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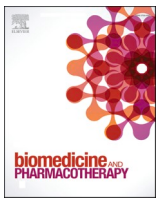

Review

Research progress of the Otubains subfamily in hepatocellular carcinoma

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ABSTRACT

In cancer research, oncogenesis can be affected by modulating the deubiquitination pathway. Ubiquitination regulates proteins post-translationally in variety of physiological processes. The Otubain Subfamily includes OTUB1 (ovarian tumor-associated proteinase B1) and OTUB2(ovarian tumor-associated proteinase B2). They are deubiquitinating enzymes, which are research hotspots in tumor immunotherapy, with their implications extending across the spectrum of tumor development. Understanding their important role in tumorigenesis, includ-ing hepatocellular carcinoma (HCC) is crucial. HCC has alarming global incidence rates and mortality statistics, ranking among the top five prevalent cancers in Malaysia[1]. Numerous studies have consistently indicated significant expression of OTUB1 and OTUB2 in HCC cells. In addition, OTUB1 has important biological functions in cancer, suggesting its important role in tumorigenesis. However, the mechanism underlying the action of OTUB1 and OTUB2 in liver cancer remains inadequately explored. Therefore, Otubain Subfamily, as potential molecular target, holds promise for advancing HCC treatments. However, further clinical studies are required to verify its efficacy and application prospects.

1. Introduction

Liver cancer, mainly hepatocellular carcinoma (HCC), starts in liver cells and makes up about 80 % of primary liver cancer instances[1]. HCC is the most common primary liver malignancy[2], ranking sixth among all malignant tumors globally[3]. In 2023, Malaysia reported 2363 cases of liver cancer, ranking the fourth cancer of mortality rate in the country [4]. This illness can be linked to various risk factors including long-term infection with hepatitis virus, consuming aflatoxin B1 through diet, chronic alcohol misuse, and cirrhosis related to genetic liver conditions [5]. Diagnosis typically involves a combination of imaging techniques such as ultrasound, CT scans, MRI, biopsy, and the assessment of serum biomarkers like alpha-fetoprotein. Treatment strategies range from curative therapies like surgery and liver transplantation to locoregional techniques such as radiofrequency ablation and transarterial chemoembolization. Systemic therapies, including targeted therapies and immunotherapy, play a crucial role. Comprehensive patient includes supportive care and ongoing surveillance[6]. In individuals with cirrhosis, HCC is often diagnosed at an advanced stage, precluding patients from receiving curative interventions[7]. Therefore, the search for novel biomarkers and potential targets for improving early diagnosis and personalized therapy of liver cancer continues to be a key area of study in the liver cancer field. Proteins have the ability to undergo post-translational changes like ubiquitination[8,9]. Enzymes called deubiquitinases (DUBs) are in charge of cleaving ubiquitin from substrates that have been ubiquitinated, which affects a variety of physiological processes in cells[10]. These enzymes are involved in the regulation of processes such as cell cycle[11], gene transcription, and DNA damage signal transduction[9,12]. Dysregulation of many DUBs has been implicated in tumorigenesis[13], including HCC. Two deubiquitinating enzymes in the Otubains subfamily are closely related to HCC. The human OTU family comprises 16 active DUBs organized into

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four distinct subfamilies[14]. One of these subfamilies includes the proteins Otubain1, which contain the OTU domain and bind to ubiquitin aldehyde, which is further subdivided into OTUB1 and OTUB2, is one of the subfamilies that stands out[14].

Two key members of the ovarian tumor domain (OTU) deubiquitinating cysteine proteases subfamily are OTUB1 and OTUB2[15]. OTUB1 has a distinctive crystal structure with dual ubiquitin binding sites, enabling it to selectively cleave polyubiquitin chains linked by Lys while facilitating substrate reactions through its active center[9,16]. There are numerous biological functions for OTUB2, which was first discovered in the ovarian tumor genes of Drosophila melanogaster[17,18]. The presence of an N-terminal in OTUB1 is the primary distinction between these proteins[19]. When the N-terminal region of OTUB1 is removed, it becomes more like OTUB2[19]. Both OTUB1 and OTUB2 exhibit interaction capabilities[20,21], their function in suppressing the expression of the RANTES and IFNB1 genes that are found naturally has been demonstrated by studies[20], thereby suggesting redundant functions in suppressing virus-triggered signaling[20]. OTUB1 exhibits high expression levels in human liver tissue, which is linked to a poor prognosis for patients[13,22], especially in HCC. This raises the possibility that OTUB1 will prove to be a useful marker for liver cancer treatment using targeted therapy[9,13,22]. The tissues and cell lines of liver cancer tumors showed a marked upregulation of OTUB2[23,24], with knockdown experiments revealing its impact on inhibiting HCC cell growth [23]. Both OTUB1 and OTUB2 act as promising biomarkers and therapeutic targets for HCC, and their involvement in various malignancies suggests their important role in disease pathogenesis. However, their precise mechanism of action in HCC remains elusive.

