Oncology Treatment Discovery, 2024, Volume 2, Issue 4
http://ojs.bbwpublisher.com/index.php/OTD
Online ISSN: 2981-8079

Comprehensive Bioinformatics Evaluation of CTNNB1 as a Diagnostic, Therapeutic and Prognostic Biomarker in Liver Hepatocellular Carcinoma

Syed Hussain Raza[1]*, Muhammad Akram[2]

[1]Department of Biochemistry and Biotechnology, The Islamia University of Bahawalpur, Bahawalpur, Pakistan
[2]Department of Microbiology, King Saud University, Saudi Arabia

Corresponding author: Syed Hussain Raza, hussainrbukhari@gmail.com

Abstract: Liver Hepatocellular Cancer (LIHC) is a fatal disease, that keeps rigorous to therapeutic approach. This study explores the expression and promoter methylation of CTNNB1 and survival analysis, to extricate its role in LIHC progression, prognosis, and therapeutic approaches. Employing UALCAN, the study examined upregulation in CTNNB1 expression in LIHC as compared to normal samples explained its role in LIHC progression. Further analysis, stratified by patient's age, gender, race, and pathological stages, revealed upregulation across all variables. Analysis of promoter methylation level of CTNNB1 in LIHC revealed hypomethylation, acknowledging upregulation of expression. Moreover, survival analysis of CTNNB1 using a KM plotter demonstrated its prognostic significance, as CTNNB1 overexpression results in the worst overall survival (OS) and vice versa. Validation via GEPIA2 affirmed elevated expression level of CTNNB1 in LIHC, further establishing its correlation with unfavorable survival outcomes. Furthermore, pathway enrichment analysis utilizing the STRING and DAVID tool identified association with genes implicated in essential signaling processes such as the Wnt signaling pathway revealing its role in LIHC progression. Subsequently, Genetic mutation analysis performed using cBioPortal demonstrated a 10% mutation of CTNNB1 in LIHC, indicating that alteration in the gene has a critical role in the development of LIHC. In conclusion, this comprehensive analysis highlights the significance of CTNNB1 upregulation in LIHC progression and its capability as a prognostic biomarker, offering valuable insight for developing targeted therapeutic strategies.

Keywords: CTNNB1; LIHC; Biomarker; Wnt signaling pathway

Online publication: December 31, 2024

1. Introduction

Cancer is a prominent health and economic issue globally, with high human mortality. As stated by studies, in 2020

about 19.3 million new cases and 10 million cancer-related deaths were reported [1–4]. There are numerous types of cancer, liver cancer is 5th most common with 905,677 cases and the second highest 830,180 mortalities worldwide in 2020 [1,5]. Liver hepatocellular cancer (LIHC) accounts for 80% of these cases [6]. Alcohol consumption, hepatitis B, hepatitis C and metabolic infection are key risk factors of LIHC [7–9]. It is a heterogeneous disease and is often diagnosed at advanced stages, which brings problems in treatment [7,10]. For LIHC early stages, the treatment includes surgery, liver transplant and immunotherapy [11]. While advanced stages of LIHC demonstrate a response to atezolizumab and bevacizumab combined therapy [12]. However, it has a 70% recurrence rate and hardily 50% of LIHC patients outlive 5 years after surgery [13,14]. Drug resistance, economic burden, less efficient target therapy, high recurrence, and late diagnosis pose challenges to treating LIHC [15–18]. Therefore, it an urgent need to identify diagnostic, prognostic and therapeutic biomarkers to tackle challenges in LIHC treatment.

In recent years, signal transduction pathway activation has been revealed to play a role in LIHC, of these pathways Wnt/β catenin pathway activation is reported to have a role in the prognosis and development of LIHC [19–21]. Catenin beta 1 (CTNNB1) is a key gene located on chromosome 3p22.1 and codes in the Wnt/β catenin pathway to act as an intracellular signal transducer [22,23]. E-cadherin is directly connected to β-catenin and forms an adhesion complex, phosphorylation degrades this complex. This complex maintains cell functions and cell-cell adhesion. Because of phosphorylation, β-catenin dislocates to the nucleus, and activation of Wnt target genes related to cell proliferation, cell cycle and carcinogenesis is triggered [24–27].

Because of lifestyle and risk factors in different geographical regions, variations in genetic mutation are identified in liver cancer. As Asia-Pacific region has a low number of LIHC as compared to the eastern Asia region with respect to HCV and HBV rates. Similarly, CTNNB1 mutation is lower in Asia and higher in Europe and America [28–30]. CTNNB1 is identified to be mutated in 20 to 40 percent of liver cancer. CTNNB1 mutation is also associated with the progression of different human cancers [29,31,32]. CTNNB1 is also associated with irruption and poor prognosis in LIHC [24]. All of these studies identified the role of CTNNB1 in the progression of LIHC. But to our knowledge, the role of CTNNB1 as a diagnostic, therapeutic and prognostic biomarker is yet to be identified. Therefore, this study aimed to the bioinformatics analysis of CTNNB1 in LIHC.

2. Material and method

2.1. UALCAN

UALCAN is a user-friendly online software based on The Cancer Genome Atlas (TCGA), employed for gene expression analysis across different tumors [33]. This study analyzed the expression and promoter methylation level of CTNNB1 in LIHC using the UALCAN database. The study employed UALCAN to execute analysis in LIHC and normal samples, together with different variables such as patient's age, gender, race and individual cancer stage.

2.2. Kaplan-Meier plotter

Kaplan-Meier (KM) plotter is an online tool that is utilized to verify the survival curve of the specified gene in cancer [34]. The study evaluates the OS of LIHC patients affected by CTBBN1 expression by Survival analysis using a KM plotter. A *p*-value less than 0.05 is considered statistically significant and the hazard ratio is calculated with a 95% interval.

