Abdul Lajis K and Zulkifle Z

Department of Biomedical Sciences, Advanced Medical and Dental Institute, Universiti Sains Malaysia, Bertam, 13200 Kepala Batas, Pulau Pinang, Malaysia.

*Corresponding author
Nurulisa Zulkifle
nurulisa@usm.my

Received 14th August 2024
Revised 20th November 2024
Accepted 31st December 2024
Published Online 10th Jan 2024

Expression and Interaction of OTU Deubiquitinases in Hepatocellular Carcinoma

Abstract **–** Deubiquitinases (DUBs) play crucial roles in cancer progression by regulating protein ubiquitination, yet their involvement in hepatocellular carcinoma (HCC) remains underexplored. This study investigates the expression and interactions of OTU (Ovarian Tumor) DUBs in HCC to evaluate their potential as diagnostic, prognostic, and therapeutic markers. Gene Expression Omnibus (GEO) microarray datasets were analyzed using GEO2R to compare gene expression profiles between HCC and normal liver tissues. Differentially expressed genes (DEGs) were identified based on stringent criteria of log fold change (logFC) > 2.0 and adjusted P-value < 0.05. The expression profiles of 16 OTU DUBs were examined, and a protein-protein interaction (PPI) network was constructed incorporating overlapping DEGs and selected OTU DUBs. Kaplan-Meier (KM) survival analysis was performed using patient data from The Cancer Genome Atlas (TCGA) to assess prognostic significance. The analysis identified *OTULIN* as the only OTU DUB meeting DEG criteria, showing significant downregulation. *OTUB1*, *OTUB2*, and *OTUD6B* exhibited near-threshold expression changes and were included for further analysis due to their potential relevance. The PPI network revealed OTUB1 and OTUB2 as integral components of the HCC interactome, connecting to key regulators such as FOXM1 and ERα, respectively. KM survival analysis demonstrated that high expression of OTUB1 and OTUB2 correlates with poorer overall survival, whereas OTULIN and OTUD6B showed no significant impact. These findings highlight the potential roles of OTUB1 and OTUB2 in modulating HCC-related pathways, positioning them as promising candidates for therapeutic exploration, despite their limitations as diagnostic or prognostic marker.

Keywords **–** *Hepatocellular Carcinoma, OTU Deubiquitinases, Biomarkers, Differentially Expressed Genes, Protein-Protein Interaction Network*

1 INTRODUCTION

Hepatocellular carcinoma (HCC) is the most prevalent form of primary liver cancer and represents a major global health challenge [1]. According to the 2020 World Health Organization report, HCC is the second leading cause of cancer-related mortality worldwide, with a rising incidence attributed to chronic liver diseases such as hepatitis B virus (HBV) and hepatitis C virus (HCV) infections, as well as non-alcoholic steatohepatitis (NASH) and alcohol-related liver damage [2]. A significant proportion of HCC cases are diagnosed at advanced stages, limiting therapeutic options and contributing to poor prognosis and high mortality rates [3]. Despite advancements in systemic therapies, including immune checkpoint inhibitors and molecularly targeted agents, the survival outcomes for HCC remain suboptimal [4]. Current molecular markers, such as alpha-fetoprotein (AFP) and des-gamma carboxyprothrombin (DCP), offer limited sensitivity and specificity, particularly for early-stage disease [5].

Ubiquitination is a dynamic post-translational modification process that regulates diverse cellular functions, including protein degradation, signal transduction, and cell cycle progression. Its reversal, mediated by deubiquitinases (DUBs), is equally crucial for maintaining cellular homeostasis [6,7]. The human genome encodes approximately 100 DUB genes, categorized into six cysteine proteases families: ubiquitin carboxyl-terminal hydrolase (UCH), ubiquitin-specific

protease (USP), ovarian tumor protease (OTU), Josephin, motif interacting with ubiquitin-containing novel DUB family (MINDY) and zinc finger with UFM1-specific peptidase domain-containing protein (ZUFSP), alongside the JAB1/MPN/MOV34 metalloenzyme (JAMM) family, a group of metalloproteases [8].

Mounting evidence suggests that alterations in ubiquitination components, especially E3 ligases and DUBs, are significantly linked to cancer development [9]. While extensively studied DUB families such as USP and UCH have revealed numerous cancer-related roles, the OTU DUB family remains relatively underexplored, particularly in HCC. However, accumulating evidence underscores their involvement in cancer progression, making them promising candidates for therapeutic and diagnostic research. The OTU DUB family consists of 16 members, including A20 (TNFAIP3), OTUB1, OTUB2, OTULIN, OTUD3, OTUD5, OTUD6B, and Cezanne (OTUD7B), each with unique roles in cellular signaling and cancer biology [10]. A20, one of the most extensively studied, functions as both a tumor suppressor and oncogene depending on the cancer type. In HCC, A20 primarily exhibits tumor-suppressive properties by negatively regulating NF-κB signaling [11]. OTUB1 stabilizes oncogenic proteins like p53 and FOXM1, promoting tumor growth, while OTUB2 supports cancer progression through the stabilization of E3 ligases and suppression of tumor-suppressive pathways [12-14]. OTULIN, a key regulator of linear ubiquitin chain assembly, is involved in maintaining immune homeostasis and preventing inflammation, with its dysregulation linked to tumorigenesis [15]. Cezanne is implicated in the degradation of NF-κB pathway components, exhibiting tumor-suppressive properties in various cancers [16]. Similarly, OTUD6B is associated with transcriptional regulation and long non-coding (lnc)RNA-mediated oncogenesis in HCC [17].