2. Crucial traits and functions of Otubain subfamily

2.1. Gene and protein structural traits

The OTUB1 and OTUB2 sequences exhibited a 70 % sequence similarity and a 48 % sequence identity when compared[15]. However, the N terminus of the two is different[25]. The OTUB1 N-terminal helix and the α1α2 loop are both heavily interacting with the proximal Ub in the OTUB1-Ub-Ubch5b-Ub configuration, and the interaction with E2 (UBC13) stabilizes the N-terminal α-helix[26,27]. The OTUB2 N-terminal tail is shorter and less organized than the proximal ubiquitin[25]. In humans, the OTUB1 gene is found on chromosome 11q13.1[28,29]. The 130 amino acid OTU domain of the protein gene is highly conserved across yeast and mammal[28,29]. In addition, due to control E2-ubiquitin conjugates, In addition to its ability to reverse Lys48 ubiquitination, OTUB1 is a strong inhibitor of other ubiquitination events[14]. OTUB2 gene is located at chromosome position 14q32.12 (Ensembl genome database[30]). The OTUB2 gene of protein that consists of 234 amino acids[31]. The larger α-helix region (α3-α10) and the smaller α-helix region (α1, α2) encircle the central five-stranded β-fold in the protein structure of OTUB2[17].

2.2. Mechanism

The deubiquitination of OTUB1 occurs through two mechanisms: direct deubiquitination and non-canonical Inhibition(Fig. 1). OTUB1 exerts its antagonistic effect on ubiquitination by directly deubiquitinating certain substrates[32]. On the other hand, it inhibits ubiquitination through noncanonical blocking of the ubiquitin transfer mediated by certain ubiquitin-conjugating enzymes (E2)[32]. Unlike OTUB1, the

