Kurdish Studies
August 2024
Volume: 12, No: 5, pp 877-888
ISSN: 2051-4883 (Print) | ISSN 2051-4891 (Online)
www.KurdishStudies.net

DOI: 10.53555/ks.v12i5.3370

Comprehensive Analysis of Cell Adhesion Molecule-Related/Down-Regulated by Oncogenes Expression and Methylation Patterns in Liver Hepatocellular Carcinoma: Prognostic Implications and Genetic Alterations

Uzma Azeem Awan[1]*, Sammina Mahmood[2], Sara Sarfaraz[3], Muhammad Amjad Bashir[4], Reem Alajmi[5], Abdullah Sethar[6], Syed Sabir Ali Shah[7], Tamseel Saleem[8], Asim Shamim[9], Hafeez Ullah[10], Muhammad Kashif Iqbal[11], Habibullah Janyaro[12]*

[1]***Department of Biological Sciences, National University of Medical Sciences, Rawalpindi, Pakistan, ma.awan@numspak.edu.pk
[2]Department of Botany, Division of Science and Technology, University of Education, Lahore, Pakistan, sammina.mahmood@ue.edu.pk
[3]Department of Bioinformatics, Faculty of Biomedical and Life Sciences, Kohsar University Murree, Pakistan, sara.sarfaraz@live.com
[4]Department of Plant Protection Faculty of Agriculture Sciences Ghazi University Dera Ghazi Khan Punjab Pakistan, amjadhajbani@hotmail.com
[5]Department of Zoology, College of Science, King Saud University, Riyadh, Saudi Arabia, Mbatool@gmail.com
[6]Livestock Breeding Services Authority (LBSA) Sindh, Livestock & Fisheries Department, Government of Sindh, Pakistan, drabdullahsethar@hotmail.com
[7]Livestock and Fisheries Department, Government of Sindh, Pakistan, drsayedsabir@gmail.com
[8]Department of Biochemistry and Physiology, Faculty of Animal Husbandry and Veterinary Sciences (AHVS) , Sindh Agriculture University, Tandojam, Pakistan, drtamseel11@gmail.com
[9]Department of Pathobiology, Faculty of Veterinary and Animal Sciences, University of Poonch, Rawalakot, Azad Kashmir Pakistan, asimshamim@upr.edu.pk
[10]Department of Microbiology, Abasyn University Peshawar, Pakistan, Hafeezullah@mail.com
[11]Institute of Continuing Education & Extension, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, Pakistan, mkashifiqbal@cuvas.edu.pk
[12*]Department of Veterinary Surgery SBBUVAS Sakrand, Pakistan, Janyaroh@gmail.com

***Corresponding Authors;** Habibullah Janyaro, Uzma Azeem Awan,
*Department of Veterinary Surgery SBBUVAS Sakrand, Pakistan, Janyaroh@gmail.com
*Department of Biological Sciences, National University of Medical Sciences, Rawalpindi, Pakistan, uzma.awan@numspak.edu.pk

Abstract
Background: The CDON gene encodes a protein from the immunoglobulin super family that is essential for developmental processes, specifically the control of cell regulation throughout embryogenesis. CDON (Cell Adhesion Molecule) is engaged in various signaling networks, including the Hedgehog signaling system, which is essential for cell growth and differentiation. **Methods:** CDON expression and methylation was determined in LIHC by using UALCAN database. Further, survival, validation and mutation analysis of CDON was determined in LIHC by utilizing KM plotter, GEPIA2.0 and cBioPortal respectively. The gene enrichment and pathway were determined in the presence of CDON by using STRING and DAVID tools. **Results:** The current research intends to look at CDON expression in LIHC tissues. In this study, we compared CDON expression to various clinic-pathological parameters in LIHC patients. CDON expression was high in LIHC patients in different clinical parameters as compared to normal control samples. Furthermore, in LIHC patients, CDON high expression was found to be associated with shorter overall and disease-free survival. A small percentage of CDON mutations in LIHC are found through genetic mutation analysis using cBioPortal; they are primarily missense and truncating variants, which suggest their fundamental role in CDON dysregulation in the context of LIHC.CDON-associated genes co-express with CDON and are engaged in a wide range of biological processes, molecular functions, and pathways, according to the results of Gene Ontology and pathway analysis. The relationship between the DEGs and the Hedgehog signaling pathway, basal cell carcinoma, pathways in cancer, axon guidance, and proteoglycans in cancer was discovered through the study of enriched KEGG pathways. This critical finding showed that CDON is involved in the pathogenesis and proliferation of LIHC tumors. **Conclusion:** The results of this research showed that CDON is involved in LIHC cancer diagnosis and prognosis. In the end, these results might aid in the creation of more potent therapeutic plans and diagnostic tools for LIHC patients

Key words: LIHC, Diagnosis, Treatment, Biomarker

Introduction

Liver cancer is the sixth most prevalent malignancy and the fourth major cause of cancer-related death worldwide, providing a substantial burden to public health (1). Hepatocellular carcinoma (HCC) makes up around 90% of all primary liver cancers (2, 3). Genetic abnormalities, cellular context, and the external environment all play vital roles in the development of HCC. Interestingly, the major risk factors for HCC differ by geography. Hepatocellular carcinoma (HCC) is a lethal liver cancer. HCC, like other malignancies, is linked to potentially alterable risk factors, including excess body weight, alcohol usage, hepatitis B virus (HBV), hepatitis C virus, non-alcohol fatty liver disease, and specific genes (4-6).The majority of HCC cases (80%) occur in Asian countries as a result of chronic HBV infection and aflatoxin exposure (7, 8). China is expected to have the highest number of HCC patients in the world by 2030, representing an 82% rise over 2016 (9).Over 120 million individuals have been found to carry the hepatitis B surface antigen (HBsAg), and in China, HBV infection is thought to be responsible for about 54% of HCC (10). In addition, the rise in fast food culture and sedentary lifestyles brought about by recent urbanization in China has made non-alcoholic fatty liver disease (NAFLD) a greater cause of hepatocellular carcinoma and cirrhosis (9, 11, 12). As of right now, radiofrequency ablation or excision, transplantation, and radioembolization are the most often used treatments for HCC. Different stages of malignancies require different approaches to treatment, however even after five years, intrahepatic dissemination and recurrence rates are still very high (13-15). Potential biomarkers were identified by Nault et al. in 2013, which caused the focus of research to change to genes associated with the pathogenesis of HCC (16, 17). Gores suggested that in order to implement various molecularly matched targeted treatments for HCC, each patient would require a customized treatment plan and be stratified based on a five-gene score (18-21). Subsequently, genetic mutations were found in HCC, including those in the TERT promoter, TP53, CTNNB1, FGF, and PTEN abnormalities, which are used to direct biomarker-matched molecularly targeted therapy of HCC. Lately, there has been a notable advancement in the study of these biomarkers (22).