This study aims to address the gap in OTU DUBs characterization by investigating their expression, interaction networks, and prognostic significance in HCC. By integrating differential gene expression analysis, protein-protein interaction (PPI) networks, and survival data, we aim to elucidate their potential as diagnostic and prognostic markers, as well as therapeutic targets.

2 MATERIALS AND METHODS

2.1 Selection of Microarray Datasets

The keyword 'hepatocellular carcinoma' was used to search the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo). The search was restricted to datasets derived from *Homo sapiens*, focusing on expression profiling by array and tissue samples. The inclusion criteria were as follows: (1) datasets must contain matched pairs of HCC and adjacent noncancerous tissue samples to ensure comparability and minimize inter-sample variability; and (2) datasets must consist of mRNA expression profiles, as these are essential for identifying transcriptional changes associated with HCC.

2.2 Gene Expression Analysis

Differential gene expression between HCC and adjacent noncancerous tissues was analyzed using GEO2R [18], an online tool designed for analyzing gene expression data in the GEO database. The Benjamini-Hochberg false discovery rate method was applied to control for multiple testing. Genes with a log fold change (logFC) > 2.0 and an adjusted (adj.) P-value < 0.05 were considered differentially expressed. Overlapping differentially expressed genes (DEGs) across datasets were identified using a Venn diagram analysis tool from Bioinformatics & Evolutionary Genomics (http://bioinformatics.psb.ugent.be/webtools/Venn/). Expression data for OTU DUBs from the GEO2R analysis were extracted and tabulated in Microsoft Excel, regardless of whether they met the differential expression criteria.

2.3 Construction of PPI Network and Cluster Analysis

Interactions between DEGs and OTU DUBs were retrieved from the Search Tool for the Retrieval of Interacting Genes (STRING) database using a confidence score threshold > 0.7, ensuring high confidence interactions [19]. The PPI network was visualized and analyzed using Cytoscape v3.8.2 [20]. Key genes were identified based on node degree, which quantifies the number of connections a gene has within the network, reflecting its centrality and potential regulatory importance. Highly connected clusters within the network were identified using the Molecular Complex Detection (MCODE) plug-in, which prioritizes clusters based on their density of interactions, providing insights into potential protein complexes or pathway components [21].

2.4 Assessing DEGs and DUBs Prognostic Value

Kaplan-Meier (KM) plotter (https://kmplot.com/analysis/) was employed to evaluate the prognostic significance of key genes and selected OTU DUBs in HCC [22]. Data from 364 patients in The Cancer Genome Atlas (TCGA) database were used for this analysis, with survival differences assessed using the log-rank test (P-value < 0.05 considered significant) [23]. Genetic alterations in these genes were further analyzed using clinical data from 369 patients in the TCGA PanCancer Atlas, accessed via the c-BioPortal platform (https://www.cbioportal.org/) [24].

3 RESULTS

3.1 Dataset Selection and Gene Expression Analysis

A search in the GEO database conducted in April 2021 yields 40,241 entries related to HCC. Of these, 22 datasets meet the inclusion criteria and are selected for further investigation: GSE166163, GSE101728, GSE84402, GSE84598, GSE76297, GSE84005, GSE76427, GSE64041, GSE57957, GSE39791, GSE46408, GSE33006, GSE31370, GSE36376, GSE25097, GSE29722, GSE14520, GSE19665, GSE65372, GSE60502, GSE12941 and GSE22058. After applying the GEO2R for differential expression analysis, two datasets (GSE166163 and GSE33006) are excluded as they did not yield genes meeting the differential expression criteria. From the remaining 20 datasets, a pattern of overlapping DEGs is observed. DEGs present in at least six datasets are prioritized, resulting in a compilation of 260 DEGs, consisting of 185 downregulated and 75 upregulated genes. The selection ensures robust identification of genes relevant to HCC. These results align with previously reported datasets [25], supporting the study's methodologies and reinforcing the consistency of observed DEGs.

3.2 Expression of OTU DUBs

Expression profiles for 16 members are extracted and only *OTULIN* meets the cut-off, showing significant downregulation with logFC=3.31 and adj. P-value=0.01. Nevertheless, *OTUB1* (logFC=1.89, adj. P-value=1.92×10^{5}), *OTUB2* (logFC=1.91, adj. P-value=1.71×10^{2}) and *OTUD6B* (logFC=1.43, adj. P-value=0.06) exhibit near-threshold upregulation and are included for further analysis due to their potential biological relevance.