2.3. GEPIA2

Gene Expression Profiling and Interactive Analysis version 2 (GEPIA2) is an online server that is used for gene expression and prognostic analysis [35]. We also employed GEPIA2 to perform expression and survival analysis of CTNNB1 in LIHC. This study implemented this technology in the box stage and sample-based analysis of CTNNB1 in LIHC. The study also examined survival analysis using the survival module of GEPIA2.

2.4. STRING database

Protein-protein interaction (PPI) networks are constructed utilizing an online database, Search Tool for the Retrieval of Interacting Genes (STRING) [36]. This study constructed the PPI network of CTNNB1 using the STRING database.

2.5. DAVID

Pathway enrichment analysis is performed using an online tool, Database for Annotation, Visualization, and Integrated Discovery (DAVID) [37]. This study uses the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) pathway enrichment analyses. Cellular component (CC), molecular function (MF), and biological process (BP) are three main categories of GO analysis.

2.6. cBioPortal

Genetic alteration of specific gene is analyzed utilizing an online tool cBioPortal. This study utilized genetic mutation of CTNNB1 in LIHC using cBioPortal.

3. Results

3.1. Analysis of CTNNB1 expression in LIHC

First, the study analyzed the expression of ETNB1 in LIHC and normal samples using the UALCAN database. The study analyzed that CTNNB1 was significantly upregulated in LIHC samples as compared to normal samples (**Figure 1**). This study coincides with previous studies that the upregulation of genes leads to the progression of cancer [38,39]. Hence, the upregulation of CTNNB1 has an association with the progression of LIHC.

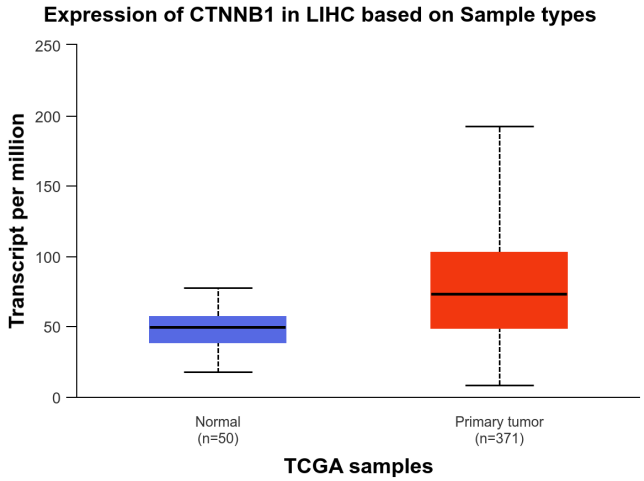

Figure 1. The expression analysis of CTNNB1 in LIHC and normal control sample using the UALCAN database.

3.2. Expression analysis of CTNNB1 in LIHC categorized according to different variables

Following the analysis, the study analyzed CTNNB1 expression in LIHC categorized according to different variables such as patient age, patient gender, patient race and individual pathological stages. First, the study examined CTNNB1 expression in LIHC based on pathological stages. The study evaluated that CTNNB1 was significantly upregulated in each of this individual cancer stages (**Figure 2A**). After that, the study evaluated that CTNNB1 expression was significantly upregulated in LIHC patients of different races (**Figure 2B**). Furthermore, the study investigated significant upregulation in LIHC patient's different age group and gender (**Figure 2C** and **Figure 2D**). Altogether, based on all these results, CTNNB1 has a role in the proliferation of LIHC.