A part of the Hedgehog signaling pathway is CDON gene. Researchers found in 2012 that mice exposed to alcohol during pregnancy and with a mutation in the CDON gene exhibit symptoms like holoprosencephaly in humans. It appears that a complicated and poorly defined combination of genetic and environmental factors is the cause of many common structural birth abnormalities (23). Holoprosencephaly (HPE) is a common developmental defect in midline patterning of the forebrain and/or mid-face (24, 25). CDON encodes a co-receptor that serves multiple functions, including the HH pathway (26, 27). CDON loss-of-function mutations have been found in human HPE patients (28-32), however these variants are rather common in the human population. Furthermore, a patient with a rare homozygous CDON mutation demonstrated retinal coloboma, a mild HPE-associated ocular condition also seen in Cdon-/- mice (33-35). As a result, more insults are probably needed for CDON mutations to cause HPE. This conclusion is supported by studies conducted on mice. Mice with the 129S6 genetic background that lack CDON exhibit a sub threshold deficiency in HH signaling, making them susceptible to HPE caused by both genetic and environmental modifiers. One such environmental modification is ethanol (EtOH) ice (36). The CDON mutation or in utero EtOH exposure has no effect on 129S6 animals. However, the combination synergized to block HH signaling in the developing forebrain, producing a complete range of HPE symptoms with great penetrancen (37).According to the theory that a threshold of HH signaling activity limits the rate of midline patterning, 129S6 CDON mice were saved from EtOH-induced HPE by genetically removing one copy of the negative route regulator Ptch1 (38).

In this research, we used bioinformatics to assess CDON mutations, expression levels, survival prognostic outcomes, and utilitarian viewpoints in the context of LIHC. We also investigated the connection between promoter methylation levels and CDON expression. We conducted this investigation using a variety of databases, including the Database for Annotation, Visualization, and Integrated Discovery (DAVID), the UALCAN portal, the Kaplan-Meier tool, the Gene Expression Profiling and Interactive Analysis GEPIA2.0, the cBioPortal, the STRING database for protein-protein interactions (PPI), and the Cancer Genome Atlas (TCGA) informational index. DAVID provides a comprehensive set of functional annotation tools to assist in interpreting the biological significance of the large gene list. The primary contribution of this study was to determine the CDON expression pattern in LIHC and its possible role in cancer development and treatment.

Materials and methods

UALCAN

The UALCAN database provides instant access to cancer multi-omics data (TCGA and MET500) collected from over 30 different cancer types, easy to its user-friendly interface and basic features (39). It uses substantial data from The Cancer Genome Atlas (TCGA) to conduct in-depth assessments of gene expression, protein abundance, and patient survival across a variety of malignant tumor types. Researchers can utilize the UALCAN user-friendly interface to study and depict gene expression patterns associated to different stages of cancer, molecular subtypes, and patient socio-demographics. This informative index was used in the current research to assess CDON expression at various stages of specified cancer development, where this gene demonstrates considerable dysregulation and has a strong link with low overall survival. The CDON promoter methylation level in LIHC was determined using the UCALAN online tool. Furthermore, we evaluated CDON promoter methylation data in a variety of clinical settings, taking into account the patient age, gender, race, and stage of cancer.

GEPIA2.0

GEPIA2.0 is a popular online tool for predicting expression and analyzing the survivability of genomic data (40). GEPIA2, which has 198 619 isoforms and 84 cancer subtypes, has expanded the measurement of gene expression from the gene to the

transcript levels. It also makes it easier to analyze a specific cancer subtype as well as the relationships between them. Furthermore, the prognosis for various cancer subtypes may differ. Furthermore, the prognosis for various cancer subtypes may differ. It is easier to look into the underlying molecular pathways of many cancer subtypes when using GEPIA2, which enables users to investigate their research to focus on each of the 84 malignant growth subtypes and to study across numerous subtypes. Furthermore, new criteria for evaluation have evolved with the increased accessibility of single-cell sequencing. The associations between CDON expression and prognosis (OS and RFS) in patients with LIHC cancer were examined using the GEPIA 2.0 data set. This study used GEPIA2.0 to investigate the association between CDON expression and the prognosis (OS and DFS) of LIHC cancer.

KM plotter

The KM plotter(41), was used to connect the durations of relapse-free survival (RFS) and overall survival (OS) in cancer patients with the transcription expression of CDON. The following data were obtained from this analysis, OS and RFS graphs, hazard ratio (HR), 95% confidence interval, and log-rank P values. When $P < 0.05$, it was considered statistically significant. This web-based platform analyzes large amounts of clinical data to investigate the influence of specific genes on a patient tendency to survive different types of tumor growth. Prognostic biomarkers can be rapidly identified and the prognostic value of gene expressions can be immediately analyzed by researchers. The primary point of interaction in KM Plotter displays Kaplan-Meier survival curves, which provide details regarding the link between gene expression and patient outcomes. The KM plotter can help researchers assess the association between patient survival and gene expression levels in a range of cancer types, including gastric, ovarian, lung, and breast cancer. The effect of CDON dysregulation on overall survival (OS) in LIHC patients was investigated in this study utilizing the KM plotter tool.

cBioPortal

The cBioPortal database, with its user-friendly features, is mostly utilized for multidimensional cancer omics data analysis (42). It offers an easy-to-use platform for analyzing large cancer genomic datasets, allowing researchers to investigate genetic alterations, pathways, and clinical consequences in a variety of cancer types. It improves the analysis of complex genomic data by offering straightforward prognostic tools and making it available to a wide variety of academics. The platform aims to handle any issues that arise from complex genomic data. The current study used this database to analyze CDON mutations during LIHC malignancy.

STRING and DAVID analysis

The Search Tool for Retrieval of Interacting Genes/Proteins (STRING)(43), was used in this investigation to extract a PPI network of CDON-enriched genes. It harnesses a wealth of data to assist scientists in deciphering the perplexing network of protein interactions. In our study, we incorporated STRING into the CDON protein production. The STRING data collection is well-known for its high availability of PPI information, coverage, and increased quality control in comparison to other datasets. STRING combines data from multiple sources, including literature and gene expression patterns, to generate a consolidated quality score for each communication. It uses PPI from both experimental and computational approaches. DAVID (44) was used to investigate the GO and KEGG terms of CDON and its enriched genes, with $P < 0.05$ indicating significant results.

Results

Expression analysis of CDON in LIHC

We examined the CDON expression in LIHC and normal control samples using the UALCAN data set (Figure 1). Following research, we found that CDON expression was up- regulated in LIHC malignant development cells than in normal control samples. This significant up-regulation showed the close relationship between CDON expression and the growth of LIHC malignant cells. This finding increases the likelihood that CDON, a LIHC therapeutic target or diagnostic marker, may be crucial in limiting the cancer ability to proliferate.