3.3 PPI Network Analysis

The analysis of interactions between 185 downregulated DEGs, 75 upregulated DEGs, and four selected OTU DUBs generates a PPI network comprising 259 nodes and 1,429 edges. Within this network, 79 isolated nodes, including OTULIN, 9 upregulated and 70 downregulated DEGs, are identified as non-interacting (Figure 1). A notable feature of the network is a dense hub comprising 48 upregulated nodes and 1,079 edges. Among these, CDC20 emerges as the node with the highest degree, followed by CCNA2, BUB1, CDK1, TOP2A, and other key regulators such as CENPF, KIF20A, AURKA, DLGAP5, CDCA8, TPX2, CCNB2, RRM2, ASPM, TTK, KIF23, PBK, and MELK, each engaging with over 50 partners. OTUB1 is directly connected to both the upregulated FOXM1, a critical component of the dense hub, and the downregulated Erα (ESR1), located outside the hub. Similarly, OTUB2 is also connected to ERα and indirectly linked to the dense hub through this interaction, indicating its potential regulatory influence on the network. Further analysis using the MCODE plugin identifies six densely connected clusters representing potential protein complexes and pathway components. However, none of these clusters include the selected OTU DUBs, and the data are not shown as they do not involve the proteins of interest.

3.4 Survival and Genetic Alteration Analysis

Kaplan-Meier (KM) survival analysis evaluates the prognostic significance of selected genes using data from 364 HCC patients in the TCGA database. Survival analysis is performed for overall survival (OS), which measures the time from diagnosis to death or censoring, defined as the last follow-up date for patients still alive. The overall cumulative survival rate in these plots spans up to 120 months (10 years), with events including death due to any cause. Figure 2 demonstrates that patients in the high expression group for the top five key DEGs (*CDC20*, *CCNA2*, *BUB1*, *CDK1*, and *TOP2A*) exhibit significantly reduced overall survival compared to those in the low-expression group for these genes (all log-rank P-value < 0.05).

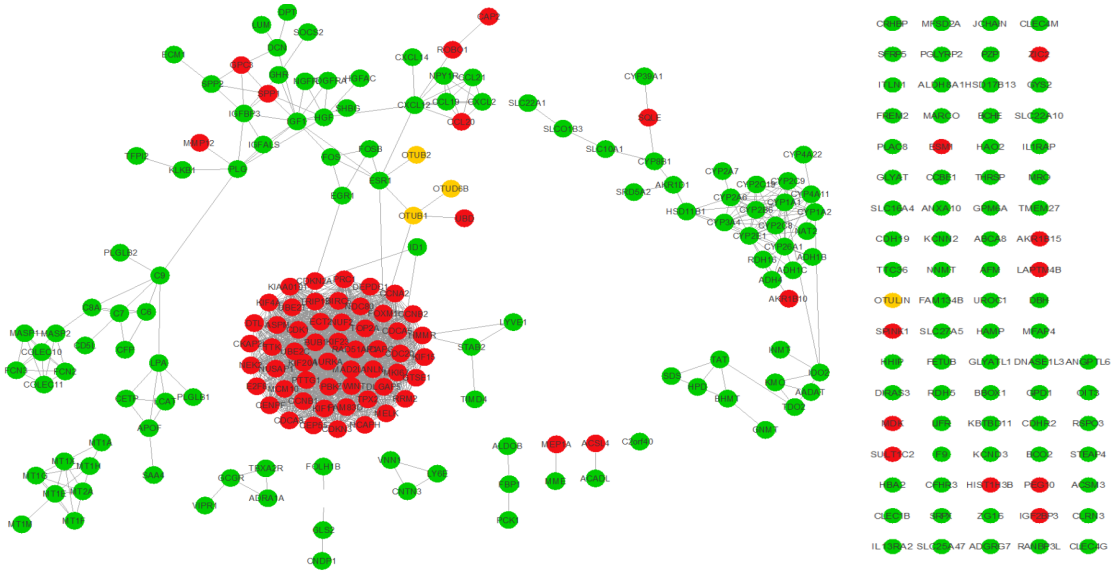

Figure 1. PPI network depicting the interactions among the DEGs and four selected OTU DUBs. Green nodes stand in for downregulated DEGs, red nodes for upregulated DEGs, and yellow nodes for the selected OTU DUBs