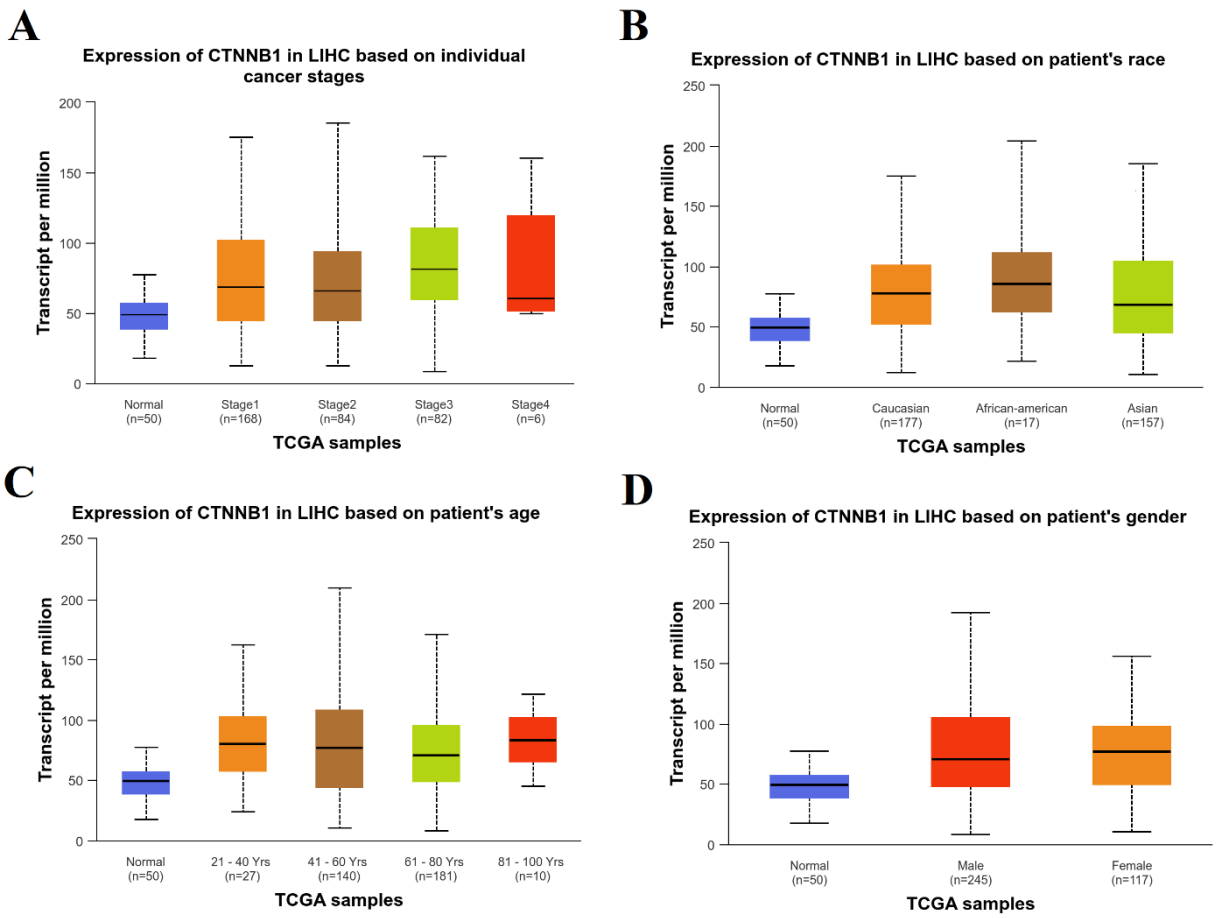

Figure 2. (A) Expression analysis of CTNNB1 in LIHC patient's individual cancer stage; (B) Expression analysis of CTNNB1 in LIHC patient's race; (C) Expression analysis of CTNNB1 in LIHC patient's age group; (D) Expression analysis of CTNNB1 in LIHC patient's gender.

3.3. Analysis of CTNNB1 promoter methylation level in LIHC and normal control sample

The study analyzed CTNNB1 promoter methylation levels in LIHC and normal control samples utilizing the UALCAN database. The study assessed that CTNNB1 was significantly hypomethylated in LIHC samples as compared to normal control samples (**Figure 3**). As per studies gene promoter methylation level and gene expression have inverse relation [40,41]. From this perspective, hypomethylation of CTNNB1 indicates upregulation in CTNNB1 expression and its role in the progression of LIHC.

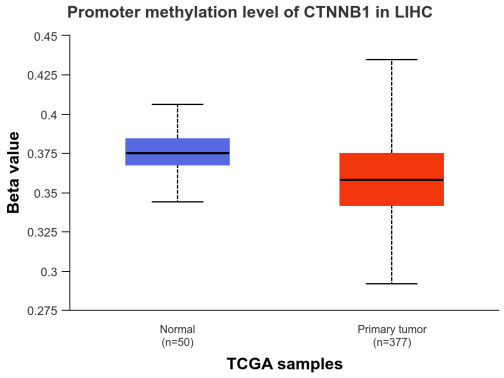

Figure 3. The promoter methylation level of CTNNB1 in LIHC and normal control samples using the UALCAN database.

3.4. Promoter methylation level of CTNNB1 in LIHC based on distinct characteristics

Subsequently, the study analyzed the promoter methylation level of CTNNB1 in LIHC based on different characteristics such as the patient's age, patient's gender, patient's race and pathological stages. Primarily, the study analyzed promoter methylation level of CTNNB1 in LIHC patient's pathological stages. The study evaluated that CTNNB1 was significantly hypomethylated in LIHC across individual cancer stages (**Figure 4A**). Next, the study assessed significant hypomethylation in CTNNB1 methylation level in LIHC based on the patient's race (**Figure 4B**). Eventually, the study examined that CTNNB1 was significantly hypomethylated in LIHC based on the patient's different age group and gender (**Figure 4C** and **Figure 4D**). This observation illuminates the hypomethylation of CTNNB1 across distinct characteristics, demonstrating its role in the progression of LIHC.

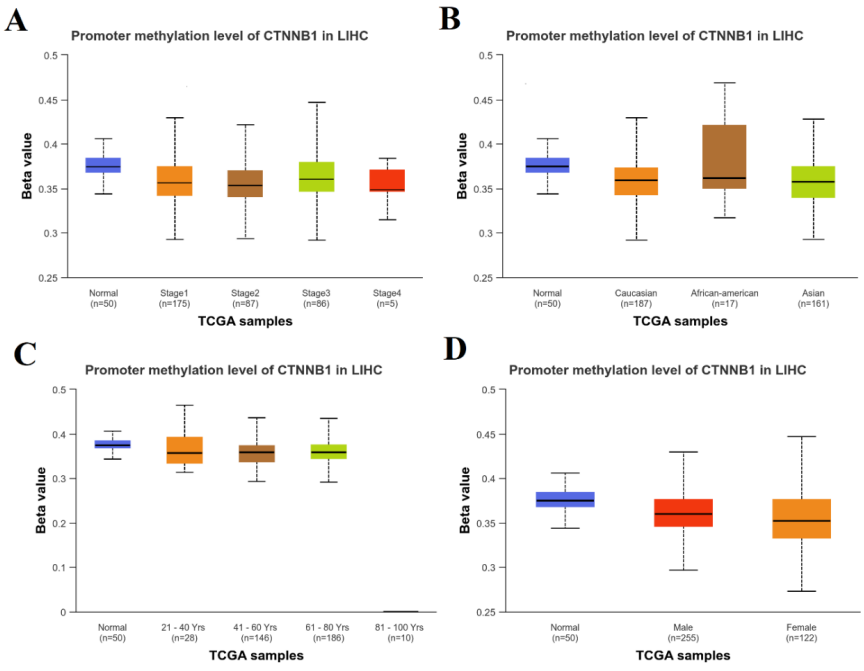

Figure 4. (A) Promoter methylation level of CTNNB1 in LIHC patient's individual cancer stage; (B) Promoter methylation level of CTNNB1 in LIHC patient's race; (C) Promoter methylation level of CTNNB1 in LIHC patient's age group; (D) Promoter methylation level of CTNNB1 in LIHC patient's gender.