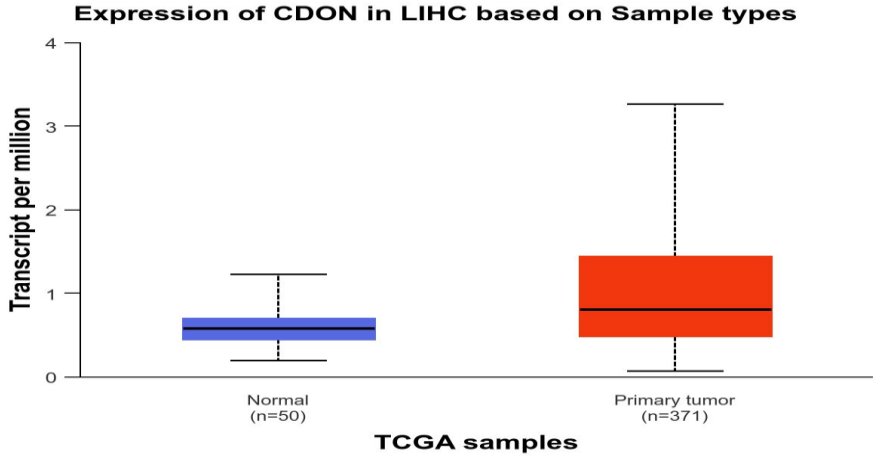

Figure 1: Expression profiling of CDON in LIHC and normal tissue samples.

Expression analysis of CDON based on different clinical parameters

Hence, we evaluated CDON in LIHC samples across a range of clinical parameters, such as the patient age, gender, and race, as well as the specific cancer stage (Figure 2). When we first examined CDON expression in various phases of cancer formation, we found that LIHC samples had significantly higher levels of CDON expression in all stages when compared to normal control samples (Figure 2A). In addition, we looked into CDON expression in LIHC patients and found that, in comparison to normal control samples, CDON expression was significantly up-regulated in each of the three racial groups, including Asian, African-American, and Caucasian (Figure 2B).Furthermore, we investigated CDON expression in LIHC cancer patients by gender, and found significant up-regulation of CDON in both male and female patients as compared to normal control samples (Figure 2C). Finally, we investigated the connection between CDON expression and patient age in LIHC. Our analysis revealed that CDON was up-regulated across several age groups among LIHC patients (Figure 2D). These findings support the importance of CDON in LIHC and emphasize its potential as a useful biomarker for diagnosis and prognosis.

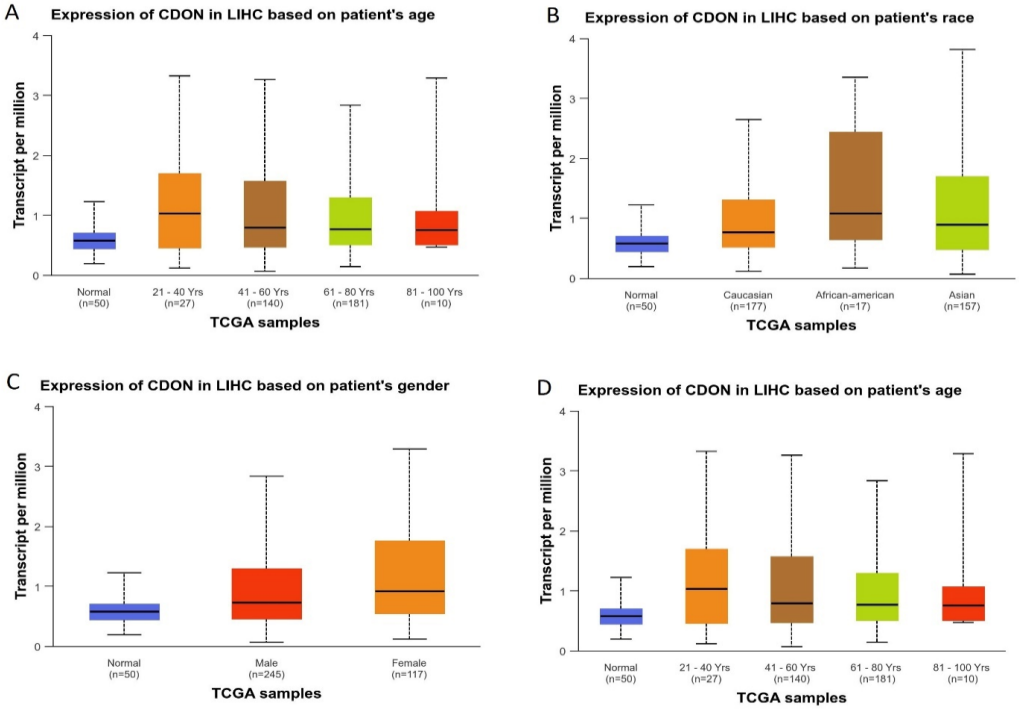

Figure 2: Expression of CDON across different clinical boundaries

Expression validation of CDON

We employed GEPIA2 to investigate the expression of CDON in LIHC cells and normal control samples. LIHC exhibited significantly higherCDON expression than normal control samples (Figure 3A). Furthermore, we used the GEPIA2 data set to investigate the association between CDON expression and different phases of cancer development. These findings revealed a significant relationship between CDON expression and LIHC patient stages. Additionally, (Figure 3B) demonstrated that in LIHC, CDON gene had the lowest expression in stage IV and the elevated expression in stage III.

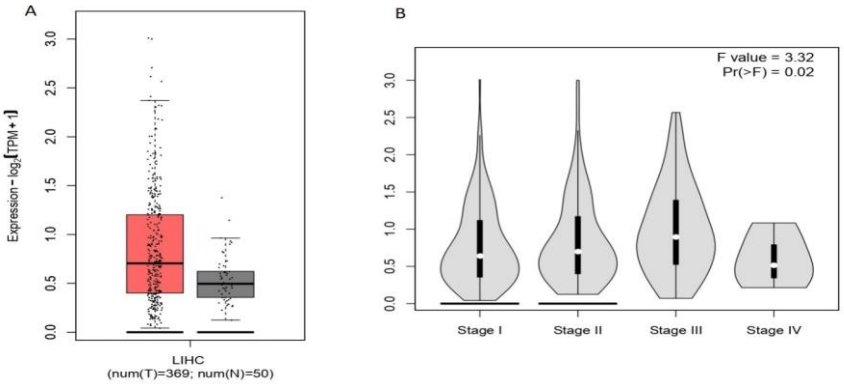

Figure 3: Validation of CDON expression across different stages of LIHC

CDON promoter methylation

We used the UALCAN online database to analyze the CDON promoter methylation levels in LIHC and normal control samples. Our findings showed that CDONwas hyper-methylated in LIHC samples compared to normal control samples (Figure 4). This finding indicates a positive relationship between CDON expression and promoter methylation in LIHC. This association highlighted CDON therapeutic potential in the pathogenesis of LIHC, indicating that it could be a target for therapeutic interventions in this cancer type.