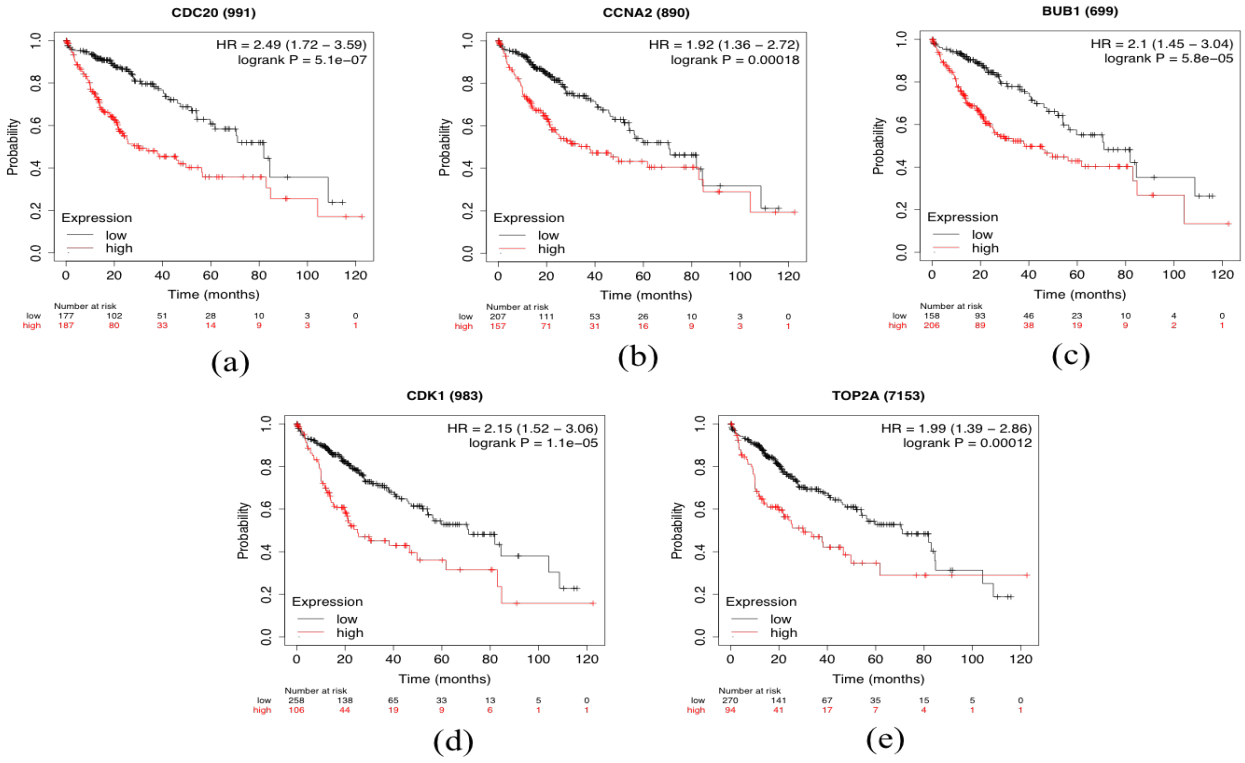

Figure 2. KM survival curves illustrating that the high expression group is associated with worse overall survival compared to the low expression group for (a) *CDC20*, (b) *CCNA2*, (c) *BUB1*, (d) *CDK1* and (e) *TOP2A*