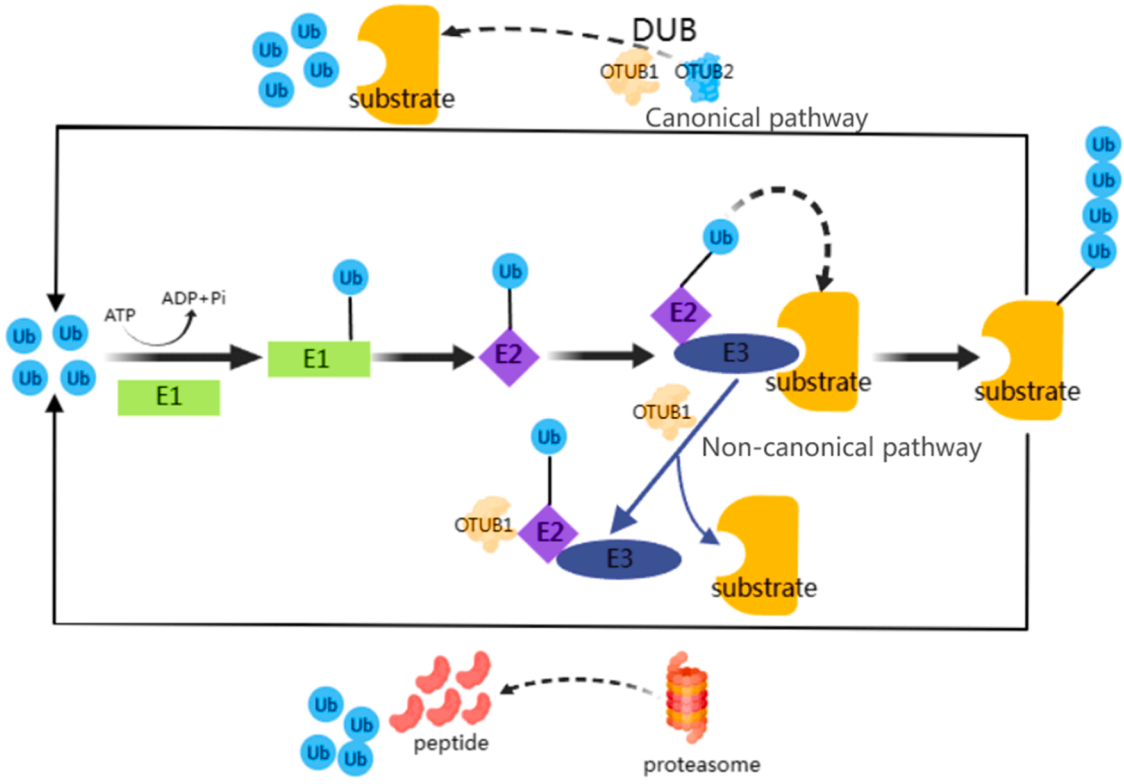

Fig. 1. Otubains two deubiquitylation pathways. Figure generated using Med Peer. (Canonical ubiquitination pathway: a. The ubiquitin activating enzyme (E1) catalyzes the formation of a ubiquitin-AMP intermediate by reacting with ATP, which is then transferred to the active site of E1. b. The ubiquitin transferase (E2) transfers ubiquitin from E1 to its own active site. c. The ubiquitin ligase enzyme(E3) facilitates the transfer of ubiquitin from E2 to the substrate protein through auxiliary action, achieving substrate ubiquitination. Canonical deubiquitination pathway: This process reverses the canonical ubiquitination pathway by the OTUB1 and OTUB2 deubiquitinating enzymes. Non-canonical deubiquitination pathway: OTUB1, which possesses an additional N-terminal α-helix compared to OTUB2, binds to the ubiquitin-containing E2, and inhibits the transfer of ubiquitin to the protein substrate.).

absence of an N-terminal helix in OTUB2 renders it incapable of recognizing and inhibiting E2Ub[27]. Therefore, OTUB2 primarily affects the life activities of the human body through the canonical deubiquitination pathway(Fig. 1). Non-canonical deubiquitination process can occur as monoubiquitination or polyubiquitination, each carries distinct functional implications based on the kind of ubiquitin lysine linkage that was applied. As an illustration, K48 chains usually indicate proteasomal breakdown, whereas K63 chains frequently control protein interactions and lysosomal targeting[33]. Unlike ubiquitin ligases, deubiquitinases (DUBs) break the isopeptide bond, which results in the partial or total removal of the polyubiquitin chain. Certain deubiquitinases (DUBs) exhibit a preference for particular types of linkages, which enables them to alter or counteract the functions of ubiquitin ligases.

In the year 2010, the Durocher lab uncovered a noteworthy finding concerning the unconventional function of OTUB1[34]. They found that when DNA is damaged, OTUB1 acts as a deubiquitinase (DUB) to inhibit K63 chromatin ubiquitination[35], contradicting previous concepts that OTUB1's DUB activity was specific to K48 linkages. They discovered that the DUB activity of OTUB1 was not necessary for the decrease in ubiquitination. A closer look showed that OTUB1 interacts with the E2 enzyme UBC13 to stop chromatin at DNA damage sites from being ubiquitinated by the ubiquitin ligase RNF168. Moreover, OTUB1 exhibited a higher binding affinity for the ubiquitin-charged form UBC13. This interaction, as well as the interaction between OTUB1 and other E2 enzymes, have been verified by later research. OTUB1 also controls the production of K63-linked polyubiquitin chains in alternative pathways by attaching to UBC13~Ub thioester[26].

In addition, OTUB2 also has the same deubiquitylation mechanism. The deubiquitylation activity-dependent manner of OTUB2 inhibits the RNF8-mediated ubiquitination process of L3MBTL1 and hinders the formation of Lys 63-linked ubiquitin chains, thereby affecting the selection of DNA repair pathways[36].