3.5. Survival analysis of CTNNB1 in LIHC

The study performed a survival analysis employing the KM plotter to assess the role of CTNNB1 expression in the overall survival (OS) of LIHC patients. The study then investigated that overexpression of CTNNB1 leads to the worst OS, similarly, lower expression of CTNNB1 leads to the worst OS as indicated by significant *P*-value and higher hazard ratio. The calculated *P*-value is 0.045 which explains the statistically significant survival difference between the two groups. Based on this, the study assessed that overexpressed CTNNB1 in LIHC relates to a high mortality rate, illustrating its potential as a prognostic biomarker.

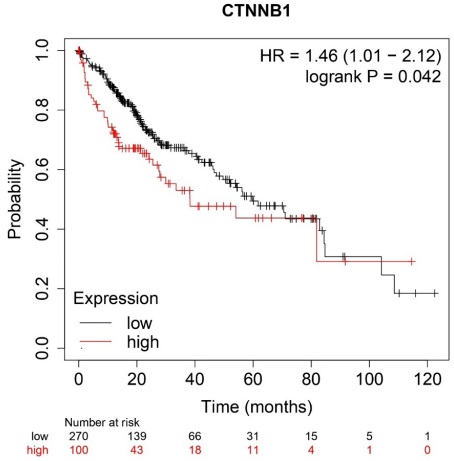

Figure 5. Survival analysis of CTNNB1 in LIHC using KM plotter.

3.6. Affirmation of CTNNB1 expression and survival analysis

The study utilized GEPIA2 to verify the detections of expression and survival analysis of CTNNB1. Firstly, the study analyzed CTNNB1 expression in LIHC and normal samples. Then, CTNNB1 was confirmed to be upregulated in the LIHC sample (**Figure 6A**), this result coincides with the previous investigation. Next, the study employed the box plot module to investigate CTNNB1 expression in LIHC pathological stages, and assessed variation but upregulation of CTNNB1 expression in various pathological stages (**Figure 6B**). This finding coincides with the previous observations, explaining that the upregulation of CTNNB1 expression leads to the progression of LIHC and has potential as a diagnostic biomarker.

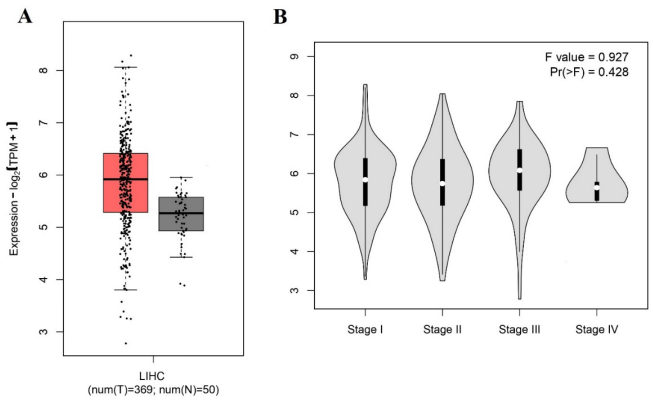

Figure 6. (A) Expression analysis of CTNNB1 in LIHC and normal control sample using GEPIA2; (B) Expression analysis of CTNNB1 in LIHC based on pathological stages using GEPIA2.

Following this, the study analyzed the impact of CTNNB1 expression on the OS of LIHC patients using the survival module of GEPIA2. The study analyzed that overexpressed CTNNB1 worst OS and lower expressed CTNNB1 had better OS (**Figure 7**). This result coincides with the previous findings, however, the difference is slightly significant because of *P*-value = 0.11. All of these findings suggest CTNNB1 overexpression has role in LIHC progression and with the worst OS of patients. This highlights the potential of CTNNB1 as diagnostic and prognostic biomarker.

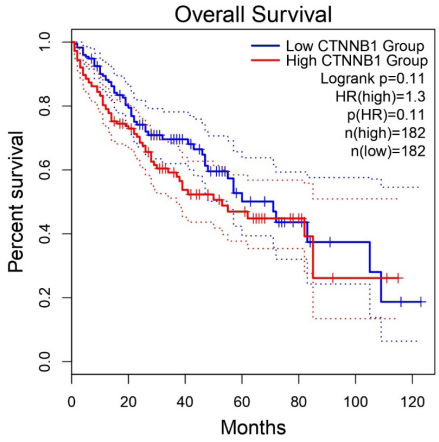

Figure 7. Survival analysis of CTNNB1 in LIHC using GEPIA2.

3.7. PPI network and pathway enrichment analysis of CTNNB1

In addition, the study widened the analysis by implicating protein-protein interaction (PPI) network construction, Gene Ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses. The study expanded the analysis to evaluate the biological functions of CTNNB1. First, the study constructed a PPI network using the STRING database and unveiled interlinked 10 gens (**Figure 8**). This explains the diversity in the biological function of CTNNB1. Based on this, the study utilized the DAVID database and observed 6 terms for cellular component (CC), biological process (BP), molecular function (MF), and KEEG pathways by performing Go and KEGG analysis (**Table 1** and **Figure 9**). Then, the study performed KEGG pathway analysis and observed that genes were interlinked in several pathways with the Wnt signaling pathway, gastric cancer, Human papillomavirus infection, endometrial cancer and adherens junction pathways. These findings illustrate the role of CTNNB1 and associated genes in different processes.