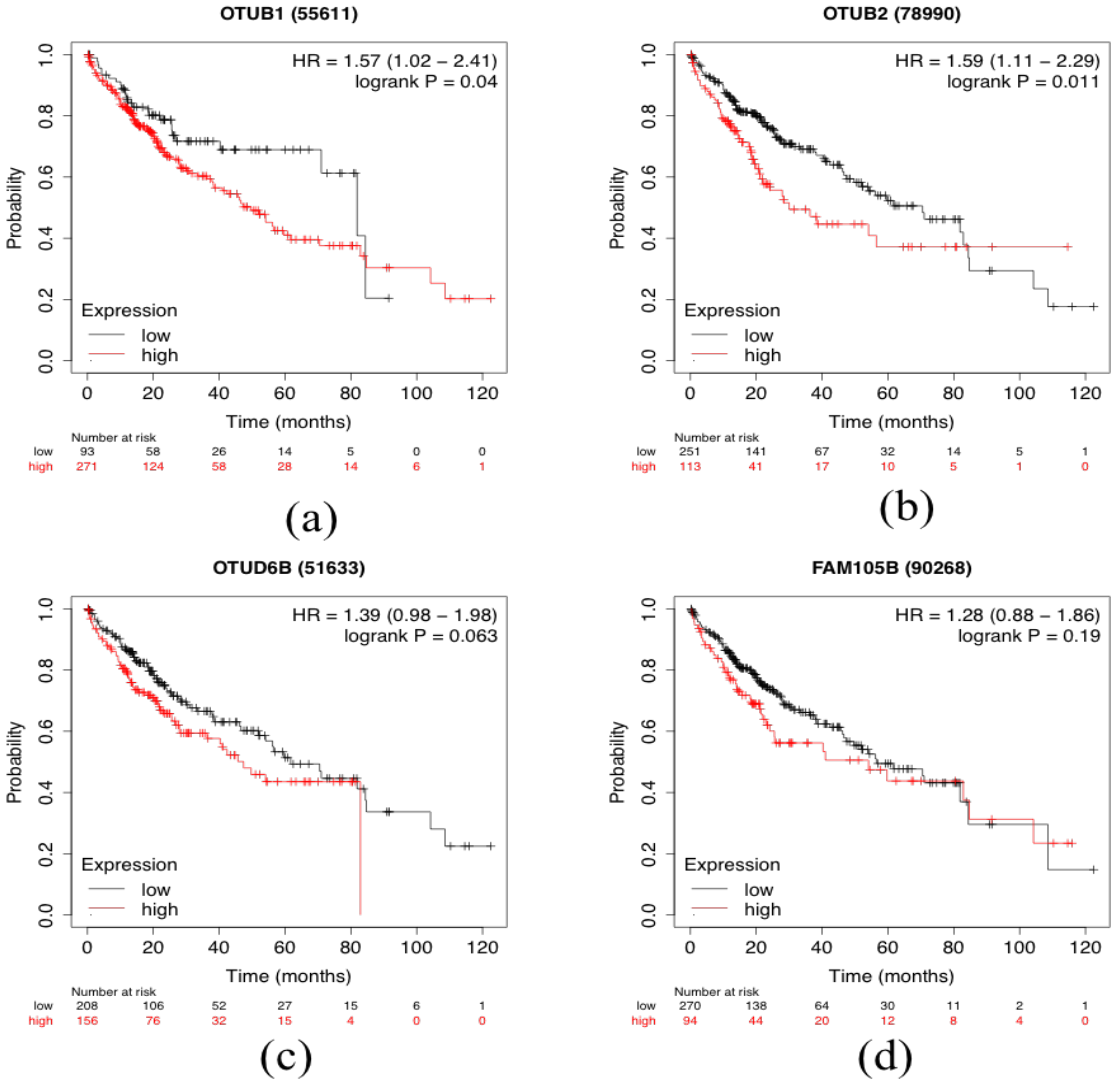

Figure 3. KM survival curves illustrating that the high expression group is associated with worse overall survival compared to the low expression group for (a) *OTUB1* and (b) *OTUB2*. In contrast, the survival curves demonstrate no significant differences between high and low expression group for (c) *OTUD6B* and (d) *FAM105B/OTULIN*

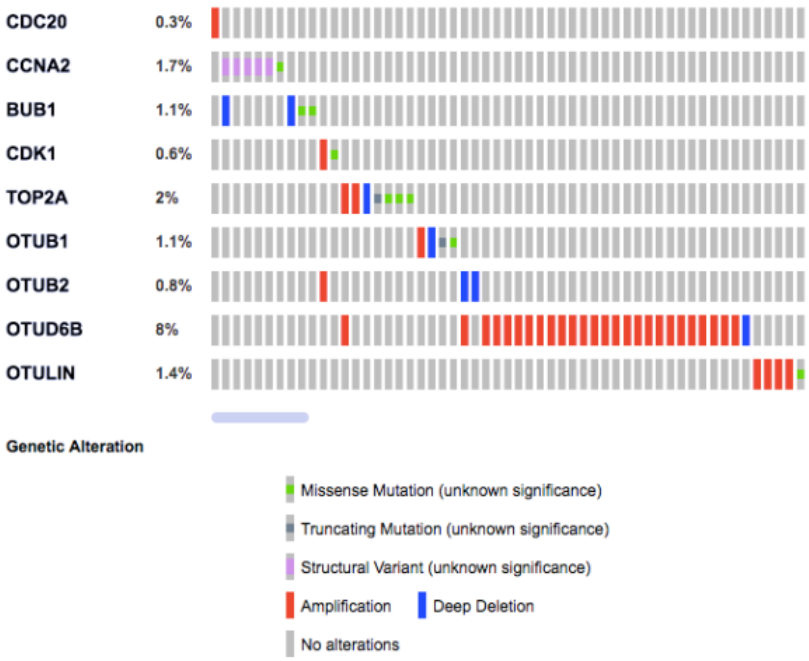

Figure 4. The most frequently altered genes in HCC sample are *OTUD6B* (8%), followed by *TOP2A* (2%), *CCNA2* (1.7%), *OTULIN* (1.4%), *BUB1* (1.1%), *OTUB1* (1.1%), *OTUB2* (0.8%), *CDK1* (0.6%) and *CDC20* (0.3%)

Similarly, patients in the high-expression group for *OTUB1* and *OTUB2* demonstrate a worse overall prognosis, reflected by shorter overall survival (all log-rank P-value < 0.05). In contrast, no statistically significant differences in overall survival were observed between high- and low-expression groups for *OTUD6B* and *OTULIN* (also known as *FAM105B*) (all log-rank P-value > 0.05) (Figure 3). Additionally, genetic alterations in the PPI network and selected OTU DUBs are analyzed using clinical data from 369 patients in the TCGA PanCancer Atlas via the cBioPortal platform. The overall genetic alteration rate was identified as 15.71%, with *OTUD6B* exhibited the highest alteration frequency at 8%, followed by *TOP2A* at 2% and *CCNA2* at 1.7% (Figure 4). These findings highlight the potential genetic instability associated with key DEGs and OTU DUBs in HCC, emphasizing their relevance in tumor progression and prognostic evaluation.

4 DISCUSSION

4.1 Expression of OTU DUBs in Cancer

Overall, the expression levels of OTU DUBs are generally low in HCC, as indicated by their relatively modest logFC values. This observation may reflect their regulatory roles rather than primary involvement in direct gene expression changes. As members of the OTU DUB family, they primarily regulate post-translational modifications, which influences protein stability and signaling cascades rather than directly impacting transcriptional activity. This is consistent with the roles of many DUBs, which act as modulators rather than primary drivers of gene expression. Despite these modest logFC values, the functional significance of OTUB1 and OTUB2 is evident from their associations with key regulators like FOXM1 and ERα in the PPI network and their correlation with poor overall survival, as revealed by KM analysis. These findings underscore the importance of focusing on functional impacts rather than expression magnitude when evaluating DUBs as therapeutic targets in HCC [26].

4.2 Role of OTULIN, OTUBs and OTUD6B in HCC

OTULIN emerges as the only OTU DUB meeting differential expression criteria, with significant downregulation observed in HCC tissues. Known for its role in regulating linear ubiquitin chains,

OTULIN functions within the linear ubiquitin assembly complex (LUBAC) to maintain immune homeostasis and prevent tumor necrosis factor (TNF)-induced apoptosis [15]. Its downregulation in HCC suggests a loss of protective mechanisms, potentially exacerbating inflammation and tumorigenesis. Despite its biological importance, OTULIN appears as an isolated node in the PPI network, reflecting the limited understanding of its molecular interactions in liver cancer. Future studies should focus on proteomic profiling and experimental validation to clarify OTULIN's role in HCC pathogenesis.