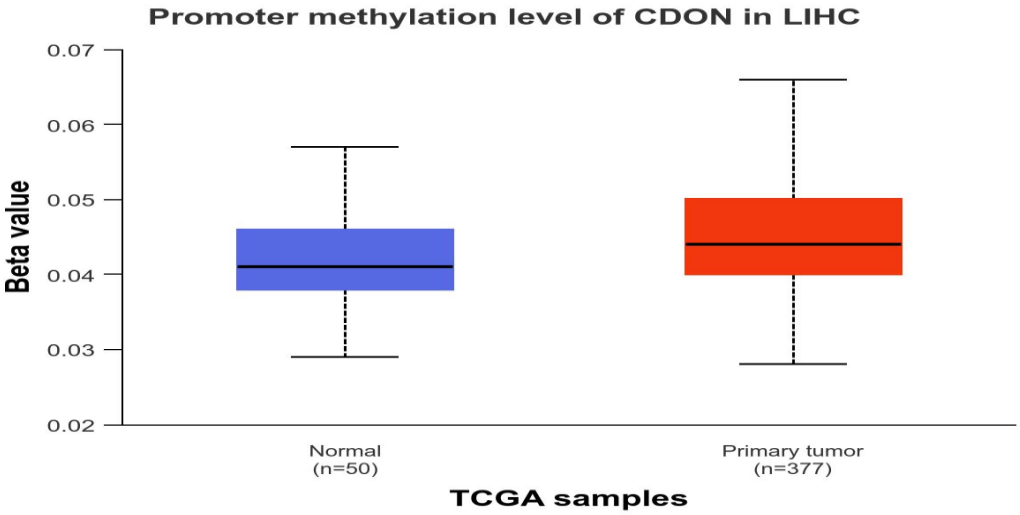

Figure 4: Promoter methylation pattern of CDON in LIHC and normal control samples

CDON promoter methylation in LIHC divided based on different clinical parameters

We studied several clinical limits to gain further understanding into the CDON promoter methylation in LIHC (Figure 5). In summary, we compared LIHC cancer progression phases to normal control samples and examined CDON promoter methylation. Importantly, changes were seen between phases; in contrast to normal control samples, all four stages showed obvious hyper-methylation (Figure 5A).In this method, we investigated CDON promoter methylation using the race of the LIHC patients as a criterion. When compared to normal control samples, we found evidence that hyper-methylation occurred in the CDON promoter region in all three racial groups: Asian, African-American, and Caucasian (Figure 5B).After that, the methylation of the CDON promoter was evaluated, and the results showed hyper-methylation in both the male and female individuals (Figure 5C). Last but not least, we looked into the CDON promoter methylation in connection to patient age and discovered that all age groups were significantly hyper-methylated when compared to normal samples, with methylation levels varying within age groups (Figure 5D).These thorough assessments demonstrate the surprising association between the CDON promoter methylation and different clinical boundaries in LIHC, which results in a consistent pattern of hyper-methylation in the CDON promoter methylation level in LIHC and highlights its potential role in the etiology of LIHC cancer.

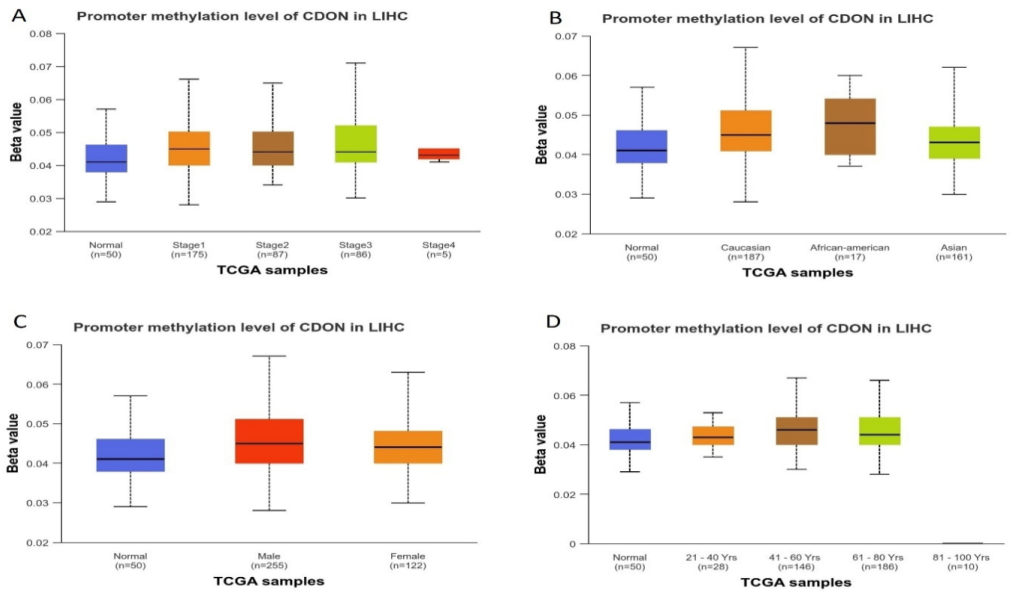

Figure 5: CDON promoter methylation pattern across different clinical parameters

Survival analysis of CDON

To further investigate the CDON expression in LIHC, we developed an assessment for overall survival (OS) and disease-free survival (DFS) using the KM plotter tool. The present investigation shows artificial relationship between patient survival outcomes and CDON gene expression. Specifically, patients with LIHC who expressed low levels of CDON had favorable overall survival (OS) than patients who expressed high levels of CDON expression (Figure 6A). Furthermore, LIHC patients with highCDON expression showed worse outcomes than those with lowCDON expression when it came to disease-free survival (DFS) study. The aforementioned studies underscore the crucial role that CDON plays in determining the survival outcomes of patients, hence featuring its potential therapeutic utility as a prognostic marker in the therapy of liver cancer and suggesting its involvement in the course and development of LIHC cancer.

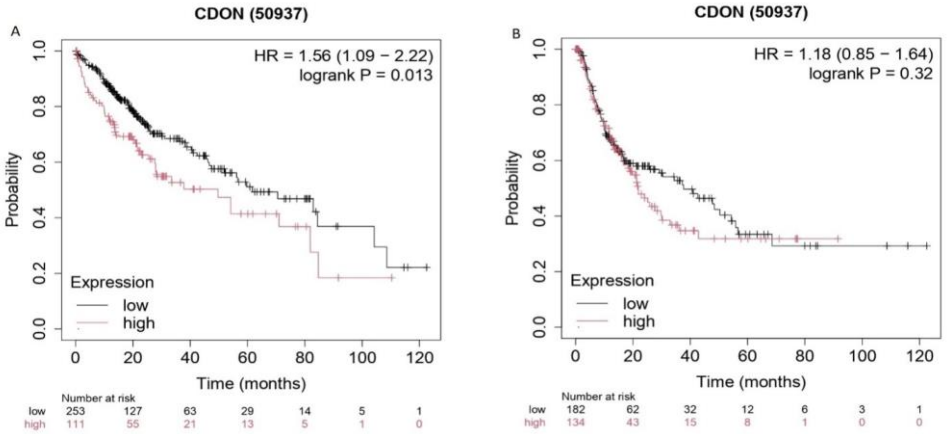

Figure 6: KM survival curve (OS, RFS) of CDON in LIHC patients

CDON survival validation

To explore the prognostic efficacy of CDON expression in LIHC tumor progression, the GEPIA2.0 informational index was used. We separated LIHC patients into two groups based on CDON expression levels: low and high. When compared to high CDON expression, low CDON expression in LIHC was associated with great overall survival (OS) (Figure 7A). Following that, we discovered, unlike the high CDON expression group, a lowCDON expression level was related with good DFS in LIHC (Figure 7B). These findings highlight the crucial role that the CDON gene plays in the initiation and spread of LIHC cancer.