Moreover, in GO analysis of CTBB1, the study analyzed substantial enrichment in biological processes such as proteasome-mediated ubiquitin-dependent protein catabolic process, negative regulation of canonical Wnt signaling pathway, positive regulation of transforming growth factor-beta.

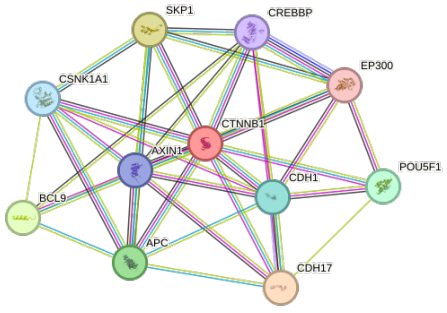

Figure 8. PPI network of CTNNB1 using STRING tool.

Receptor signaling pathway, canonical Wnt signaling pathway, N-terminal peptidyl-lysine acetylation, and macromolecular complex assembly (**Figure 9B**). Subsequently, in regard to cellular component, the study examines pivotal enrichment including beta-catenin destruction complex, catenin complex, lateral plasma membrane, Wnt signalosome, adherens junction, and transcription factor complex (**Figure 9C**). Furthermore, the study observed molecular function linked with CTNNB1 such as beta-catenin binding, RNA polymerase II sequence-specific DNA binding transcription factor binding, transcription coactivator activity, ubiquitin protein ligase binding, p53 binding, histone acetyltransferase activity (H3-K27 specific) (**Figure 9D**). These findings shed light on understanding the role of CTNNB1 and associated genes in different pathways and biological processes.

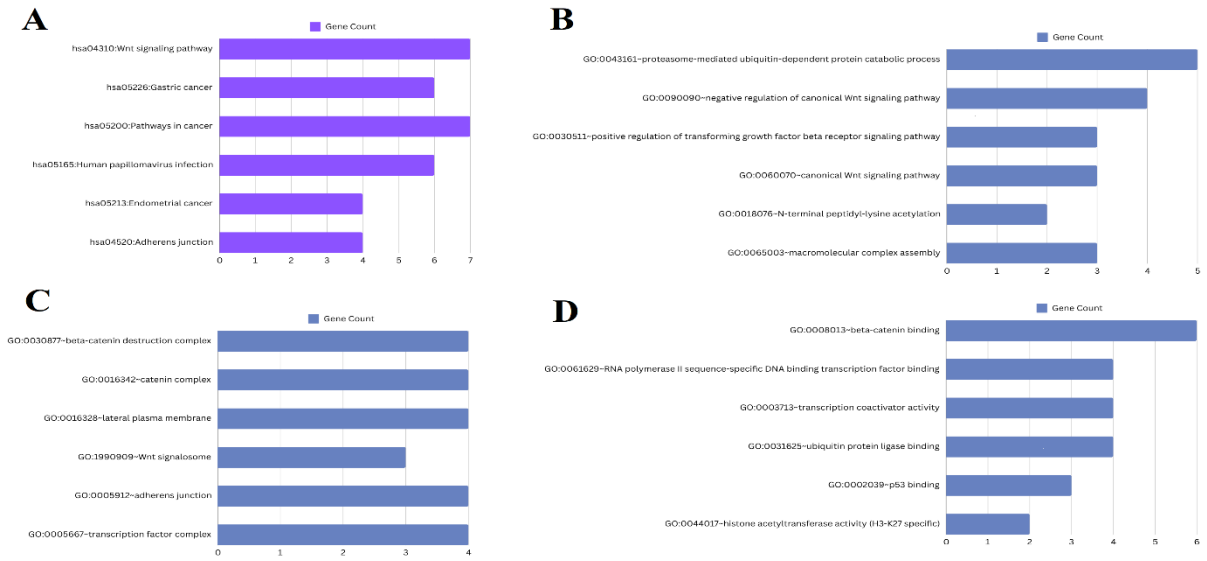

Figure 9. Kegg and GO analysis of CTNNB1 enriched genes utilizing the DAVID tool.

Table 1. Result of gene enrichment analysis

Gene term	Gene count	Genes	*P*-value
BP			
GO0043161: proteasome-mediated ubiquitin-dependent protein catabolic process	5	APC, CSNK1A1, AXIN1, CTNNB1, SKP1	3.736089222847483E-6
GO0090090: negative regulation of canonical Wnt signaling pathway	4	APC, CSNK1A1, AXIN1, CTNNB1	5.3413295934421694E-5
GO0030511: positive regulation of transforming growth factor beta receptor signaling pathway	3	CREBBP, AXIN1, EP300	1.3497253797827575E-4
GO0060070: canonical Wnt signaling pathway	3	BCL9, AXIN1, CTNNB1	0.0012638592484148154
GO0018076: N-terminal peptidyl-lysine acetylation	2	CREBBP, EP300	0.0015572278568399716
GO0065003: macromolecular complex assembly	3	CREBBP, APC, AXIN1	0.0023052682893945405
CC			
GO0030877: beta-catenin destruction complex	4	APC, CSNK1A1, AXIN1, CTNNB1	2.377141141438321E-8
GO0016342: catenin complex	4	APC, CDH1, CTNNB1, CDH17	4.102589855782143E-7
GO0016328: lateral plasma membrane	4	APC, CDH1, AXIN1, CTNNB1	8.05942485285713E-6

Table 1 (Continued)