Although OTUB1 and OTUB2 fail to meet strict DEG criteria, their near-threshold upregulation and connections in the PPI network underscore their functional relevance in HCC. OTUB1 directly connects to FOXM1, a key oncogenic transcription factor in the PPI network, implicating its role in driving cell cycle regulation and tumor proliferation. FOXM1 is a known oncogenic driver in HCC, controlling downstream effectors such as KIF4A and miR-34a [27,28]. In addition to FOXM1, OTUB1 also connects to ERα, a transcription factor known to suppress oncogenic pathways, including Wnt/β-catenin and YAP signaling [29,30]. This dual connection highlights OTUB1's potential role in stabilizing both oncogenic and tumor-suppressive regulators within the HCC interactome, adding complexity to its role in cancer progression. Similarly, OTUB2 interacts with ERα and indirectly links to the dense hub through this interaction. While the reduced expression of ESR1 in HCC likely diminishes its tumor-suppressive effects, OTUB2's stabilization of proteins such as PJA1 may counteract this by promoting metastasis and proliferation [14]. These interactions position OTUB1 and OTUB2 as critical modulators within both oncogenic and tumor-suppressive signaling cascades, reinforcing their candidacy for targeted therapy.

The marginal upregulation of OTUD6B, paired with its high alteration frequency (8%), suggests a significant role in HCC. Elevated levels of OTUD6B-AS1, a tumor-associated lncRNA, are associated with poor patient outcomes, implicating OTUD6B in the regulation of transcriptional networks that promote proliferation and invasiveness [17]. Although the exact molecular mechanisms remain unclear, its link to apoptotic and growth pathways warrants further exploration. Targeting lncRNA-regulated networks may offer novel therapeutic opportunities.

4.3 Diagnostic, Prognostic and Therapeutic Potential of OTUB1 and OTUB2

The diagnostic utility of OTUB1 and OTUB2 in HCC is limited by their low logFC values, making them less effective as standalone biomarkers. However, their inclusion in multi-marker panels alongside established diagnostic markers like AFP or glypican-3 could enhance diagnostic sensitivity and specificity, particularly in distinguishing HCC from non-cancerous liver conditions. Prognostically, their significant association with poor overall survival supports their use in predicting patient outcomes and stratifying patients for clinical trials. Therapeutically, OTUB1 and OTUB2 represent promising targets due to their pivotal roles in stabilizing key oncogenic regulators, including FOXM1 and ERα. By targeting these interactions, novel inhibitors could disrupt the signaling pathways that drive tumor proliferation and metastasis. Furthermore, combining such inhibitors with existing treatments, such as immune checkpoint inhibitors or molecularly targeted therapies, may result in synergistic effects, improving overall treatment efficacy. Thus, OTUB1 and OTUB2 emerge as multifaceted targets in HCC, offering promise in diagnostics, prognostics, and therapeutic interventions.

4.4 Future Directions

While this study highlights the relevance of OTU DUBs in HCC, it also highlights the need for further research. The reliance on transcriptomic and STRING-based interaction data limits the contextual specificity of findings. Advanced techniques such as single-cell RNA sequencing, spatial transcriptomics, and co-immunoprecipitation assays are essential to uncover cell-type-specific and interaction-specific roles of OTU DUBs. Furthermore, exploring the regulatory roles of lncRNAs, particularly those associated with OTU DUBs, may uncover novel mechanisms driving liver cancer progression.

5 CONCLUSION

This study successfully met its main and specific objectives through comprehensive bioinformatics analyses. Key genes such as *CDC20*, *CCNA2*, *CDK1*, *BUB1*, and *TOP2A* were identified, supporting our existing understanding of the molecular mechanisms underlying HCC development and progression.

The study also shed light on the role of OTU DUBs, specifically OTUB1, OTUD6B, and OTULIN, in the diagnosis, prognosis, and potential treatment of HCC. The relatively low logFC values of these genes pose challenges for using them as reliable standalone markers but in the PPI network, OTUB1 and OTUB2 are strategically positioned to modulate the HCC interactome through their interactions with FOXM1 and ERα, suggesting their potential in therapeutic interventions. The current study highlights the need for further research on OTU DUBs, particularly given their promising yet underexplored role in HCC. The upregulation of *OTUB1*, *OTUB2*, and *OTUD6B*, along with the downregulation of *OTULIN*, merits deeper investigation to unravel the complex molecular mechanisms of HCC.

Availability of Data and Material

The datasets generated and analyzed during the current study are available from the corresponding author on request.

Competing Interests

The authors declare that they have no competing interests.

Funding

This work was supported by Universiti Sains Malaysia (USM) through the RUI Grant (1001.CIPPT.8012340).