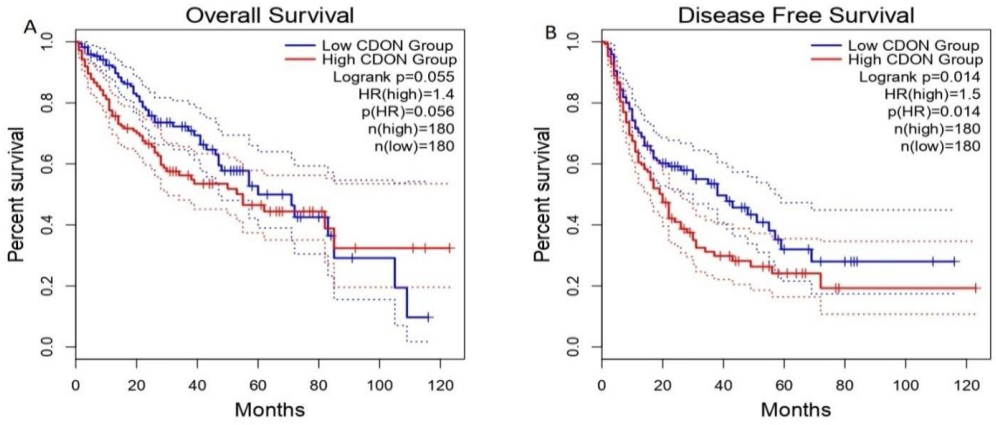

Figure 7: Survival curve (OS, RFS) of CDON inLIHC patients

Mutation analysis of CDON

We used cBioPortal to investigate the CDON genetic alterations in LIHC patients in greater depth. Our data demonstrate that only 8% of LIHC samples depicted genetic abnormalities in CDON. Truncating and missense mutations were among the genomic alterations examined in LIHC (Figure 8). Despite the fact that genetic abnormalities in CDON are infrequent in LIHC, our findings suggest that truncating and missense mutations may be crucial to CDON dysregulation in LIHC.

Figure 8: Oncoplot of CDON in LIHC cancer

CDON PPI network

The STRING software was used to investigate the functional and structural connections between CDON, DEG proteins. The development of PPI networks revealed links between the CDON hub gene and ten other genes, including BOC, CDH15, CDH2, SHH, PTCH2, IHH, DHH, GAS1, LRP2, and PTCH1, highlighting the CDON gene diversity. This implies that CDON serves a range of functions, interacts strongly with related genes, and is essential for numerous biological processes.

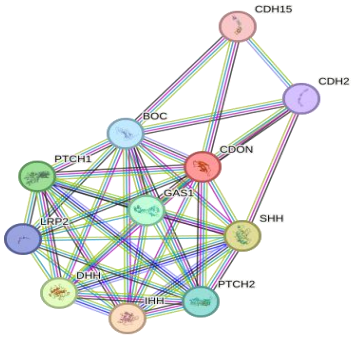

Figure 9: Protein-protein Interactions of CDON

Gene ontology (GO), and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis

The functional annotation of DEGs was performed using the DAVID online tool. Terms denoting biological processes, molecular activities, and cellular components associated with KEGG pathways were utilized to study the KEGG pathway-enriched genes and their potential GO (Gene Ontology) classification.The gene ontology BP analysis revealed that the GEGs are enriched in smoothened signaling pathway (GO:0007224), cell fate specification (GO:0001708), self-proteolysis (GO:0097264), somite development (GO:0061053), smooth muscle tissue development (GO:0048745) Table 1(Figure 10A). The gene ontology CC analysis depicted the DEGs are involved in plasma membrane (GO:0005886), Golgi apparatus (GO:0005794), axonal growth cone (GO:0044295), collagen-containing extracellular matrix (GO:0062023), catenin complex (GO:0016342) Table 2 (Figure 10B). By examining MF, we found that DEGs from the complex PPI network were enriched in patched binding (GO:0005113), cholesterol-protein transferase activity (GO:0140853), calcium ion binding (GO:0005509), peptidase activity (GO:0008233), transferase activity (GO:0016740) Table 3 (Figure 10C). The analysis of KEGG enrichment pathways showed that DEGs are involved in Hedgehog signaling pathway (hsa04340), Basal cell carcinoma (hsa05217), Pathways in cancer (hsa05200), Axon guidance (hsa04360), Proteoglycans in cancer (hsa05205) Table.4 (Figure .10D).

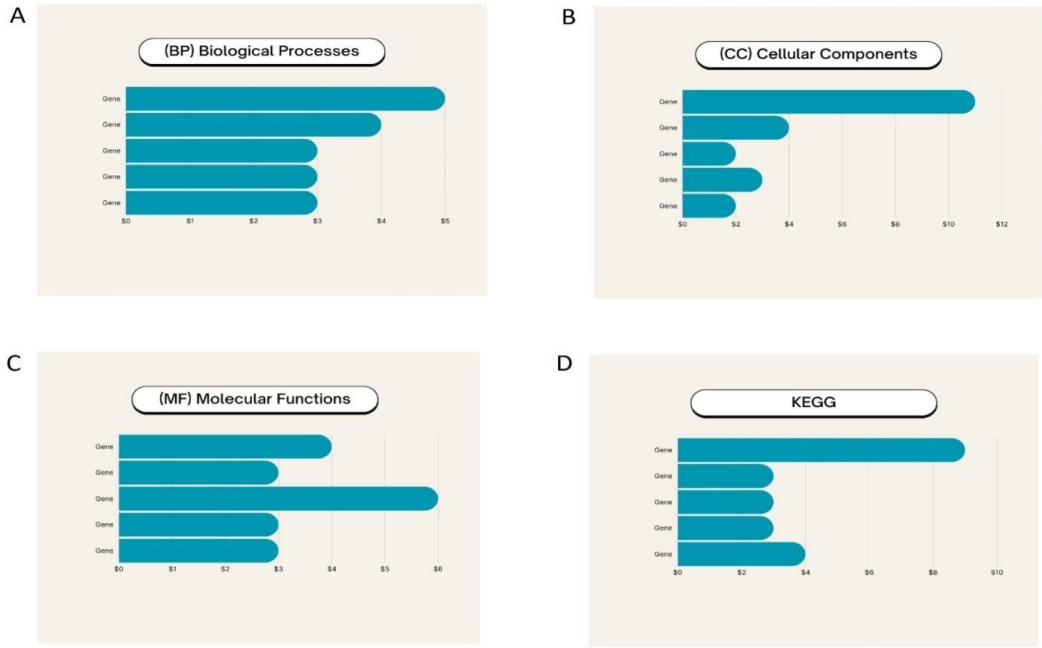

Figure 10: GO and KEGG analysis of CDON by DAVID tool

Table.1 gene enrichment analysis

BP			
Gene Term	**Gene Count**	**Genes**	**P-value**
GO:0007224~smoothened signaling pathway	05	SHH, DHH, PTCH1, IHH, CDON	6.599495653743032E-8
GO:0001708~cell fate specification	04	SHH, DHH, IHH, CDON	4.180669505577206E-7
GO:0097264~self proteolysis	03	SHH, DHH, IHH	1.5212625574481203E-5
GO:0061053~somite development	03	SHH, PTCH1, IHH	2.418228423201806E-5
GO:0048745~smooth muscle tissue development	03	SHH, PTCH1, IHH	2.418228423201806E-5