There are three essential residues in the catalytic domain of OTUB1: Asp(D)268, His(H)265, and Cys(C)91[37]. In contrast, the catalytic domain of OTUB2 is composed of Cys 51, His 224, and Asn 226[17]. In the protein-protein interaction (PPI) network, both OTUB1 and OTUB2 exhibit extensive interactions with numerous proteins related to various cellular functions(Figs. 2 and 3). These proteins play critical roles in

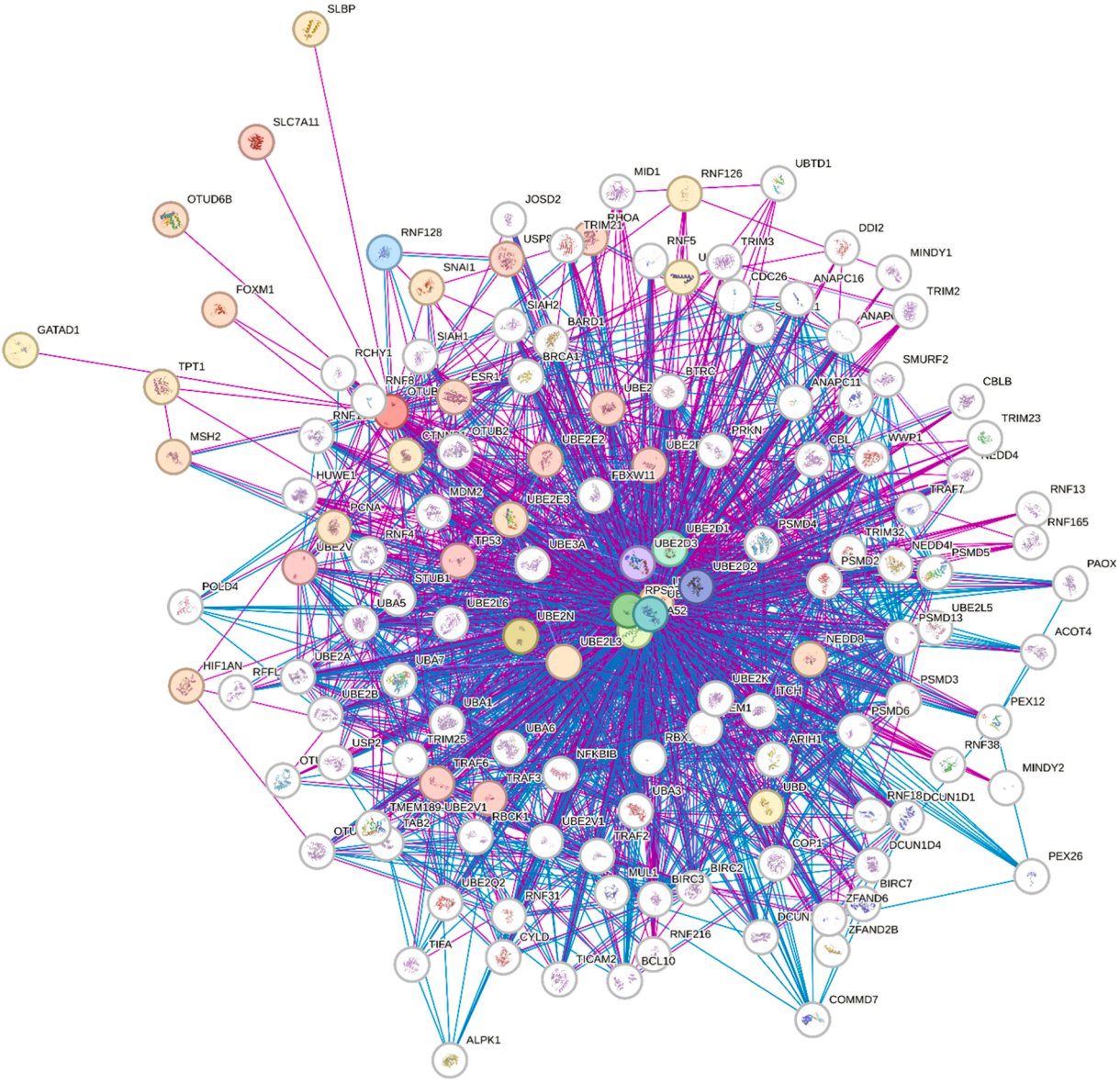

Fig. 2. OTUB1 protein-protein interaction network constructed in String(confidence=0.400). Nodes indicate a protein; edges show the interaction sources (purple line=experiment data; bule line=databases). The diagram displays up to 100 interactors. (OTUB1 protein (human) - STRING interaction network (string-db.org)).

