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Identification of Potential Hub Genes and Biological Mechanism in Nonalcoholic Fatty Liver Disease and Gallbladder Cancer via Integrated Bioinformatics Analysis

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is a widespread liver disorder linked to metabolic conditions and an increased risk of extrahepatic cancers while Gallbladder cancer (GBC) is one of the most common biliary tract malignancies which shares key risk factors with NAFLD, including obesity and gallstone disease. However, the molecular association between NAFLD and GBC remains unexplored. Therefore, the present study was undertaken to identify the common molecular signatures underlying NAFLD and GBC using an *in silico* computational biology-based approach. RNA-seq datasets for NAFLD (GSE126848) and GBC (GSE139682) were retrieved from the GEO database, common DEGs between the two diseases were identified using GEO2R and analysed using various plugins of Cytoscape. The analysis identified 361 common DEGs and the PPI network, constructed using STRING, consisted of 307 nodes and 517 edges. Seven hub genes i.e., *CDC20, CCNA2, TOP2A, BIRC5, CDK1, NUSAP1,* and *CCNB1*, that were found to be predominantly involved in the cell cycle pathway. This study demonstrates that the molecular factors and mechanisms driving the onset and progression of liver diseases such as NAFLD and GBC are the dysregulation of the cell cycle and the hub genes identified in the presented study can be further explored for therapeutic intervention of these diseases.

Key words: *Nonalcoholic fatty liver disease (NAFLD), Gallbladder cancer (GBC), Differential gene expression (DEG), computational biology, In-silico, Cell cycle*

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is amongst the most common causes of chronic liver disease worldwide. The prevalence of NAFLD is notably higher than previously estimated and continues to increase rapidly. Recent estimates indicate that the condition affects 32% of the adult population and is notably higher in males with 40% of males, compared to 26% of the female population affected (Riazi *et al*., 2022). Nonalcoholic fatty liver disease (NAFLD) is a condition characterized by the accumulation of excess fat in the liver without the influence of alcohol consumption or other known secondary causes of chronic liver disease. It differentiates from alcoholic liver disease by the absence of excessive alcohol consumption and a strong association with

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metabolic comorbidities such as type 2 diabetes, high cholesterol, hypertension, and obesity (Akshintala *et al*., 2019). NAFLD is an umbrella term encompassing a range of liver conditions, from the relatively benign nonalcoholic fatty liver (NAFL) to the more severe nonalcoholic steatohepatitis (NASH). Around 20% of people with NAFLD develop NASH, which can progress further to advanced stages, fibrosis, cirrhosis, and hepatocellular carcinoma (HCC) in some cases (Kawanaka *et al*., 2021). Recent research indicates that NAFLD is linked to a moderately higher long-term risk of developing extrahepatic cancers, particularly gastrointestinal, breast, and gynaecological cancers, over a median follow-up period of nearly six years (Mantovani *et al*., 2022). NAFLD has also been reported to be linked to gallstone disease and cholecystectomy, suggesting these may increase the risk of developing NAFLD (Kichloo *et al*., 2021).

Gallbladder cancer (GBC) is a rare type of cancer overall, but is the most common malignancy of the biliary tract, making up 80% to 95% of all cases (Hundal and Shaffer, 2014). According to the World Cancer Research Fund, there were 122,491 new cases of gallbladder cancer in 2022, with China reporting the highest number of cases, followed by India. It is an aggressive malignancy originating in the epithelial lining of the gallbladder often associated with chronic inflammation, gallstones, obesity, cholelithiasis, and other biliary tract disorders. Due to its asymptomatic nature in its early stages, it is frequently diagnosed at an advanced stage, contributing to its poor prognosis (Hundal and Shaffer, 2014).

NAFLD and GBC have been reported to share common risk factors (Hundal and Shaffer, 2014). Research indicates that obesity is associated with insulin resistance, which contributes to fat accumulation in the liver. This accumulation can lead to inflammation and liver damage, increasing the risk of NAFLD and its progression to NASH (Nogueira and Cusi, 2024). A study by Slouha *et al.* (2023) found that NAFLD and gallstone disease have a bidirectional relationship, where each condition can increase the likelihood of developing the other (Slouha et al., 2023). Another study by Konyn *et al.* (2023) reported that having gallstone disease increases the possibility of developing NAFLD (Konyn et al., 2023). In another study, gallstones were linked to a higher risk of mortality from hepatobiliary cancers, particularly liver / intrahepatic cancer and gallbladder cancer, regardless of other influencing factors (Ryu et al., 2016). Individuals wisth liver diseases, gallstones, or pancreatic conditions have a higher risk of developing biliary tract cancers than those without these conditions and identified an association between NAFLD and an increased risk of developing cholangiocarcinoma and GBC (Kamal *et al*., 2021; Park *et al*., 2021). Despite the shared common risk factors and the established clinical relationship between gallstones and NAFLD (Ryu et al., 2016; Kamal *et al*., 2021), there are currently no studies on the molecular association between NAFLD and GBC. Therefore, the present study was undertaken to identify common molecular signatures between Nonalcoholic fatty liver disease and Gallbladder cancer using a computational biology approach, which could be further used to develop therapeutic interventions.

2. Materials and methods

2.1. Data acquisition

To find the association between NAFLD and GBC, the RNA-seq datasets having data on clinical features and gene expressions were selected from the Gene Expression Omnibus (GEO) database, respectively (Clough *et al*., 2024; Suppli *et al*., 2019 ; Xu *et al*., 2019).

2.2. Data pre-processing and DEG identification

Differential gene expression analysis tool GEO2R that employs the “DESeq2” R package to identify DEGs in RNA-seq data was used followed by an online Venn diagram-generating tool “Venny” to find the common DEGs between NAFLD and GBC (Clough *et al*., 2024; Suppli *et al*., 2019 ; Xu *et al*., 2019; Oliveros, 2007).