Authors' Contributions

NZ: Conceptualization, Funding acquisition, Supervision, Writing - review & editing

KAL: Data curation, Investigation, Formal analysis, Writing - original draft

Abbreviations / Gene Symbols

A20: TNF alpha-induced protein 3 (TNFAIP3)
ASPM: Assembly factor for spindle microtubules
AURKA: Aurora kinase A
BUB1: Mitotic checkpoint serine/threonine-protein kinase BUB1
CCNA2: Cyclin-A2
CCNB2: Cyclin-B2
CDC20: Cell division cycle 20
CDCA8: Cell division cycle associated 8
CDK1: Cyclin-dependent kinase 1
CENPF: Centromere protein F
DLGAP5: Disks large-associated protein 5
ESR1: Estrogen receptor 1
FOXM1: Forkhead box protein M1
GAS6-AS2: GAS6 antisense RNA 2
KIF: Kinesin family member
MELK: Maternal embryonic leucine zipper kinase
NF-κB: Nuclear factor-kappa B
OTUB1: OTU deubiquitinase B1
OTUB2: OTU deubiquitinase B2
OTUD6B: OTU domain-containing protein 6B
OTUD7B: OTU domain-containing protein 6B
OTULIN: OTU domain-containing deubiquitinase with linear linkage specificity
p53: Tumor protein p53
PBK: PDZ-binding kinase
PJA1: Praja ring finger ubiquitin ligase 1RIPK: Receptor-interacting serine/threonine-protein kinase
RRM2: Ribonucleoside reductase regulatory subunit M2
TNF: Tumor necrosis factor
TOP2A: DNA topoisomerase 2-alpha
TPX2: TPX2 microtubule nucleation factor
TTK: TTK protein kinase
Wnt: Wingless-related integration site
YAP: Yes-associated protein

REFERENCES

[1] Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, & Bray F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: a cancer journal for clinicians*. 2021;**71**(3):209–249. https://doi.org/10.3322/caac.21660

[2] Llovet JM, Kelley RK, Villanueva A, Singal AG, Pikarsky E, Roayaie S, Lencioni R, Koike K, Zucman-Rossi J, & Finn RS. Hepatocellular carcinoma. *Nature reviews. Disease primers*. 2021;**7**(1):6. https://doi.org/10.1038/s41572-020-00240-3

[3] Marrero JA, Kulik LM, Sirlin CB, Zhu AX, Finn RS, Abecassis MM, Roberts LR, & Heimbach JK. Diagnosis, Staging, and Management of Hepatocellular Carcinoma: 2018 Practice Guidance by the American Association for the Study of Liver Diseases. *Hepatology (Baltimore, Md.)*. 2018;**68**(2):723–750. https://doi.org/10.1002/hep.29913

[4] Villanueva A. Hepatocellular Carcinoma. *The New England journal of medicine*. 2019;**380**(15):1450–1462. https://doi.org/10.1056/NEJMra1713263

[5] Tzartzeva K, Obi J, Rich NE, Parikh ND, Marrero JA, Yopp A, Waljee AK, & Singal AG. Surveillance Imaging and Alpha Fetoprotein for Early Detection of Hepatocellular Carcinoma in Patients With Cirrhosis: A Meta-analysis. *Gastroenterology*. 2018;**154**(6):1706–1718.e1. https://doi.org/10.1053/j.gastro.2018.01.064

[6] Komander D & Rape M. The ubiquitin code. *Annual review of biochemistry*. 2012;**81**:203–229. https://doi.org/10.1146/annurev-biochem-060310-170328