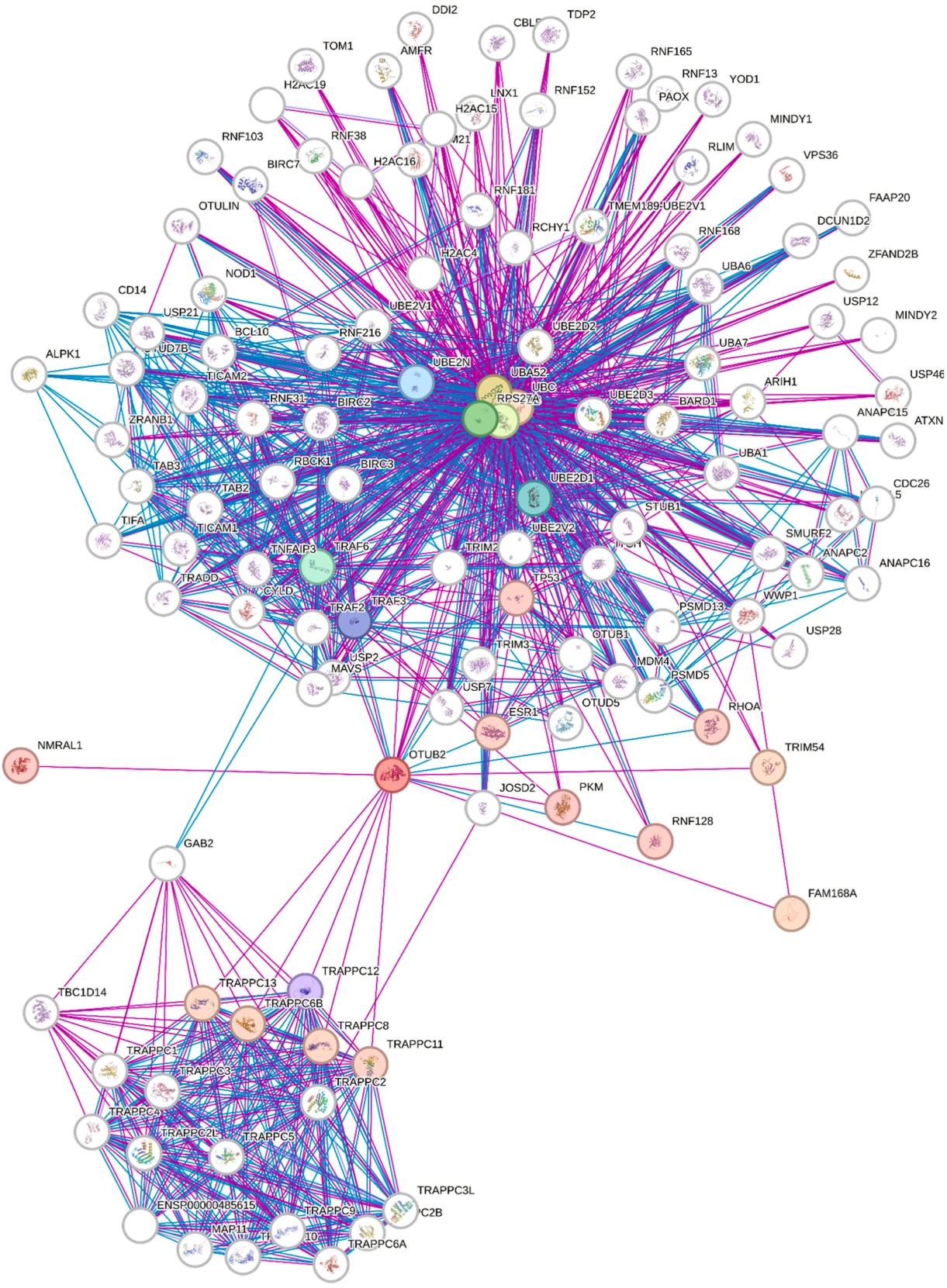

Fig. 3. OTUB2 protein-protein interaction network constructed in String(confidence=0.400). Nodes indicate a protein; edges show the interaction sources (purple line=experiment data; bule line=databases). The diagram displays up to 100 interactors. (OTUB2 protein (human) - STRING interaction network (string-db.org)).