2.3. PPI network construction, analysis & gene enrichment

Common DEGs were then input to STRING (Search Tool for the Retrieval of Interacting Genes / Proteins) to construct a Protein-Protein Interaction (PPI) network followed by analysis using Cytoscape (Szklarczyk *et al*., 2023; Majeed and Mukhtar, 2023). Plugin Cytocluster was used for cluster analysis, which uses ClusterOne algorithm, and then, Cytohubba was used for identification of top ten hub genes according to the MCC (Maximal Clique Centrality) method following using NetworkAnalyzer for validation of the hub genes based on degree (Li *et al*., 2017; Chin *et al*., 2014). Functional enrichment visualization tool from STRING database was employed to perform KEGG pathway analysis of top seven genes based on libraries of known functions, such as biological

ontologies, pathways, and diseases and outputs in the form of bar graphs representing the role of input genes in the pathways (Szklarczyk *et al*., 2023). The flowchart of the research methodology is shown in Figure 1.

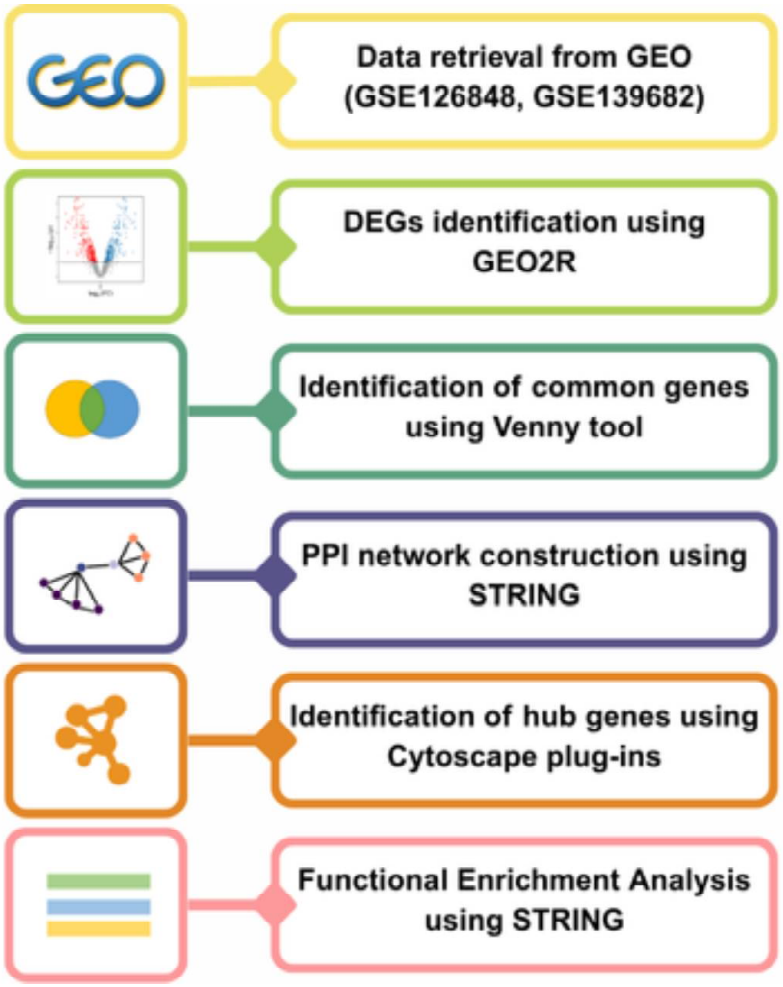


Figure 1: Flowchart of the research methodology

Table 1: Overview of datasets and dysregulated genes

Disease	Dataset	Diseased	Normal	Tissue	Dysregulated genes	Up-regulated genes	Down-regulated genes
Nonalcoholic fatty liver disease	GSE126848	16	14	Liver	1604	609	995
Gallbladder cancer	GSE139682	10	10	Gallbladder	3218	1804	1414

3. Results

3.1. Data acquisition, processing and DEG identification

The RNA-seq datasets for NAFLD-NASH (GSE126848) and GBC (GSE139682) from GEO database were processed using GEO2R. To identify differential gene expression between disease and normal states, p-value <0.05 and log2FC > 1 was defined for up-regulated genes, and $p < 0.05$ and log2FC < 1 was defined for down-regulated genes (Table 1) (McDermaid *et al*., 2019). Volcano plots representing distribution of up-regulated and down-regulated genes was generated by GEO2R (Figure 2) and 361 common DEGs were identified (Figure 3).