Gene term	Gene count	Genes	*P*-value
GO1990909: Wnt signalosome	3	APC, AXIN1, CTNNB1	1.6623380306006684E-5
GO0005912: adherens junction	4	APC, CDH1, CTNNB1, CDH17	8.257006264142433E-5
GO0005667: transcription factor complex	4	CREBBP, EP300, CTNNB1, POU5F1	1.45523934603438E-4
MF			
GO0008013: beta-catenin binding	6	BCL9, APC, CDH1, AXIN1, EP300, SKP1	5.435587111301412E- 10
GO0061629: RNA polymerase II sequence-specific DNA binding transcription factor binding	4	CREBBP, EP300, CTNNB1, POU5F1	1.0554759656852471E-4
GO0003713: transcription coactivator activity	4	BCL9, CREBBP, EP300, CTNNB1	3.082509233862163E-4
GO0031625: ubiquitin protein ligase binding	4	APC, AXIN1, CTNNB1, POU5F1	4.918766119255133E-4
GO0002039: p53 binding	3	CREBBP, AXIN1, EP300	6.151526152403066E-4
GO0044017: histone acetyltransferase activity (H3-K27 specific)	2	CREBBP, EP300	0.001058901316322019
KEGG			
hsa04310: Wnt signaling pathway	7	CREBBP, APC, CSNK1A1, AXIN1, EP300, CTNNB1, SKP1	4.8186637813062545E-9
hsa05226: Gastric cancer	6	APC, CDH1, CSNK1A1, AXIN1, CTNNB1, CDH17	1.6789194931954612E-7
hsa05200: Pathways in cancer	7	CREBBP, APC, CDH1, AXIN1, EP300, CTNNB1, SKP1	3.6991021891065767E-6
hsa05165: Human papillomavirus infection	6	CREBBP, APC, CSNK1A1, AXIN1, EP300, CTNNB1	8.778542088270608E-6
hsa05213: Endometrial cancer	4	APC, CDH1, AXIN1, CTNNB1	2.3261089609916448E-5
hsa04520: Adherens junction	4	CREBBP, CDH1, EP300, CTNNB1	9.605435077106246E-5

3.8. Genetic mutation of CTNNB1 in LIHC

The study then investigated the genetic mutation of CTNNB1 and the role of this mutation in LIHC progression using cBioPortal. The analysis revealed a 10% mutation of CTNNB1 in LIHC. These observed mutations include in-frame mutation, missense mutation, splice mutation, truncating mutations, structural variant and amplification (**Figure 10**). These findings suggest that genetic mutation of CTNNB1 has a significant role in the proliferation of LIHC. That explains the potential of CTNNB1 as a therapeutic biomarker in LIHC.

Figure 10. Genetic mutation of CTNNB1 in LIHC.

4. Discussion

Cancer is a fatal disease that has been in focus of thorough scientific investigation for decades [42]. In this disease, uncontrolled cell division results in various types of malignancies [43]. While liver cancer is the 5th most common cancer with 2nd most mortalities worldwide, 90% of liver cancers are liver hepatocellular carcinoma (LIHC) [1]. LIHC is mostly diagnosed at advance stages, it shows resistance to chemotherapy, target therapy and therapeutic strategies. Therefore, there is an urgent need to discover biomarkers. This study used bioinformatics tools to evaluate CTNNB1's potential as a diagnostic, prognostic and therapeutic biomarker in LIHC. CTNNB1 is a gene that is located on human chromosome 3p21–22, it regulates various signaling pathways such as Wnt/β-catenin and Hippo pathway. CTNNB1 mutation is associated with the progression of different cancers [44]. CTNNB1 variation disturbs the signaling pathways which leads to the progression of LIHC and other cancers. This study performed expression analysis, survival analysis, gene enrichment analysis and gene mutation of CTNNB1 in LIHC using bioinformatics tools.

The study utilized the UALCAN database to analyze CTNNB1 expression in LIHC and evaluated that CTNNB1 was significantly overexpressed in LIHC as compared to normal samples. Moreover, this study analyzed CTNNB1 expression based on various variables like individual cancer stage, patient's age, gender and patient's race, unveiling significant overexpression. All of these findings proposed that CTNNB1 has a role in the progression and development of LIHC.

In addition, this study analyzed promoter methylation levels of CTNNB1 in LIHC UALCAN. The study analyzed hypomethylation in the CTNNB1 promoter region in LIHC as compared to the normal control sample. This hypomethylation of CTNNB1 supports upregulation in expression, as previously it's been stated that genes promoter methylation level and expression have an inverse relation [45]. Further, hypomethylation of CTNNB1 was investigated in LIHC when analyzed based on different parameters such as LIHC pathological stages, patient age, gender and race. These findings acknowledge previous analysis that hypomethylation regulates the upregulation of CTNNB1 expression and plays a role in the progression of LIHC.

Moreover, the study performed a survival analysis of CTNNB1 in LIHC by employing a KM plotter, which revealed that overexpression is associated with the worst OS of LIHC patients. The study checked the validation of the result by utilizing GEPIA2 to perform expression and survival analysis. This study investigated that CTNNB1 was overexpressed in LIHC in contrast with normal samples. Expression analysis in LIHC pathological stages using GEPIA2 was also supports upregulation of CTNNB1. Moreover, survival analysis with GEPIA2 revealed that overexpression is linked with worst OS and lower expression is linked with better OS. But, the difference between these groups is not statistically significant. These similarities in results proposed that CTNNB1 have role in development and progression of LIHC.

A group of ten genes directly linked with CTNNB1 was analyzed in protein-protein interaction (PPI) network analysis. While in pathway enrichment analysis, Gene Ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis were performed. KEGG analysis identified enriched pathways associated with CTNNB1 and linked genes such as the Wnt signaling pathway, gastric cancer, Human papillomavirus infection, endometrial cancer and adherens junction. GO analysis revealed pathways specially enriched linked with CTNNB1 including negative regulation of canonical Wnt signaling pathway, positive regulation of transforming growth factor beta receptor signaling pathway, canonical Wnt signaling pathway, beta-catenin destruction complex, catenin complex, lateral plasma membrane, Wnt signalosome, adherens junction, transcription factor complex, beta-catenin binding, RNA polymerase II sequence-specific DNA binding transcription factor binding,

transcription coactivator activity, ubiquitin protein ligase binding, p53 binding and histone acetyltransferase activity (H3-K27 specific). These pathways accounts for especially negative regulation of canonical Wnt signaling pathway, p53 binding, beta-catenin destruction complex and such others are recognized for their role in LIHC progression.

