNNB1, STAT5B, STAT3,* and *EGFR* genes are effective in regulating DNA templated transcription. In addition, *BCL2, BRCA1, CDKN1A, CDKN2A, ERBB2,* and *EGFR* genes play a role in the regulation of cell growth, MDM2 contributes to the negative regulation of DNA template transcription, and MDM2*, BCL2, CTNNB1, ERBB2,* and *EGFR* contribute to the negative regulation of apoptosis.

In terms of GO molecular functions, *MDM2, HSP90AA1, BCL2, BRCA1, CDKN1A,* and *EGFR* genes are important for ubiquitin protein ligase binding; *EP300, BCL2, BRCA1, TCF7L2, STAT3,* and *EGFR* genes are important for DNA binding; and *MDM2* and *HSP90AA1* genes are important for ubiquitin-like protein ligase binding. Additionally, EP300*, BCL2, TCF7L2,* and *STAT3* genes were associated with DNA-binding transcription factor binding, *BRCA1, TCF7L2,* and *STAT3* genes were associated with transcription cis-regulatory region binding, and *BRCA1, TCF7L2,* and *STAT3* genes were associated with transcription regulatory region nucleic acid binding.

Finding better treatments for the disease and understanding the underlying processes that cause disease progression are the main objectives of molecular research on HCC samples. Activated p53 triggers correct reactions in cells in response to biological stressors, including DNA repair, genetic stability, cell cycle arrest, and the elimination of DNA-damaged cells. The oncogenic protein MDM2 is a crucial cellular p53 antagonists. By stimulating the breakdown of p53, MDM2 inhibits p53 function. The processes underlying MDM2-p53 interactions are more intricate than initially believed, according to available research. Nutlin-3 may be useful for therapy because it stops p53 from binding to MDM2, which makes p53 more stable and increases its accumulation in cells (14).

Most research has focused on EP300's function as a histone acetyltransferase, which modifies chromatin structure to affect transcription. However, we still don't fully understand how EP300 functions as a transcriptional regulator of epithelial-to-mesenchymal transition (EMT). High EP300 expression in HCC tissues is associated with an increased risk of poor prognosis following EMT (15).

Hsp90a, encoded by the *HSP90AA1* gene, is the major cytosolic chaperone in eukaryotes. It functions in cell protection and intracellular signalling, controls protein homeostasis folding and the assembly of secretory polypeptides in the endoplasmic reticulum, and modulates the post-translational translocation of proteins across organelle membranes. Toraih et al. found that late HCC patients had statistically significantly higher *HSP90AA1* expression compared to early HCC patients (16). In response to DNA double-strand breaks, *ATM* acts as a crucial mediator. In their study, Patra et al. demonstrated that hepatocytes and chronic liver biopsy samples infected with HCV had greater ATM expression (17). Compared with HCC patients, those with wild-type TP53 and low p53 target expression have a notable increase in both copy number and expression of the p53 inhibitor protein MDM4 (18).

Bcl-2 suppressed programmed cell death and enhanced cell viability, conferring resistance to detrimental influences. Genes linked to *Bcl-2* are believed to control cell death and may contribute to the initiation and spread of cancer. Li et al. found that *STAT3, MMP-2, and Bcl-2* expression was significantly induced in peripheral blood mononuclear cells isolated from patients with HCV infection and in HCV-infected cell cultures. They also showed that HCV regulates *MMP-2* and *Bcl-2* by activating the STAT3 signalling cascade (19). Diao et al. found that distinct BRCA1-activated networks were identified in higher HCC tissues compared with lower tumour-free hepatitis/cirrhotic tissues arising from HBV or HCV infection (20). Researchers found a link between an unfavourable prognosis of HCC and reduced levels of breast cancer 2 *(BRCA2)* and cyclin-dependent kinase inhibitor 1A *(CDKN1A)* interacting protein (BCCIP) (21).

CDKN2A is a well-recognised gene that inhibits tumour growth and generates the p16-INK4a protein, which controls the cell cycle by preventing tumour progression. It hinders the activities of cyclin-dependent kinases 4 and 6, which are crucial in regulating the cell cycle by impeding the transition from G1 to S phase, thereby aiding in the prevention of cancer. Extensive CpG methylation downregulates CDKN2A expression in HCV-induced HCC (22). Ling et al. investigated the impact of *TCF7L2* gene polymorphisms on HCC risk in a cohort of patients with cirrhosis. This is significant because the *TCF7L2* gene is associated with cancer risk and plays a crucial role in the Wnt signalling pathway. Their research showed that differences in three single-nucleotide polymorphisms (rs290481, rs290487, and rs290489) near the 3' end of the *TCF7L2* gene may increase the risk of HCC. Three SNPs form the basis of a haplotype that significantly distinguishes between patients at low and high risk of HCC (23).

SYK is an innovative biomarker of HCC that plays a vital role in immune cell signalling pathways. Research indicates that HBV or HCV infection leads to significant increases in *SYK* and cytokine expression in hepatocytes. The two major isoform of SYK, SYK (L) and SYK (S), play different roles in HCC development. Healthy liver tissue contains SYK (L), but HCC significantly reduces it. On the other hand, non-tumour tissue showed significantly lower SYK (S) expression. Moreover, SYK (L) suppresses

the proliferation and invasion of HCC cells, whereas SYK (S) has oncogenic activities and promotes the invasion and metastasis of HCC cells (24).

Src is an oncogene, and its overexpression and high activity appear to be involved in the progression of various tumour types, including HCC (25). Physiological processes, such as cell survival and proliferation, rely on *Src* to maintain proper cellular homeostasis. Src regulates the cytoskeleton and cellular morphology, as well as preserving intercellular connections, cell-matrix adhesion, and mobility. Src signalling is essential for the regulation of cellular processes such as proliferation, invasion, migration, angiogenesis, and treatment resistance in liver cancer (26).

Hepatitis B infection is one of the most common differentially expressed genes linked to liver cancer and hepatitis C. Researchers have concluded that the KEGG pathway links it to gastrointestinal stromal tumours (GIST), including colorectal and stomach cancers, as well as various cancer pathways, including prostate, non-small cell lung, and endometrial cancers. According to pathway enrichment analysis data, finding that the Homo sapiens h gleevec pathway inhibits cell growth may help identify new drugs that can be used to improve treatment effectiveness, especially when gleevec (imatinib) is used to treat metastatic malignant GISTs.

In conclusion, our study investigated the roles of common differential genes in liver cancer and hepatitis C infection via bioinformatic analysis of publicly available databases. In hepatitis C-associated liver cancer, the expression and functions of genes related to biological processes, molecular functions, and cellular components were determined. Experimental studies are needed to determine whether these common genes are associated with liver cancer and hepatitis C. To validate our findings, experimental transcriptomic methods should investigate the expression of genes significantly upregulated in hepatitis C-related liver cancer, and proteomic methods should investigate changes in protein levels.

CONCLUSION

Our study found many hub genes, particularly *MDM2, TP53, EP300, HSP90AA1, ATM,* and *MDM4,* to be highly connected in HCC using the genomic association method in the context of the PPI network. These results are significant prognostic biomarkers of HCC and may offer guidance for HCC treatment.

Our study, which is based on bioinformatics results, can be supported by experimental studies that identify common DEGs and related pathways to detect the molecular mechanisms underlying the pathogenesis of HCC.

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