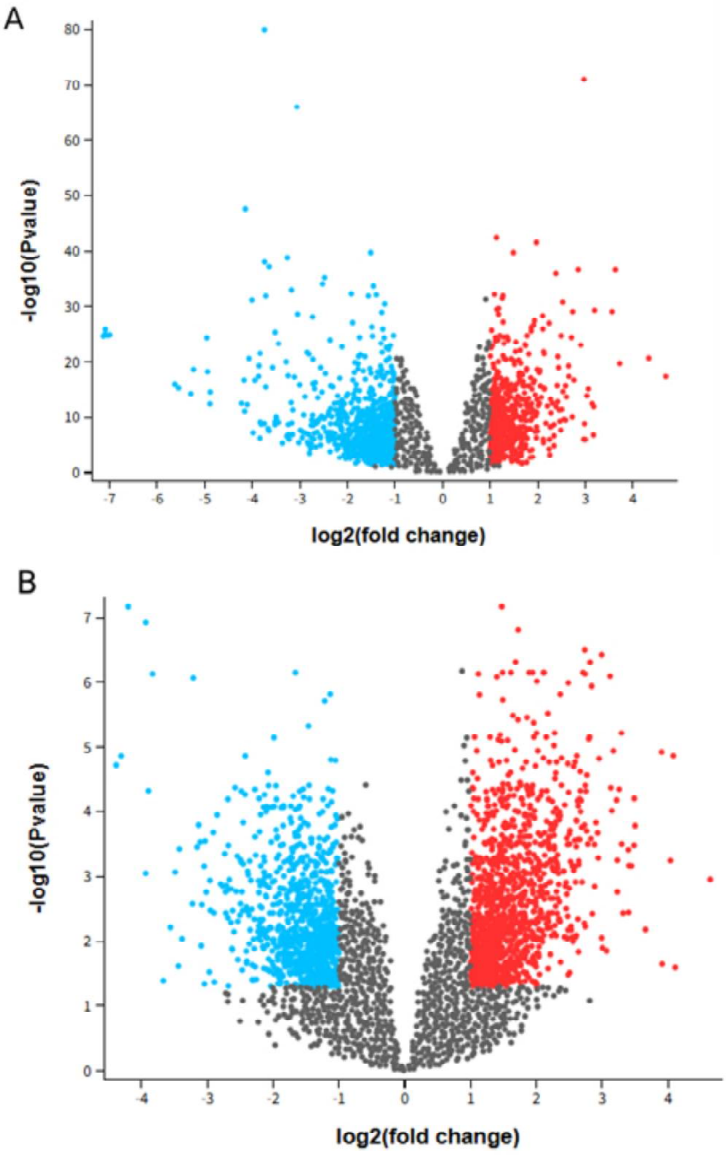


Figure 2: Volcano plots distribution of up-regulated genes (Red) and down-regulated genes (Blue) in (A.) Nonalcoholic Fatty Liver disease (GSE126848) and (B.) Gallbladder cancer (GSE139682)

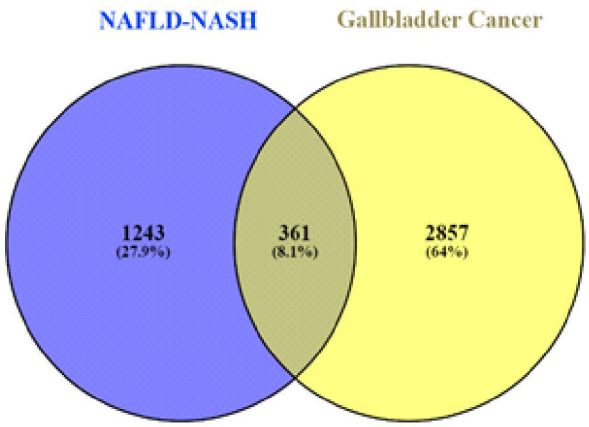


Figure 3: Identification of 361 common DEGs in NAFLD-NASH (Blue) and GBC (Yellow) using Venny tool

3.2. PPI analysis and hub gene identification

The PPI network of common DEGs was built in STRING (minimum required interaction score: high confidence 0.700, k-means clustering) and the resulting network contained 307 nodes (genes) and 517 edges (interaction between nodes) (Figure 4). CytoCluster plugin of cytoscape identified 23 clusters, from which seven were selected based on their p-value (Table 2). The selected clusters were merged (Figure 5), and the most significant genes *CDC20, CCNA2, TOP2A, BIRC5, CDK1, NUSAP1, KIF20A, CCNB1, TPX2* and *CENPF* were identified from the network (Figure 6A). Further validation using NetworkAnalyzer plugin of Cytoscape gave top ten hub genes: *CDK1, CDC20, CCNB1, TOP2A, CCNA2, BIRC5, PLK1, CENPF, NUSAP1* and *TPX2* (Table 3, Figure 6B).

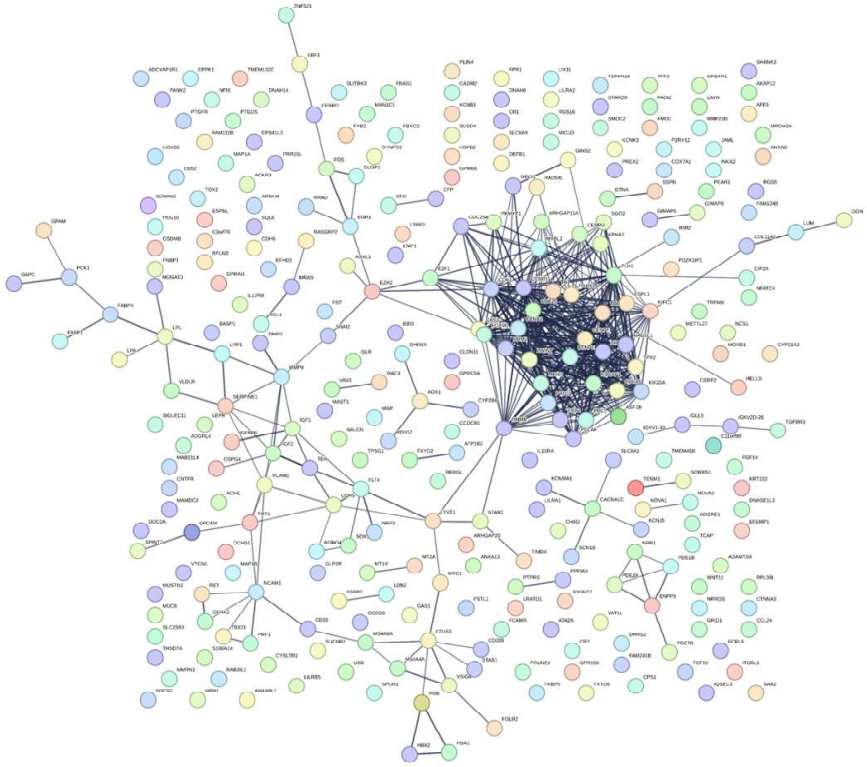


Figure 4: PPI Network Generated using STRING with 307 nodes (genes) and 517 edges (interaction between node) (confidence score 0.700)