Table.2: gene enrichment analysis

CC			
Gene Term	**Gene Count**	**Genes**	**P-value**
GO:0005886~plasma membrane	11	SHH, CDH2, DHH, PTCH1, PTCH2, BOC, IHH, GAS1, LRP2, CDH15, CDON	1.8808316653355096E-6
GO:0005794~Golgi apparatus	04	SHH, PTCH1, LRP2, CDH15	0.013226411975849076
GO:0044295~axonal growth cone	02	PTCH1, BOC	0.015218597705081632
GO:0062023~collagen-containing extracellular matrix	03	SHH, CDH2, CDON	0.015440879640466823
GO:0016342~catenin complex	02	CDH2, CDH15	0.021341376180874214

Table.3: gene enrichment analysis

MF			
Gene Term	**Gene Count**	**Genes**	**P-value**
GO:0005113~patched binding	04	SHH, DHH, PTCH1, IHH	5.598289177966452E-9
GO:0140853~cholesterol-protein transferase activity	03	SHH, DHH, IHH	7.243886351838069E-7
GO:0005509~calcium ion binding	06	SHH, CDH2, DHH, IHH, LRP2, CDH15	1.8229073197385505E-5
GO:0008233~peptidase activity	03	SHH, DHH, IHH	0.0011179293507000453
GO:0016740~transferase activity	03	SHH, DHH, IHH	0.0014053838001314948

Table.4: gene enrichment analysis

KEGG			
Gene Term	**Gene Count**	**Genes**	**P-value**
hsa04340:Hedgehog signaling pathway	09	SHH, DHH, PTCH1, PTCH2, BOC, IHH, GAS1, LRP2, CDON	6.857985949016537E-17
hsa05217:Basal cell carcinoma	03	SHH, PTCH1, PTCH2	0.0021681711629743003
hsa04360:Axon guidance	03	SHH, PTCH1, BOC	0.017373192446623643
hsa05205:Proteoglycans in cancer	03	SHH, PTCH1, IHH	0.021109930814925966
hsa05200:Pathways in cancer	06	SHH, PTCH1, IHH GAS1, LRP2, CDON	4.857985949016537E-17

Discussion

In this research article, using a range of online bioinformatics tools, we evaluated CDON expression, prognosis, methylation, survival, mutations, and gene enrichment to perform an evaluation in LIHC. Furthermore, the Liver Hepatocellular Carcinoma with differentially expressed significant data was validated using overall survival and DFS. As a putative regulator in the pathogenesis of LIHC, the data demonstrated the significance of CDON expression for human health and suggested a potential correlation between CDON expression and the spread of LIHC cancer.

Common DEGs take role in HCC cell division and proliferation. Common DEGs and sub-networks were linked to signaling pathways, including the cell cycle and metabolic pathways. Cancer cells undergo adaptive metabolic reprogramming to maintain a certain metabolic state that supports their unchecked proliferation under the regulation of many carcinogenic pathways (45). The most recent study used integrated proteogenomic analysis of paired tumor and surrounding liver tissues to identify liver-specific metabolic reprogramming in HBV-related HCC (46). Furthermore, given the evidence that the epidemics of obesity, diabetes, and metabolic syndrome were identified as contributing factors to the prevalence of HCC(9).Changes in metabolic pathways are not only caused by the course of HCC, but they may also contribute to its genesis. The recovery of aberrant metabolism offers a new approach to the prevention, diagnosis, and treatment of HCC in China. Previous studies have shown that *CDK1/CCNB1* inhibits the p53 signaling pathway and regulate the development of HCC (47). Studies on hepatic carcinogenesis have demonstrated that epigenetic and miRNA changes associated with the progression of precancerous lesions to HCC (48, 49) miRNAs are short non-coding RNAs (~22 nucleotides) that regulate the expression of many target genes (50). In HCC, tumor suppressor genes may be epigenetically silenced by histone changes, such as histone H3 lysine 9 (H3K9) methylation and tri-methylation of H3K27, as well as DNA hyper-methylation of CpG island promoters and histone deacetylation (51, 52). Recently, long, non-coding RNAs have also been found to be differentially expressed in HCC and have been implicated in HCC pathogenesis (53).

According to research, CDON must interact with SHH ligand and other SHH receptor components, particularly PTCH1, at the cell surface for appropriate signaling to occur, and disruption of these interactions can result in HPE (28, 54).Defects in numerous SHH [MIM 600725]-signaling pathway components are the most well-studied etiology of HPE-like abnormalities. Studies on mice have demonstrated that the expression of Gas1, CDON, and Boc may alter Hh. Interestingly, separate Holoprosencephaly phenotypes result from the loss of either Gas1 or CDON in mice(55).A variety of extracerebral issues are commonly associated with mutations in CDON. Different CDON variants result in a broad variety of aberrant phenotypes. Hepatic cholestasis, biliary atresia, or dark, thick eyebrows with synophrys have been described in addition to disorders of the central nervous system (56). Dominant inheritance has been connected to mutations in CDON (28). It has been discovered that CDON is the first HPE gene to cause coloboma with a recessive inheritance pattern, in contrast to the dominant pattern observed in HPE. A new heterozygous missense mutation in the CDON gene was related to PSIS, unilateral facial, and abducens nerve paralysis. Typically, CDON causes the microform kind of Holoprosencephaly. All of the traits of a dependence receptor are present in CDON: it elicits apoptosis when its ligand SHH is absent, it is cleaved at aspartic acid residues by a protease-like enzyme, and it triggers apoptosis that is dependent on caspase-9 through a domain that is exposed as a result of this proteolytic cleavage (57). According to new research on the dependency receptor DCC, SHH/CDON plays a critical regulatory role in the development of cancer (58). CDON expression depends on the conventional Hedgehog canonical route, even though CDON pro-apoptotic function seems to be entirely independent of it.Premigratory neural folds, NPB, and developing mouse neural folds exhibit the conserved cell-surface protein CDON(59).The maturing mouse neural folds, NPB, and premigratory NCCs exhibit the conserved cell-surface protein CDON. Recently, CDON has also been linked to the development of zebrafish optic vesicles as a decoy receptor for Shh. In addition to acting in a Patched-independent manner to pattern the eye in a proximo-distal manner, CDON also adversely controlsShh signaling.