regulating cellular homeostasis, stress response, and signal transduction. Notably, OTUB1 and OTUB2 also interact with other members of the OTU family. For example, OTUB1 interacts with OTUD6B, and OTUB2 interacts with YOD1. These interactions further highlight the crucial roles of OTUB1 and OTUB2 in cellular physiology and regulatory mechanisms.

2.3. Biological functions

Otubains have become important modulators of many physiological processes, such as DNA damage response and immunological signaling, in the recent past[38]. Through controlling TRAF3 and TRAF6 deubiquitination, it has been discovered that OTUB1 and OTUB2 restrict virus-induced activation of type I interferons (IFNs) during the early innate antiviral response[20]. OTUB1 is important for the development of lung tissue and bone. In both embryonic and adult lung tissue, OTUB1 gene deletion enhances mTOR signaling, which is critical for lung cell proliferation, lung development, adult lung tissue homeostasis, and respiratory regulation[39]. Reduced body length is the result of impaired bone development caused by a generalized deletion of OTUB1 [40]. During this process, OTUB1 inhibits the ubiquitination of FGFR2 through an atypical mechanism, which affects bone formation[40]. OTUB1 participates in DNA repair pathways[37]. Higurashi et al. discovered that the upregulation of FOXM1 in liver cancer cells lacking mitochondrial DNA was facilitated by OTUB1[41]. Likewise, OTUB2 could also have an impact on the choice of DNA repair pathways and help regulate DNA damage-dependent ubiquitination[36]. In addition, it regulates NK and CD8 T cell activation by deubiquitinating and stabilizing related proteins[42]. It is also involved in PD-L1-mediated immune escape[42]. Meanwhile, numerous cancer cells exhibit high levels of OTUB1 expression[42]. By affecting the immune cells of the body, it causes cancer cells to proliferate and migrate.

3. The roles of deubiquitinases in liver cancer

An enzyme cascade consisting of E1, E2, and E3 enzymes, is known as ubiquitination[43]. It regulates various biological processes by modifying protein substrates[44]. It can also affect post-translational modification, thereby regulating critical cellular functions such as kinase activation, DNA repair, transcriptional regulation[9,44], and cell cycle modulation[45,46]. These physiological processes are closely related to the occurrence and progression of cancer[47–49]. Interestingly, many ubiquitinations exhibit dual roles, acting as both tumor suppressors and promoters, suggesting potential therapeutic targets [50]. Studies have shown that a large number of DUBs are involved in the development and migration of hepatocellular carcinoma. For instance, CYLD, a K63 bond-specific deubiquitinating enzyme[51], contributes to HCC cell apoptosis resistance by improving NF-κB activity [52]. Similarly, USP9x, a protease specific to X-linked ubiquitin[53], influences hepatocyte epithelial-mesenchymal transition by modulating miR-26b-induced suppression of endogenous USP9X expression[54]. Additionally, ubiquitin carboxyl-terminal hydrolase L1 (UCHL1)[55], a ubiquitin located at the carboxyl terminal increases p21 levels in HCC cells, causing G2/M phase halt and suppressing cell growth[55]. Hydrolase 37 (UCH37), through PRP19 (an essential RNA splicing factor) deubiquitination, enhances the migration and invasion of HCC cells[56]. A20, part of the family of deubiquitinated cysteine proteases called Ovarian Tumor (OTU), removes mono-ubiquitin from altered proteins [57]. The excessive expression of A20 greatly inhibits the growth, movement, and transformation of HCC cells, in laboratory settings and in living organisms[58,59]. Furthermore, among the protective DUBs shielding substrate proteins from degrading is ubiquitin-specific protease 7(USP7)[60]. USP7, up-regulated in HCC tissues, directly promotes tumor cell proliferation and invasion by reversing ubiquitination[61].