While cBioPortal revealed 10% of genetic alteration of CTNNB1 in LIHC. These observed mutations include in-frame mutation, missense mutation, splice mutation, truncating mutations, structural variant and amplification. These results suggest that CTNNB1 mutation has strong role in the progression of LIHC. Comprehensively, these findings suggest that CTNNB1 has potential as a diagnostic, prognostic and therapeutic biomarker in LIHC.

5. Conclusion

In overview, our detailed examination of CTNNB1 expression, prognostic significance, and genetic mutations in LIHC utilizing UALCAN, GEPIA2, KM plotter, and cBioPortal uncovered significant understanding. This study discovered a correlation between higher CTNNB1 expression levels and the progression of LIHC. These findings illuminate CTNNB1's potential as a diagnostic, prognostic, and therapeutic biomarker in LIHC, emphasizing its pivotal role in disease management.

Disclosure statement

The authors declare no conflict of interest.

References

[1] Sung H, Ferlay J, Siegel RL, et al., 2021, Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: A Cancer Journal for Clinicians, 71(3): 209–249.

[2] Tao S, Ye X, Pan L, et al., 2021, Construction and Clinical Translation of Causal Pan-Cancer Gene Score Across Cancer Types. Frontiers in Genetics, 12: 784775.

[3] Andre F, Mardis E, Salm M, et al., 2014, Prioritizing Targets for Precision Cancer Medicine. Annals of Oncology, 25(12): 2295–2303.

[4] Sial N, Saeed S, Ahmad M, et al., 2021, Multi-Omics Analysis Identified TMED2 as a Shared Potential Biomarker in Six Subtypes of Human Cancer. International Journal of General Medicine, 2021: 7025–7042.

[5] Anwanwan D, Singh SK, Singh S, et al., 2020, Challenges in Liver Cancer and Possible Treatment Approaches. Biochimica et Biophysica Acta (BBA)-Reviews on Cancer, 1873(1): 188314.

[6] Bray F, Ferlay J, Soerjomataram I, et al., 2018, Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: A Cancer Journal for Clinicians, 68(6): 394–424.

[7] Balogh J, Victor III D, Asham EH, et al., 2016, Hepatocellular Carcinoma: A Review. Journal of Hepatocellular Carcinoma, 2016: 41–53.

[8] Llovet JM, Castet F, Heikenwalder M, et al., 2022, Immunotherapies for Hepatocellular Carcinoma. Nature Reviews Clinical Oncology, 19(3): 151–172.

[9] Park JW, Chen M, Colombo M, et al., 2015, Global Patterns of Hepatocellular Carcinoma Management from Diagnosis to Death: The BRIDGE Study. Liver International, 35(9): 2155–2166.

[10] Heimbach JK, Kulik LM, Finn RS, et al., 2018, AASLD Guidelines for the Treatment of Hepatocellular Carcinoma.

Hepatology, 67(1): 358–380.

[11] Liu CY, Chen KF, Chen PJ, 2015, Treatment of Liver Cancer. Cold Spring Harbor Perspectives in Medicine, 5(9): a021535.

[12] Finn RS, Qin S, Ikeda M, et al., 2020, Atezolizumab Plus Bevacizumab in Unresectable Hepatocellular Carcinoma. New England Journal of Medicine, 382(20): 1894–1905.

[13] Dhir M, Melin AA, Douaiher J, et al., 2016, A Review and Update of Treatment Options and Controversies in the Management of Hepatocellular Carcinoma. Annals of Surgery, 263(6): 1112–1125.

[14] Poon D, Anderson BO, Chen LT, et al., 2009, Management of Hepatocellular Carcinoma in Asia: Consensus Statement from the Asian Oncology Summit 2009. The Lancet Oncology, 10(11): 1111–1118.

[15] Ouyang X, Fan Q, Ling G, et al., 2020, Identification of Diagnostic Biomarkers and Subtypes of Liver Hepatocellular Carcinoma by Multi-Omics Data Analysis. Genes (Basel), 11(9): 1051.

[16] Kaur H, Bhalla S, Raghava GPS, 2019, Classification of Early and Late Stage Liver Hepatocellular Carcinoma Patients from Their Genomics and Epigenomics Profiles. PLoS One, 14(9): e0221476.

[17] Huang A, Yang XR, Chung WY, et al., 2020, Targeted Therapy for Hepatocellular Carcinoma. Signal Transduction and Targeted Therapy, 5(1): 146.

[18] Zhang W, Yang C, Hu Y, et al., 2023, Comprehensive Analysis of the Correlation of the Pan-Cancer Gene HAUS5 with Prognosis and Immune Infiltration in Liver Cancer. Scientific Reports, 13(1): 2409.

[19] Khalaf AM, Fuentes D, Morshid AI, et al., 2018, Role of Wnt/β-Catenin Signaling in Hepatocellular Carcinoma, Pathogenesis, and Clinical Significance. Journal of Hepatocellular Carcinoma, 2018: 61–73.

[20] Li QM, Zhang FQ, Li YF, et al., 2017, Influence of Polymorphisms in the Wnt/β-Catenin Pathway Genes on Hepatocellular Carcinoma Risk in a Chinese Han Population. Medicine, 96(12): e6127.