Table 2: Highly connected clusters selected bsed on their *p*-value < 0.05

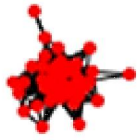
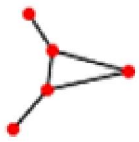
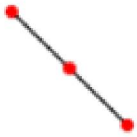
Cluster	Nodes	Cluster	Nodes
	Nodes: 40 Density: 0.512 Quality: 0.978 P-Value: 0.000		Nodes: 5 Density: 0.500 Quality: 0.714 P-Value: 0.014
	Nodes: 5 Density: 0.700 Quality: 1.000 P-Value: 0.003		Nodes: 3 Density: 1 Quality: 0.750 P-Value: 0.030

Table 2 (Cont.)

Cluster	Nodes	Cluster	Nodes
	Nodes: 4 Density: 0.500 Quality: 1.000 P-Value: 0.009		Nodes: 3 Density: 0.667 Quality: 1.000 P-Value: 0.030
	Nodes: 4 Density: 0.500 Quality: 1.000 P-Value: 0.010		

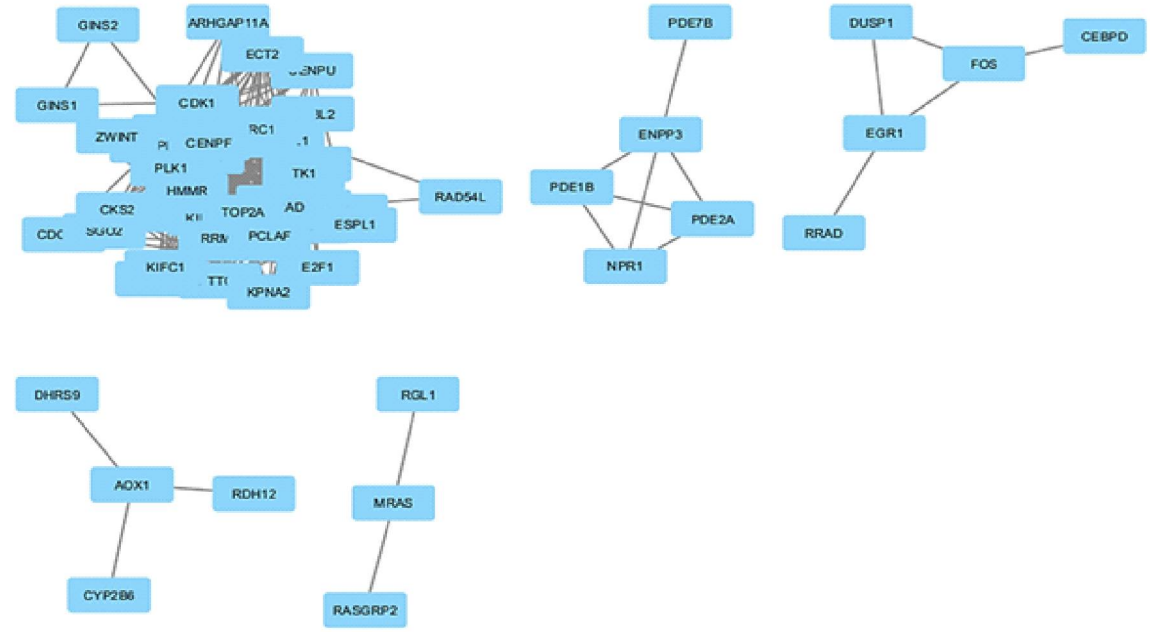


Figure 5: Network obtained after integration of the seven clusters obtained from CytoCluster

Table 3: Top ten hub genes obtained based on their degree using network analyzer

Gene	Degree	Gene	Degree
CDK1	38	BIRC5	30
CDC20	35	PLK1	29
CCNB1	34	CENPF	28
TOP2A	33	NUSAP1	28
CCNA2	33	TPX2	27

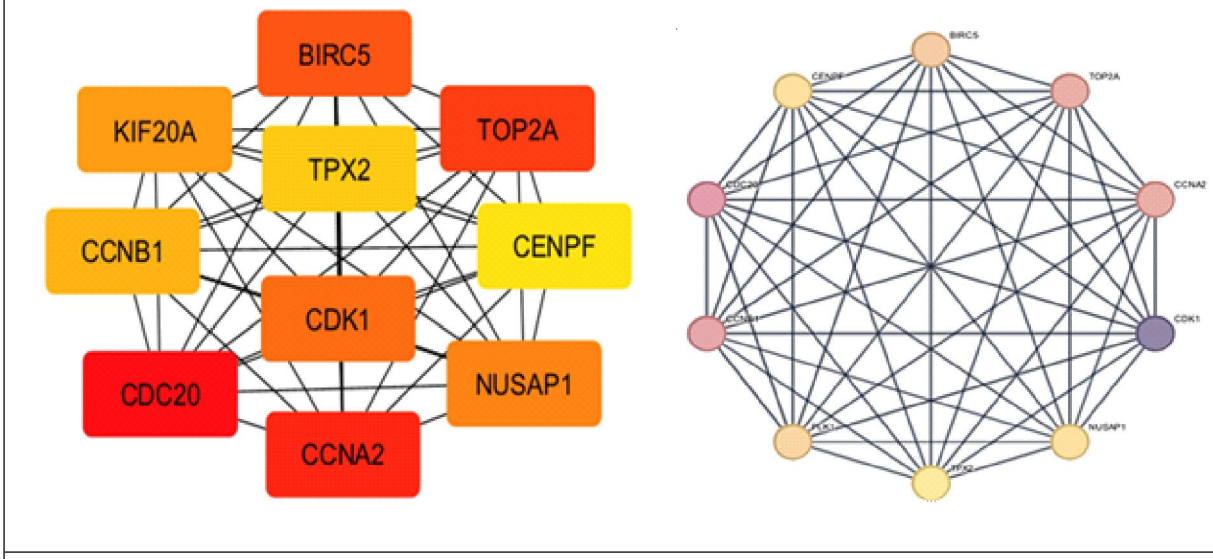


Figure 6: Top ten hub-genes identified and ranked using CytoHubba on subnetwork obtained from CytoCluster (A) and NetworkAnalyzer on STRING network (B)