[7] Swatek KN & Komander D. Ubiquitin modifications. *Cell research*. 2016;**26**(4):399–422. https://doi.org/10.1038/cr.2016.39
[8] Nijman SM, Luna-Vargas MP, Velds A, Brummelkamp TR, Dirac AM, Sixma TK & Bernards R. A genomic and functional inventory of deubiquitinating enzymes. *Cell*. 2005;**123**(5):773–786. https://doi.org/10.1016/j.cell.2005.11.007
[9] Popovic D, Vucic D & Dikic I. Ubiquitination in disease pathogenesis and treatment. *Nature medicine*. 2014;**20**(11):1242–1253. https://doi.org/10.1038/nm.3739
[10] Du J, Fu L, Sui Y, et al. The function and regulation of OTU deubiquitinases. Front. Med. 2020;14:542–563 (2020). https://doi.org/10.1007/s11684-019-0734-4
[11] Shi Y, Wang X, Wang J, Wang X, Zhou H & Zhang L. The dual roles of A20 in cancer. *Cancer Letters*. 2021;**511**:26–35. https://doi.org/10.1016/j.canlet.2021.04.017
[12] Sun XX, Challagundla KB & Dai MS. Positive regulation of p53 stability and activity by the deubiquitinating enzyme Otubain 1. *The EMBO Journal*. 2012;**31**(3):576–592. https://doi.org/10.1038/emboj.2011.434
[13] Zhou K, Mai H, Zheng S, Cai W, Yang X, Chen Z & Zhan B. OTUB1-mediated deubiquitination of FOXM1 up-regulates ECT-2 to promote tumor progression in renal cell carcinoma. *Cell & bioscience*. 2020;**10**:50. https://doi.org/10.1186/s13578-020-00408-0
[14] Hu G, Yang J, Zhang H, Huang Z & Yang H. OTUB2 Promotes Proliferation and Migration of Hepatocellular Carcinoma Cells by PJA1 Deubiquitylation. *Cellular and Molecular Bioengineering*. 2022;**15**(3):281–292. https://doi.org/10.1007/s12195-022-00720-4
[15] Keusekotten K, Elliott PR, Glockner L, Fiil BK, Damgaard RB, Kulathu Y, Wauer T, Hospenthal MK, Gyrd-Hansen M, Krappmann D, Hofmann K & Komander D. OTULIN antagonizes LUBAC signaling by specifically hydrolyzing Met1-linked polyubiquitin. *Cell*. 2013;**153**(6):1312–1326. https://doi.org/10.1016/j.cell.2013.05.014
[16] Enesa K, Zakkar M, Chaudhury H, Luong leA, Rawlinson L, Mason JC, Haskard DO, Dean JL & Evans PC. NF-kappaB suppression by the deubiquitinating enzyme Cezanne: a novel negative feedback loop in pro-inflammatory signaling. *The Journal of biological chemistry*. 2008;**283**(11):7036–7045. https://doi.org/10.1074/jbc.M708690200
[17] Kong S, Xue H, Li Y, Li P, Ma F, Liu M & Li W. The long noncoding RNA OTUD6B-AS1 enhances cell proliferation and the invasion of hepatocellular carcinoma cells through modulating GSKIP/Wnt/β-catenin signalling via the sequestration of miR-664b-3p. *Experimental Cell Research*. 2020;**395**(1):112180. https://doi.org/10.1016/j.yexcr.2020.112180
[18] Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, Marshall KA, Phillippy KH, Sherman PM, Holko M, Yefanov A, Lee H, Zhang N, Robertson CL, Serova N, Davis S & Soboleva A. NCBI GEO: archive for functional genomics data sets--update. *Nucleic Acids Research*. 2013;**41**(Database issue):D991–D995. https://doi.org/10.1093/nar/gks1193
[19] Szklarczyk D, Kirsch R, Koutrouli M, Nastou K, Mehryary F, Hachilif R, Gable AL, Fang T, Doncheva NT, Pyysalo S, Bork P, Jensen LJ & von Mering C. The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. *Nucleic Acids Research*. 2023;**51**(D1):D638–D646. https://doi.org/10.1093/nar/gkac1000
[20] Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, Amin N, Schwikowski B & Ideker T. Cytoscape: a software environment for integrated models of biomolecular interaction networks. *Genome Research*. 2003;**13**(11):2498–2504. https://doi.org/10.1101/gr.1239303
[21] Bader GD & Hogue CW. An automated method for finding molecular complexes in large protein interaction networks. *BMC bioinformatics*. 2003;**4**:2. https://doi.org/10.1186/1471-2105-4-2
[22] Győrffy B. Integrated analysis of public datasets for the discovery and validation of survival-associated genes in solid tumors. *Innovation (Cambridge (Mass.)*. 2004;**5**(3): 100625. https://doi.org/10.1016/j.xinn.2024.100625
[23] Tomczak K, Czerwińska P & Wiznerowicz M. The Cancer Genome Atlas (TCGA): an immeasurable source of knowledge. *Contemporary oncology (Poznan, Poland)*. 2015;**19**(1A):A68–A77. https://doi.org/10.5114/wo.2014.47136
[24] Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E, Antipin Y, Reva B, Goldberg AP, Sander C & Schultz N. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. *Cancer discovery*. 2012;**2**(5):401–404. https://doi.org/10.1158/2159-8290.CD-12-0095
[25] Che Rosli AF, Abdul Razak SR & Zulkifle N. Bioinformatics analysis of differentially expressed genes in liver cancer for identification of key genes and pathways. *Malaysian Journal of Medicine and Health Sciences*. 2019;**15**(SP2):18-24
[26] Módos D, Bulusu KC, Fazekas D, Kubisch J, Brooks J, Marczell I, Szabó PM, Vellai T, Csermely P, Lenti K, Bender A & Korcsmáros T. Neighbours of cancer-related proteins have key influence on pathogenesis and could increase the drug target space for anticancer therapies. *NPJ Systems Biology and Applications*. 2017;**3**:2. https://doi.org/10.1038/s41540-017-0003-6
[27] Hu G, Yan Z, Zhang C, Cheng M, Yan Y, Wang Y, Deng L, Lu Q & Luo S. FOXM1 promotes hepatocellular carcinoma progression by regulating KIF4A expression. *Journal of Experimental & Clinical Cancer Research : CR*. 2019;**38**(1):188. https://doi.org/10.1186/s13046-019-1202-3
[28] Song BN & Chu IS. A gene expression signature of FOXM1 predicts the prognosis of hepatocellular carcinoma. *Experimental & Molecular Medicine*. 2018;**50**(1):e418. https://doi.org/10.1038/emm.2017.159
[29] Bhat M, Pasini E, Pastrello C, Angeli M, Baciu C, Abovsky M, Coffee A, Adeyi O, Kotlyar M & Jurisica I. Estrogen Receptor 1 Inhibition of Wnt/β-Catenin Signaling Contributes to Sex Differences in Hepatocarcinogenesis. *Frontiers in Oncology*. 2021;**11**:777834. https://doi.org/10.3389/fonc.2021.777834
[30] Jeon Y, Yoo JE, Rhee H, Kim YJ, Il Kim G, Chung T, Yoon S, Shin B, Woo HG & Park YN. YAP inactivation in estrogen receptor alpha-positive hepatocellular carcinoma with less aggressive behavior. *Experimental & Molecular Medicine*. 2021;**53**(6):1055–1067. https://doi.org/10.1038/s12276-021-00639-2