The UALCAN database was utilized in the current evaluation to locate CDON expression in LIHC. Research has demonstrated that CDON expression is up-regulated across a range of cancer stages, different cancer development types, age, gender, and racial groupings. The main goal of the flow outcome is to demonstrate that, in relation to the tumor growth, LIHC tissues showed noticeably higher levels of CDON expression than normal control samples. Furthermore, when comparing LIHCpatients with low and high CDON expression, our analysis of the KM plotter tool revealed that patients with low CDON expression had good disease-free survival while patients with high CDON expression had worse overall survival.The CDON gene diversity was also shown by the STRING and DAVID tools research, which also showed how it interacts with other genes to be an essential component of a number of biological pathways and processes. According to our findings, the expression level of CDON in LIHC was a separate negative prognostic factor. Further evaluations should look on the prognostic role of CDON expression throughout the course of cancer development.

Conclusion

Our research indicates a strong correlation between promoter methylation, genetic alterations, and low overall survival in LIHC and CDON up-regulation. By effectively utilizing multiple public datasets, such as UALCAN, TCGA, cBioPortal, STRING, DAVID, and KM plotter, we have clarified the prognostic, therapeutic, and diagnostic functions of CDON in LIHC. To confirm and validate these results and investigate the underlying mechanisms causing CDON dysregulation in LIHC, more research is necessary. In the end, these results might aid in the creation of more potent therapeutic plans and diagnostic tools for LIHC patients.

Conflict of interest

None

Acknowledgement
None

References

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424.
2. Llovet JM, Zucman-Rossi J, Pikarsky E, Sangro B, Schwartz M, Sherman M, et al. Hepatocellular carcinoma. Nat Rev Dis Primers. 2016;2(16018):18.
3. World Medical Association Declaration of Helsinki. Ethical principles for medical research involving human subjects. Bull World Health Organ. 2001;79(4):373-4.
4. Islami F, Goding Sauer A, Miller KD, Siegel RL, Fedewa SA, Jacobs EJ, et al. Proportion and number of cancer cases and deaths attributable to potentially modifiable risk factors in the United States. CA Cancer J Clin. 2018;68(1):31-54.
5. Degasperi E, Colombo M. Distinctive features of hepatocellular carcinoma in non-alcoholic fatty liver disease. Lancet Gastroenterol Hepatol. 2016;1(2):156-64.
6. Hameed A, Condò C, Tauseef I, Idrees M, Ghazanfar S, Farid A, et al. Isolation and characterization of a cholesterol-lowering bacteria from Bubalus bubalis raw milk. Fermentation. 2022;8(4):163.
7. Forner A, Llovet JM, Bruix J. Hepatocellular carcinoma. Lancet. 2012;379(9822):1245-55.
8. Usman M, Hameed Y, Ahmad M, Iqbal MJ, Maryam A, Mazhar A, et al. SHMT2 is associated with tumor purity, CD8+ T immune cells infiltration, and a novel therapeutic target in four different human cancers. Current Molecular Medicine. 2023;23(2):161-76.
9. Estes C, Anstee QM, Arias-Loste MT, Bantel H, Bellentani S, Caballeria J, et al. Modeling NAFLD disease burden in China, France, Germany, Italy, Japan, Spain, United Kingdom, and United States for the period 2016-2030. J Hepatol. 2018;69(4):896-904.
10. Guo X, Wu J, Wei F, Ouyang Y, Li Q, Liu K, et al. Trends in hepatitis B virus resistance to nucleoside/nucleotide analogues in North China from 2009-2016: A retrospective study. Int J Antimicrob Agents. 2018;52(2):201-9.
11. Khalil T, Okla M, Al-Qahtani W, Ali F, Zahra M, Shakeela Q, et al. Tracing probiotic producing bacterial species from gut of buffalo (Bubalus bubalis), South-East-Asia. Brazilian Journal of Biology. 2022;84:e259094.
12. Usman M, Hameed Y, Ahmad M. Does human papillomavirus cause human colorectal cancer? Applying Bradford Hill criteria postulates. ecancermedicalscience. 2020;14.
13. Kulik L, El-Serag HB. Epidemiology and Management of Hepatocellular Carcinoma. Gastroenterology. 2019;156(2):477-91.
14. Sial N, Rehman JU, Saeed S, Ahmad M, Hameed Y, Atif M, et al. Integrative analysis reveals methylenetetrahydrofolate dehydrogenase 1-like as an independent shared diagnostic and prognostic biomarker in five different human cancers. Bioscience Reports. 2022;42(1):BSR20211783.
15. Zhang L, Sahar A, Li C, Chaudhary A, Yousaf I, Saeedah M, et al. A detailed multi-omics analysis of GNB2 gene in human cancers. Brazilian Journal of Biology. 2022;84:e260169.
16. Nault JC, De Reyniès A, Villanueva A, Calderaro J, Rebouissou S, Couchy G, et al. A hepatocellular carcinoma 5-gene score associated with survival of patients after liver resection. Gastroenterology. 2013;145(1):176-87.
17. Mao J, Huang X, Okla MK, Abdel-Maksoud MA, Mubarak A, Hameed Z, et al. [Retracted] Risk Factors for TERT Promoter Mutations with Papillary Thyroid Carcinoma Patients: A Meta-Analysis and Systematic Review. Computational and Mathematical Methods in Medicine. 2022;2022(1):1721526.
18. Gores GJ. Decade in review-hepatocellular carcinoma: HCC-subtypes, stratification and sorafenib. Nat Rev Gastroenterol Hepatol. 2014;11(11):645-7.
19. Hameed Y, Ejaz S. TP53 lacks tetramerization and N-terminal domains due to novel inactivating mutations detected in leukemia patients. Journal of Cancer Research and Therapeutics. 2021;17(4):931-7.
20. Yasir M, Nawaz A, Ghazanfar S, Okla M, Chaudhary A, Al WH, et al. Anti-bacterial activity of essential oils against multidrug-resistant foodborne pathogens isolated from raw milk. Brazilian Journal of Biology. 2022;84:e259449.
21. Khan M, Hameed Y. Discovery of novel six genes-based cervical cancer-associated biomarkers that are capable to break the heterogeneity barrier and applicable at the global level. Journal of Cancer Research and Therapeutics. 2023.
22. Khemlina G, Ikeda S, Kurzrock R. The biology of Hepatocellular carcinoma: implications for genomic and immune therapies. Mol Cancer. 2017;16(1):017-0712.
23. Kretzschmar M, Doody J, Timokhina I, Massagué J. A mechanism of repression of TGFbeta/ Smad signaling by oncogenic Ras. Genes Dev. 1999;13(7):804-16.
24. Muenke M, Solomon B, Odent S, editors. Holoprosencephaly. American Journal of Medical Genetics Part C: Seminars in Medical Genetics; 2010.
25. Usman M, Hameed Y. GNB1, a novel diagnostic and prognostic potential biomarker of head and neck and liver hepatocellular carcinoma. Journal of Cancer Research and Therapeutics. 2023.
26. Lu M, Krauss RS. N-cadherin ligation, but not Sonic hedgehog binding, initiates Cdo-dependent p38α/β MAPK signaling in skeletal myoblasts. Proceedings of the National Academy of Sciences. 2010;107(9):4212-7.
27. Sanchez-Arrones L, Cardozo M, Nieto-Lopez F, Bovolenta P. Cdon and Boc: Two transmembrane proteins implicated in cell-cell communication. Int J Biochem Cell Biol. 2012;44(5):698-702.