After a cross-comparison between the highly significant genes obtained using Cytohubba and NetworkAnalyzer, seven hub genes *i.e.*, *CDC20, CCNA2, TOP2A, BIRC5, CDK1, NUSAP1,* and *CCNB1 w*ere shortlisted

3.3. Functional enrichment analysis

Functional Enrichment using STRING database showed that the seven hub genes identified in the present study were involved KEGG pathways of the Cell cycle, Progesterone-mediated oocyte maturation, and oocyte meiosis (Figure 7).

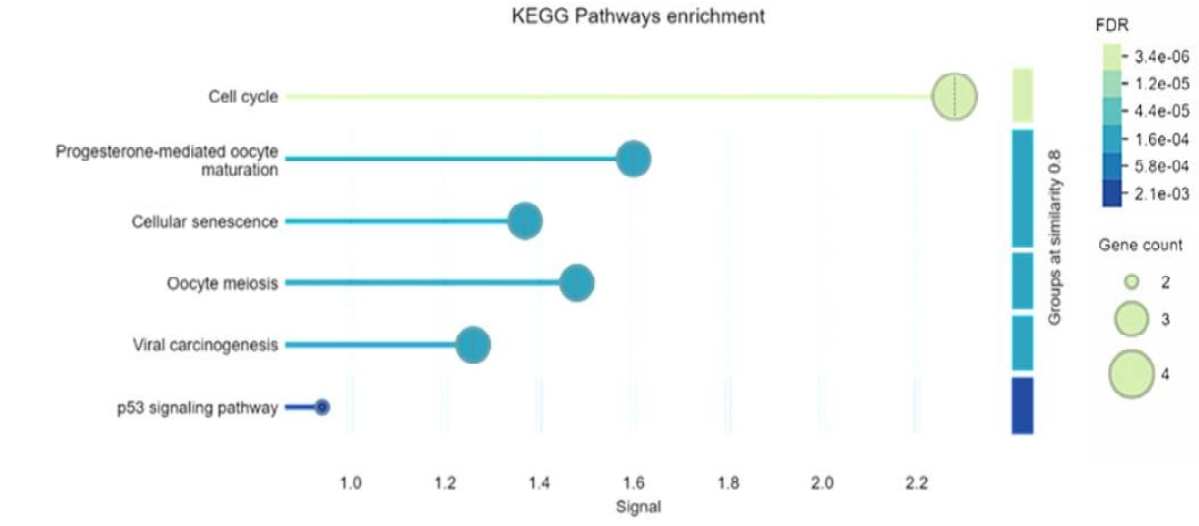


Figure 7: KEGG pathway Enrichment analysis of seven hub genes using STRING database

4. Discussion

This study identified seven hub genes associated with NAFLD and GBC namely, *CDC20, CCNA2, TOP2A, BIRC5, CDK1, NUSAP1,* and *CCNB1* using DEG analysis. *CDC20, CCNA2, CDK1,* and *CCNB1* have major

roles in the cell cycle regulation. *CCNA2* (Cyclin-A2) regulates G1/S and G2/M transitions by forming complexes with CDK1 or CDK2, promoting cell cycle progression (Pagano et al., 1992). *CCNB1* (Cyclin-B1) binds to CDK1 in the G2 phase, forming the maturation-promoting factor (MPF) complex, crucial for mitotic entry (Gavet and Pines, 2010). *CDK1* (Cyclin-dependent kinase 1) interacts with multiple cyclins, including Cyclins A2 and B1 to drive the cell cycle forward, especially during G2 and M phases (Gong and Ferrell, 2010). *CDC20* (Cell division cycle protein 20 homolog) activates the Anaphase-Promoting Complex (APC/C), a large E3 ubiquitin ligase that targets Cyclin B1 for degradation, leading to the inactivation of CDK1 in the cell cycle (Li and Zhang, 2009).

In a study by Wang *et al.* (2006), *CCNB1* has been linked to growth inhibition through G2/M phase cell cycle arrest, indicating its potential as a viable target for future anti-cancer therapies (Wang *et al*., 2006). *CCNA2* and *CCNB1* are reported to be involved in the pathogenesis of liver cirrhosis and its progression to hepatocellular carcinoma (HCC) with a high expression of *CCNA2* and *CCNB1* in elderly GBC patients and *CCNB1* was found to be upregulated in advanced GBC (Sun *et al*., 2023; Zhou *et al*., 2023; Kumar *et al*., 2020). *CCNA2* dysregulation has been implicated in various cancers, driving tumor progression, facilitating metastasis, and contributing to chemoresistance, establishing it as a prominent candidate for cancer prognostic biomarker development (Jiang *et al*., 2022). Yang *et al.* (2023) found differential expression of *CCNA2* in NAFLD (Yang *et al*., 2023).