[21] Pez F, Lopez A, Kim M, et al., 2013, Wnt Signaling and Hepatocarcinogenesis: Molecular Targets for the Development of Innovative Anticancer Drugs. Journal of Hepatology, 59(5): 1107–1117.

[22] Chen L, Zhou Q, Liu J, et al., 2021, CTNNB1 Alteration is a Potential Biomarker for Immunotherapy Prognosis in Patients with Hepatocellular Carcinoma. Frontiers in Immunology, 12: 759565.

[23] Xu C, Xu Z, Zhang Y, et al., 2022, β-Catenin Signaling in Hepatocellular Carcinoma. The Journal of Clinical Investigation, 132(4): e154515.

[24] Chen J, Liu J, Jin R, et al., 2014, Cytoplasmic and/or Nuclear Expression of β-Catenin Correlate with Poor Prognosis and Unfavorable Clinicopathological Factors in Hepatocellular Carcinoma: A Meta-Analysis. PLoS One, 9(11): e111885.

[25] Kim M, Lee HC, Tsedensodnom O, et al., 2008, Functional Interaction Between Wnt3 and Frizzled-7 Leads to Activation of the Wnt/β-Catenin Signaling Pathway in Hepatocellular Carcinoma Cells. Journal of Hepatology, 48(5): 780–791.

[26] Cui J, Zhou X, Liu Y, et al., 2003, Wnt Signaling in Hepatocellular Carcinoma: Analysis of Mutation and Expression of Beta-Catenin, T-Cell Factor-4 and Glycogen Synthase Kinase 3-Beta Genes. Journal of Gastroenterology and Hepatology, 18(3): 280–287.

[27] MacDonald BT, Tamai K, He X, 2009, Wnt/β-Catenin Signaling: Components, Mechanisms, and Diseases. Developmental Cell, 17(1): 9–26.

[28] Javanmard D, Najafi M, Babaei MR, et al., 2020, Investigation of CTNNB1 Gene Mutations and Expression in Hepatocellular Carcinoma and Cirrhosis in Association with Hepatitis B Virus Infection. Infectious Agents and Cancer, 15: 37.

[29] Tornesello ML, Buonaguro L, Tatangelo F, et al., 2013, Mutations in TP53, CTNNB1 and PIK3CA Genes in Hepatocellular Carcinoma Associated with Hepatitis B and Hepatitis C Virus Infections. Genomics, 102(2): 74–83.

[30] Saitta C, Lanza M, Bertuccio A, et al., 2015, Evaluation of CTNNB1 and TP53 Variability in Patients with Hepatocellular Carcinoma and Occult Hepatitis B Virus Infection. Cancer Genetics, 208(10): 513–516.

[31] Chen SP, Wu CC, Huang SY, et al., 2012, β-Catenin and K-ras Mutations and RASSF1A Promoter Methylation in Taiwanese Colorectal Cancer Patients. Genetic Testing and Molecular Biomarkers, 16(11): 1277–1281.

[32] Nusse R, Clevers H, 2017, Wnt/β-Catenin Signaling, Disease, and Emerging Therapeutic Modalities. Cell, 169(6): 985–999.

[33] Chandrashekar DS, Bashel B, Balasubramanya SAH, et al., 2017, UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. Neoplasia, 19(8): 649–658.

[34] Győrffy B, Surowiak P, Budczies J, et al., 2013, Online Survival Analysis Software to Assess the Prognostic Value of Biomarkers Using Transcriptomic Data in Non-Small-Cell Lung Cancer. PLoS One, 8(12): e82241.

[35] Tang Z, Kang B, Li C, et al., 2019, GEPIA2: An Enhanced Web Server for Large-Scale Expression Profiling and Interactive Analysis. Nucleic Acids Research, 47(W1): W556–W560.

[36] Mering C, Huynen M, Jaeggi D, et al., 2003, STRING: A Database of Predicted Functional Associations Between Proteins. Nucleic Acids Research, 31(1): 258–261.

[37] Shannon P, Markiel A, Ozier O, et al., 2003, Cytoscape: A Software Environment for Integrated Models of Biomolecular Interaction Networks. Genome Research, 13(11): 2498–2504.

[38] Da M, Zhuang J, Zhou Y, et al., 2021, Role of Long Noncoding RNA Taurine-Upregulated Gene 1 in Cancers. Molecular Medicine, 27(1): 51.

[39] Asakawa M, Itoh M, Suganami T, et al., 2019, Upregulation of Cancer-Associated Gene Expression in Activated Fibroblasts in a Mouse Model of Non-Alcoholic Steatohepatitis. Scientific Reports, 9(1): 19601.

[40] Anastasiadi D, Esteve-Codina A, Piferrer F, 2018, Consistent Inverse Correlation Between DNA Methylation of the First Intron and Gene Expression Across Tissues and Species. Epigenetics & Chromatin, 11(1): 37.

[41] Razin A, Cedar H, 1991, DNA Methylation and Gene Expression. Microbiological Reviews, 55(3): 451–458.

[42] Polverini PJ, 2002, Angiogenesis in Health and Disease: Insights into Basic Mechanisms and Therapeutic Opportunities. Journal of Dental Education, 66(8): 962–975.

[43] Olivier M, Hollstein M, Hainaut P, 2010, TP53 Mutations in Human Cancers: Origins, Consequences, and Clinical Use. Cold Spring Harbor Perspectives in Biology, 2(1): a001008.

[44] Ledinek Ž, Sobočan M, Knez J, 2022, The Role of CTNNB1 in Endometrial Cancer. Disease Markers, 2022(1): 1442441.

[45] Luczak MW, Jagodziński PP, 2006, The Role of DNA Methylation in Cancer Development. Folia Histochemica et Cytobiologica, 44(3): 143–154.