28. Bae GU, Domené S, Roessler E, Schachter K, Kang JS, Muenke M, et al. Mutations in CDON, encoding a hedgehog receptor, result in holoprosencephaly and defective interactions with other hedgehog receptors. Am J Hum Genet. 2011;89(2):231-40.
29. Roessler E, Hu P, Marino J, Hong S, Hart R, Berger S, et al. Common genetic causes of holoprosencephaly are limited to a small set of evolutionarily conserved driver genes of midline development coordinated by TGF-β, hedgehog, and FGF signaling. Hum Mutat. 2018;39(10):1416-27.
30. Freier TA. Isolation and characterization of unique cholesterol-reducing anaerobes: Iowa State University; 1991.
31. Dong Y, Wu X, Xu C, Hameed Y, Abdel-Maksoud MA, Almanaa TN, et al. Prognostic model development and molecular subtypes identification in bladder urothelial cancer by oxidative stress signatures. Aging (Albany NY). 2024;16(3):2591.
32. Identification of Key Biomarkers for the Future Applications in Diagnostics and Targeted Therapy of Colorectal Cancer. Current Molecular Medicine. 2022.
33. Berkun L, Slae M, Mor-Shaked H, Koplewitz B, Eventov-Friedman S, Harel T. Homozygous variants in MAPRE2 and CDON in individual with skin folds, growth delay, retinal coloboma, and pyloric stenosis. Am J Med Genet A. 2019;179(12):2454-8.
34. Pineda-Alvarez DE, Solomon BD, Roessler E, Balog JZ, Hadley DW, Zein WM, et al. A broad range of ophthalmologic anomalies is part of the holoprosencephaly spectrum. American journal of medical genetics Part A. 2011;155(11):2713-20.
35. Zhang W, Mulieri PJ, Gaio U, Bae GU, Krauss RS, Kang JS. Ocular abnormalities in mice lacking the immunoglobulin superfamily member Cdo. Febs J. 2009;276(20):5998-6010.
36. Hong M, Krauss RS, editors. Modeling the complex etiology of holoprosencephaly in mice. American Journal of Medical Genetics Part C: Seminars in Medical Genetics; 2018: Wiley Online Library.
37. Hong M, Krauss RS. Cdon mutation and fetal ethanol exposure synergize to produce midline signaling defects and holoprosencephaly spectrum disorders in mice. PLoS Genet. 2012;8(10):11.
38. Hong M, Krauss RS. Rescue of holoprosencephaly in fetal alcohol-exposed Cdon mutant mice by reduced gene dosage of Ptch1. PLoS One. 2013;8(11).
39. Chandrashekar DS, Bashel B, Balasubramanya SAH, Creighton CJ, Ponce-Rodriguez I, Chakravarthi B, et al. UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses. Neoplasia. 2017;19(8):649-58.
40. Tang Z, Li C, Kang B, Gao G, Zhang Z. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res. 2017;45(W1):W98-W102.
41. Maciejczyk A, Szelachowska J, Czapiga B, Matkowski R, Hałoń A, Györffy B, et al. Elevated BUBR1 expression is associated with poor survival in early breast cancer patients: 15-year follow-up analysis. J Histochem Cytochem. 2013;61(5):330-9.
42. Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2012;2(5):401-4.
43. von Mering C, Huynen M, Jaeggi D, Schmidt S, Bork P, Snel B. STRING: a database of predicted functional associations between proteins. Nucleic Acids Res. 2003;31(1):258-61.
44. Huang DW, Sherman BT, Tan Q, Collins JR, Alvord WG, Roayaei J, et al. The DAVID Gene Functional Classification Tool: a novel biological module-centric algorithm to functionally analyze large gene lists. Genome Biol. 2007;8(9):2007-8.
45. Ding Z, Ericksen RE, Escande-Beillard N, Lee QY, Loh A, Denil S, et al. Metabolic pathway analyses identify proline biosynthesis pathway as a promoter of liver tumorigenesis. J Hepatol. 2020;72(4):725-35.
46. Gao Q, Zhu H, Dong L, Shi W, Chen R, Song Z, et al. Integrated Proteogenomic Characterization of HBV-Related Hepatocellular Carcinoma. Cell. 2019;179(2):561-77.
47. Qin G, Tu X, Li H, Cao P, Chen X, Song J, et al. Long Noncoding RNA p53-Stabilizing and Activating RNA Promotes p53 Signaling by Inhibiting Heterogeneous Nuclear Ribonucleoprotein K deSUMOylation and Suppresses Hepatocellular Carcinoma. Hepatology. 2020;71(1):112-29.
48. Hellebrekers DM, Griffioen AW, van Engeland M. Dual targeting of epigenetic therapy in cancer. Biochim Biophys Acta. 2007;1:76-91.
49. Tischoff I, Tannapfe A. DNA methylation in hepatocellular carcinoma. World J Gastroenterol. 2008;14(11):1741-8.
50. Saito Y, Hibino S, Saito H. Alterations of epigenetics and microRNA in hepatocellular carcinoma. Hepatol Res. 2014;44(1):31-42.
51. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010;363(8):711-23.
52. Brahmer J, Reckamp KL, Baas P, Crinò L, Eberhardt WE, Poddubskaya E, et al. Nivolumab versus Docetaxel in Advanced Squamous-Cell Non-Small-Cell Lung Cancer. N Engl J Med. 2015;373(2):123-35.
53. Liu YR, Tang RX, Huang WT, Ren FH, He RQ, Yang LH, et al. Long noncoding RNAs in hepatocellular carcinoma: Novel insights into their mechanism. World J Hepatol. 2015;7(28):2781-91.
54. Kang JS, Mulieri PJ, Miller C, Sassoon DA, Krauss RS. CDO, a robo-related cell surface protein that mediates myogenic differentiation. J Cell Biol. 1998;143(2):403-13.
55. Echevarría-Andino ML, Allen BL. The hedgehog co-receptor BOC differentially regulates SHH signaling during craniofacial development. Development. 2020;147(23):189076.
56. Tenzen T, Allen BL, Cole F, Kang JS, Krauss RS, McMahon AP. The cell surface membrane proteins Cdo and Boc are components and targets of the Hedgehog signaling pathway and feedback network in mice. Dev Cell. 2006;10(5):647-56.
57. Goldschneider D, Mehlen P. Dependence receptors: a new paradigm in cell signaling and cancer therapy. Oncogene. 2010;29(13):1865-82.

58. Castets M, Broutier L, Molin Y, Brevet M, Chazot G, Gadot N, et al. DCC constrains tumour progression via its dependence receptor activity. Nature. 2011;482(7386):534-7.
59. Mulieri PJ, Okada A, Sassoon DA, Mcconnell SK, Krauss RS. Developmental expression pattern of the cdo gene. Developmental dynamics: an official publication of the American Association of Anatomists. 2000;219(1):40-9.