Cyclin-dependent kinase 1 (*CDK1)* drives nascent DNA synthesis and contributes to chemotherapeutic resistance, underscoring its potential as a promising therapeutic target in cancer treatment (Liao *et al*., 2017). Impaired cell division due to *CDK1* dysfunction can lead to liver inflammation and fibrosis in NAFLD and its dysregulation can disrupt lipid metabolism, contributing to fat accumulation in the liver. The upregulated expression of phosphorylated *CDK1* and *CCNB1* in cholangiocarcinoma cells, suggests that *CDK1* may have a role in gallbladder cancer (Caldez et al., 2020; Yamamura et al., 2020). *CDC20* acts as an oncogene by promoting epithelial-mesenchymal transition, suppressing apoptosis, inhibiting immune infiltration, and modulating signaling pathways, playing a crucial role in the development and progression of human cancers (Xian et al., 2023). *CDC20* was differentially expressed in NAFLD (Yang *et al*., 2023) and was found to be upregulated in advanced GBC (Kumar *et al*., 2020).

DNA topoisomerase 2-alpha (*TOP2A*) alters DNA topology by introducing a transient double-stranded break in one DNA molecule, allowing an intact DNA strand to pass through the break, and subsequently resealing the cleaved strands (Wyles *et al*., 2007). Inhibiting *TOP2A* has been shown to increase the expression of Fatty Acid Synthase, indicating a link between *TOP2A* activity and lipid biosynthesis pathways (Menendez et al., 2006). *TO2A* was upregulated in NAFLD patients with its enhanced expression in GBC tissues facilitating the proliferation and metastasis of GBC via the PI3K/Akt/mTOR pathway (Liu *et al*., 2020; Lyu et al., 2020). Survivin or *BIRC5* promotes cell proliferation and prevents apoptosis (Wheatley and Altieri, 2019). Expression of survivin in hepatic stellate cells is crucial for their initial activation and the development of liver with reports of a positive correlation of *BIRC5* with GBC (Sharma *et al*., 2021; Salman et al., 2016). *NUSAP1* is a microtubule-associated protein that can bundle and stabilize microtubules (Ribbeck *et al*., 2006). Zeng *et al*. (2022), reported a high expression of *NUSAP1* in both human hepatic cell lines and NAFLD models at mRNA and protein levels (Zeng *et al*., 2022) and Yan *et al.* (2020) reported dysregulation of NUSAP1 in Cholangiocarcinoma (Yan *et al*., 2020), indicating a potential relation with GBC.

Cancer development results from mutations or dysregulations in genes controlling the cell cycle that cause perturbation in cell growth and division regulation leading to increased apoptosis, which in turn causes more inflammation and fibrosis in the liver. The interaction between cell cycle regulators and metabolic pathways is significant in NAFLD as well as GBC (Caldez *et al.*, 2020; Wencong *et al*., 2020). In various studies related to NAFLD and GBC, *CDC20, CCNA2, TOP2A, BIRC5, CDK1, NUSAP1*, and *CCNB1* have been implicated to be involved, with *CDK1* and *TOP2A* are reported in both diseases. Therefore, *CDK1* and *TOP2A* can be further investigated as a therapeutic target to prevent one condition from leading to another.

Our study highlights the common genes and pathways that link NAFLD with a higher risk of GBC. Although this study aimed to map key genes and pathways in NAFLD and GBC, the current findings are observational based on experimental data sets of RNA-seq analysis and haven't been confirmed by clinical or experimental studies. Validating these findings could help develop treatments for NAFLD and GBC.

5. Conclusion

This *in silico* network systems biology study investigated the molecular association in terms of genes and pathways between NAFLD and GBC. Genes common amongst the conditions namely *CDC20, CCNA2, TOP2A, BIRC5, CDK1, NUSAP1,* and *CCNB1* were identified that were found to be involved in the cell cycle and also in fat metabolism, suggesting their dysregulation leading to these conditions. Since *CDK1* and *TOP2A* have been previously reported in various clinical studies to be involved in NAFLD and GBC, they can be further explored as targets for therapeutic intervention to prevent either condition or one condition leading to another. Further experimental validation and clinical studies are essential to confirm these findings and could serve as a key focus for future research.

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Author contributions

Tammanna R. Sahrawat and Sushant: conceived and designed the study; Sushant: collected the data, performed the analysis, and wrote the first draft; Tammanna R. Sahrawat and Sushant: interpreting the data and major revisions of the first draft. Both authors reviewed and edited the manuscript and approved the submission.

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Availability of data and materials

The datasets generated or analysed during this study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The study did not involve human subjects or animal models and therefore no ethical clearance was required.

Conflicts of interests

The authors declare no conflict of interest.

References

Akshintala, D., Chugh, R., Amer, F. and Cusi, K. (2019). Nonalcoholic fatty liver disease: the overlooked complication of type 2 diabetes. *Endotext [Internet]*.

Caldez, M.J., Bjorklund, M. and Kaldis, P. (2020). Cell cycle regulation in NAFLD: when imbalanced metabolism limits cell division. *Hepatology International*, 14(4): 463-474.

Chin, C.H., Chen, S.H., Wu, H.H., Ho, C.W., Ko, M.T. and Lin, C.Y. (2014). CytoHubba: Identifying hub objects and sub-networks from complex interactome. *BMC Systems Biology*, 8: 1-7.

Clough, E., Barrett, T., Wilhite, S.E., Ledoux, P., Evangelista, C., Kim, I.F., Tomashevsky, M., Marshall, K.A., Phillippy, K.H., Sherman, P.M. and Lee, H. (2024). NCBI GEO: archive for gene expression and epigenomics data sets: 23-year update. *Nucleic Acids Research*, 52(D1): D138-144.

Gavet, O. and Pines, J. (2010). Progressive activation of CyclinB1-Cdk1 coordinates entry to mitosis. *Developmental Cell*, 18(4): 533-543.

Gong, D. and Ferrell, Jr., J.E. (2010). The roles of cyclin A2, B1, and B2 in early and late mitotic events. *Molecular Biology of the Cell*, 21(18): 3149-61.

Hundal, R. and Shaffer, E.A. (2014). Gallbladder cancer: epidemiology and outcome. *Clinical Epidemiology*, 7: 99-109.

Jiang, A., Zhou, Y., Gong, W., Pan, X., Gan, X., Wu, Z., Liu, B., Qu, L. and Wang, L. (2022). CCNA2 as an immunological biomarker encompassing tumor microenvironment and therapeutic response in multiple cancer types. *Oxidative Medicine and Cellular Longevity*, 2022(1): 5910575.

Kamal, H., SadrAzodi, O., Engstrand, L. and Brusselaers, N. (2021). Association between proton pump inhibitor use and biliary tract cancer risk: A Swedish populationbased cohort study. *Hepatology*, 4(4): 2021-31.

Kawanaka, M., Nishino, K., Morimoto, Y., Ishii, K., Tanikawa, T., Urata, N., Suehiro, M., Sasai, T., Haruma, K. and Kawamoto, H. (2021). Progression from nonalcoholic fatty liver to nonalcoholic steatohepatitis cirrhosis confirmed by liver histology after 14 years. *Internal Medicine*, 60(9): 1397-1401.

Kichloo, A., Solanki, S., Haq, K.F., Dahiya, D., Bailey, B., Solanki, D., Singh, J., Albosta, M., Wani, F., Aljadah, M. and Shah, H. (2021). Association of non-alcoholic fatty liver disease with gallstone disease in the United States hospitalized patient population. *World Journal of Gastrointestinal Pathophysiology*, 12(2): 14.

Konyn, P., Alshuwaykh, O., Dennis, B.B., Cholankeril, G., Ahmed, A. and Kim, D. (2023). Gallstone disease and its association with nonalcoholic fatty liver disease, all-cause and cause-specific mortality. *Clinical Gastroenterology and Hepatology*, 21(4): 940-948.

Kumar, A., Gupta, R., Mathur, N., Iyer, V.K., Thulkar, S., Prasad, C.P., Das, P., Rani, L., Maqbool, M., Shukla, N.K. and Pal, S. (2020). Microarray based gene expression profiling of advanced gall bladder cancer. *Experimental Oncology*, 42(4): 277-284.

Li, M. and Zhang, P. (2009). The function of APC / C Cdh1 in cell cycle and beyond. *Cell Division*, 4: 1-7.

Li, M., Li, D., Tang, Y., Wu, F., Wang, J. (2017). CytoCluster: a cytoscape plugin for cluster analysis and visualization of biological networks. *International Journal of Molecular Sciences*, 18(9): 1880.

Liao, H., Ji, F., Geng, X., Xing, M., Li, W., Chen, Z., Shen, H. and Ying, S. (2017). CDK1 promotes nascent DNA synthesis and induces resistance of cancer cells to DNA-damaging therapeutic agents. *Oncotarget*, 8(53): 90662.

Liu, J., Lin, B., Chen, Z., Deng, M., Wang, Y., Wang, J., Chen, L., Zhang, Z., Xiao, X., Chen, C. and Song, Y. (2020). Identification of key pathways and genes in nonalcoholic fatty liver disease using bioinformatics analysis. *Archives of Medical Science*, 16(2): 374-85.

Lyu, W.J., Shu, Y.J., Liu, Y.B. and Dong, P. (2020). Topoisomerase II alpha promotes gallbladder cancer proliferation and metastasis through activating phosphatidylinositol 3-kinase / protein kinase B / mammalian target of rapamycin signaling pathway. *Chinese Medical Journal*, 133(19), 2321-9.

Majeed, A. and Mukhtar, S. (2023). Protein–protein interaction network exploration using cytoscape. In *Protein-Protein Interactions: Methods and Protocols,* 419-427. New York, NY: Springer US.

Mantovani, A., Petracca, G., Beatrice, G., Csermely, A., Tilg, H., Byrne, C.D. and Targher, G. (2022). Non-alcoholic fatty liver disease and increased risk of incident extrahepatic cancers: A meta-analysis of observational cohort studies. *Gut*, 71(4): 778-788.

McDermaid, A., Monier, B., Zhao, J., Liu, B. and Ma, Q. (2019). Interpretation of differential gene expression results of RNA-seq data: review and integration. *Briefings in Bioinformatics*, 20(6): 2044-54.

Menendez, J.A., Vellon, L. and Lupu, R. (2006). DNA topoisomerase IIá (TOP2A) inhibitors up-regulate fatty acid synthase gene expression in SK-Br3 breast cancer cells: In vitro evidence for a L / P 'functional amplicon' involving FAS, Her-2 / neu and TOP2A genes. *International Journal of Molecular Medicine*, 18(6): 1081-1087.

Nogueira, J.P. and Cusi, K. (2024). Role of insulin resistance in the development of nonalcoholic fatty liver disease in people with type 2 diabetes: from bench to patient care. *Diabetes Spectrum*, 37(1): 20-28.

Oliveros, J.C. Venny. (2007). An interactive tool for comparing lists with Venn Diagrams. http: / / bioinfogp. cnb. csic. es / tools / venny / index. html

Pagano, M., Pepperkok, R., Verde, F., Ansorge, W. and Draetta, G. (1992). Cyclin A is required at two points in the human cell cycle. *The EMBO Journal*, 11(3): 961-71.

Park, J.H., Hong, J.Y., Kwon, M., Lee, J., Han, K., Han, I.W., Kang, W. and Park, J.K. (2021). Association between non-alcoholic fatty liver disease and the risk of biliary tract cancers: A South Korean nationwide cohort study. *European Journal of Cancer*, 150: 73-82.

Riazi, K., Azhari. H., Charette. J.H., Underwood, F.E., King, J.A., Afshar, E.E., Swain, M.G., Congly, S.E., Kaplan, G.G., Shaheen, A.A. (2022). The prevalence and incidence of NAFLD worldwide: A systematic review and meta-analysis. *The Lancet Gastroenterology & Hepatology*, 7(9): 851-861.

Ribbeck, K., Groen, A.C., Santarella, R., Bohnsack, M.T., Raemaekers, T., Köcher, T., Gentzel, M., Görlich, D., Wilm, M., Carmeliet, G. and Mitchison, T.J. (2006). NuSAP, a mitotic RanGTP target that stabilizes and cross-links microtubules. *Molecular Biology of the Cell*, 17(6): 2646-60.

Ryu, S., Chang, Y., Yun, K.E., Jung, H.S., Shin, J.H. and Shin, H. (2016). Gallstones and the risk of gallbladder cancer mortality: a cohort study. *Official journal of the American College of Gastroenterology | ACG*, 111(10): 1476-1487.

Salman, T., Argon, A., Kebat, T., Vardar, E., Erkan, N. and , A. (2016). The prognostic significance of survivin expression in gallbladder carcinoma. *Apmis*, 124(8): 633-638.

Sharma, S., Ghufran, S.M., Das, B., Roy, B., Ghose, S. and Biswas, S. (2021). Survivin expression is essential for early activation of hepatic stellate cells and fibrosis progression in chronic liver injury. *Life Sciences*, 287: 120119.

Slouha, E., Biput, S.J., Kalloo, A.E. and Gorantla, V.R. (2023). Non-alcoholic fatty liver disease and gallstones: a systematic review. *Cureus*, 15(9).

Sun, Q., An, R., Li, J., Liu, C., Wang, M., Wang, C. and Wang, Y. (2023). The role of CXCL8 and CCNB1 in predicting hepatocellular carcinoma in the context of cirrhosis: implications for early detection and immune-based therapies. *Journal of Cancer Research and Clinical Oncology*, 149(13): 11471-11489.

Suppli, M.P., Rigbolt, K.T., Veidal, S.S., Heebøll, S., Eriksen, P.L., Demant, M., Bagger, J.I., Nielsen, J.C., Oró, D., Thrane, S.W. and Lund, A. (2019). Hepatic transcriptome signatures in patients with varying degrees of nonalcoholic fatty liver disease compared with healthy normal-weight individuals. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 316(4): G462-472.

Szklarczyk, D., Kirsch, R., Koutrouli, M., Nastou, K., Mehryary, F., Hachilif, R., Gable, A.L., Fang, T., Doncheva, N.T., Pyysalo, S. and Bork, P. (2023). The STRING database in 2023: Protein–protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic Acids Research*, 51(D1): D638-646.

Wang, G., Chen, H., Huang, M., Wang, N., Zhang, J., Zhang, Y., Bai, G., Fong, W.F., Yang, M. and Yao, X. (2006). Methyl protodioscin induces G2 / M cell cycle arrest and apoptosis in HepG2 liver cancer cells. *Cancer Letters*, 241(1): 102-109.

Wencong, M., Jinghan, W., Yong, Y., Jianyang, A., Bin, L., Qingbao, C., Chen, L. and Xiaoqing, J. (2020). FOXK1 promotes proliferation and metastasis of gallbladder cancer by activating AKT / mTOR signaling pathway. *Frontiers in Oncology*, 10: 545.

Wheatley, S.P. and Altieri, D.C. (2019). Survivin at a glance. *Journal of Cell Science*, 132(7): jcs223826.

Wyles, J.P., Wu, Z., Mirski, S.E. and Cole, S.P. (2007). Nuclear interactions of topoisomerase II á and â with phospholipid scramblase 1. *Nucleic Acids Research*, 35(12): 4076-4085.

Xian, F, Zhao, C., Huang, C., Bie, J. and Xu, G. (2023). The potential role of CDC20 in tumorigenesis, cancer progression and therapy: A narrative review. *Medicine*, 102(36): e35038.

Xu, S., Zhan, M., Jiang, C., He, M., Yang, L., Shen, H., Huang, S., Huang, X., Lin, R., Shi, Y. and Liu, Q. (2019). Genome-wide CRISPR screen identifies ELP5 as a determinant of gemcitabine sensitivity in gallbladder cancer. *Nature Communications*, 10(1): 5492.

Yamamura, M., Sato, Y., Takahashi, K., Sasaki, M. and Harada, K. (2020). The cyclin dependent kinase pathway involving CDK1 is a potential therapeutic target for cholangiocarcinoma. *Oncology Reports*, 43(1): 306-317.

Yan, Y., Zhao, L., Su, H., Li, G. and Jiang, S. (2020). Identification of Hub Genes and Pathways With Immune Cell Infiltration in Cholangiocarcinoma by Weighted Gene Co-expression Network Analysis.

Yang, Y., Ge, F., Qin, S., Luo, C., Xiao, X., Bai, Z. and Tang, C. (2023). Identification the shared pathogenesis between chronic hepatitis B and non-alcoholic fatty liver disease: Evidence from transcriptome data. *Gastroenterology & Endoscopy*, 1(4): 190-198.

Zeng, T., Chen, G., Qiao, X., Chen, H., Sun, L., Ma, Q., Li, N., Wang, J., Dai, C. and Xu, F. (2022). NUSAP1 could be a potential target for preventing NAFLD progression to liver cancer. *Frontiers in Pharmacology*, 13: 823140.

Zhou, H., Chen, J., Jin, H. and Liu, K. (2023). Genetic characteristics and clinical-specific survival prediction in elderly patients with gallbladder cancer: a genetic and population-based study. *Frontiers in Endocrinology*, 14: 1159235.

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