In addition, certain deubiquitinating enzymes exhibit potential implications for the HCC prognosis and could serve as promising prognostic markers in future studies. Elevated expression levels of OTUB1 and USP11 have been correlated with reduced overall survival in HCC cases. OTUB1 and OTUB2, both members of the OUT subfamily of deubiquitinating enzymes, exhibit significant expression in HCC cells [13,15,62]. However, the underlying mechanisms of their potential as a therapeutic target in HCC remain unclear. This is of great significance for the treatment and diagnosis of HCC.

4. The Role of Otubain subfamily in cancer

4.1. OTUB1 in cancer

OTUB1 can inhibit K48-linked ubiquitin and participate in DNA repair and immune signaling[63–66]. As per the latest findings, patients suffering from ovarian cancer, renal cell carcinoma, glioma, thyroid carcinoma, gastric cancer, colon cancer, liver cancer, breast cancer, lung cancer, prostate cancer, esophageal squamous cell carcinoma, gastric cancer, and multiple myeloma have highly expressed OTUB1 in their tumor tissues[13,67–76] (Fig. 4).

In HCC, increased OTUB1 expression promotes cell invasion and migration by regulating the TGF-β pathway[13]. In ovarian and breast cancer, OTUB1 enhances tumor development and chemotherapy resistance by suppressing the degradation of FOXM1 ubiquitination[69,77]. Its upregulation in lung adenocarcinoma cells inhibits RAS ubiquitination, and promotes cancer progression[78]. In breast tumors, OTUB1 drives growth by increasing aerobic glycolysis through Myc-mediated HK2 expression[79]. In esophageal squamous cell cancer (ESCC), the overexpression of OTIB1 promotes cancer progression and metastasis by regulating the stability of Snail[73]. Conversely, in endometrial cancer, OTUB1 deubiquitinates estrogen receptor alpha, hindering the cancer cell proliferation[80]. In lung cancer, OTUB1 enhances the regulation of DNA damage and repair by deubiquitinating and stabilizing CHK1, thereby promoting the development of lung adenocarcinoma and increasing the resistance to radiotherapy[81]. In glioma cells, the presence of OTUB1 increases the migration by stabilizing SLC7A11 protein, consequently inhibiting ferroptosis[72,82,83]. It also blocks the ubiquitination of EYA1 in thyroid cancer cells (PTC) cells, affects the expression of EYA1 protein, and promotes cell proliferation[71]. In prostate cancer, overexpression of OTUB1 mediates cell invasion by activating RhoA and promotes tumor growth in vivo[68]. In colon cancer, OTUB1 is also highly expressed[28]. Moreover, in colorectal cancer, the regulatory relationship between ERRα and OTUB1 is observed, promoting EMT and cell migration[84]. In gastric cancer, OTUB1 is significantly expressed[85], binding to YAP and deubiquitinate YAP protein at multiple lysine sites, thereby enhancing the activity of Hippo/YAP axis, which promotes proliferation and metastasis[85]. CST1 also can exert regulatory control over GPX4 protein stability via OTUB1, thereby suppressing ferroptosis while facilitating metastasis and invasion in gastric cancer, without affecting proliferation[86]. In Renal cell carcinoma, OTUB1 promotes tumor progression and invasion by regulating the ubiquitination of FOXM1 and upregulating ECT-2[75]. In addition, the OTUB1/c-Maf axis represents a promising therapeutic target for multiple myeloma(MM) and induces MM apoptosis[76] (Table 1).

OTUB1 is implicated in the inhibition of apoptosis (c-IAP) protein, an E3 ubiquitin ligase that directly binds to and inhibits caspases[88,89]. The ubiquitination of c-IAP-l, which controls the NF-kB and MAPK signaling pathways as well as tumor necrosis factor (TNF)-dependent cell death, is a target for OTUB1[89]. The down-regulation of OTUB1 expression promotes caspase activation and cell death stimulated by TWEAK and IAP antagonists and enhances c-IAP-l degradation to promote cell apoptosis[34,88,89]. In addition, OTUB1-stabilized MDMX could activate mitochondria-associated apoptosis by enhancing p53 phosphorylation at S46 (p53S46) through mitochondrial localization [90]. NEDD4L is an E3 ubiquitin ligase that downregulates TGF-β signaling by targeting Smad2 for degradation[91,92]. OTUB1 inhibits

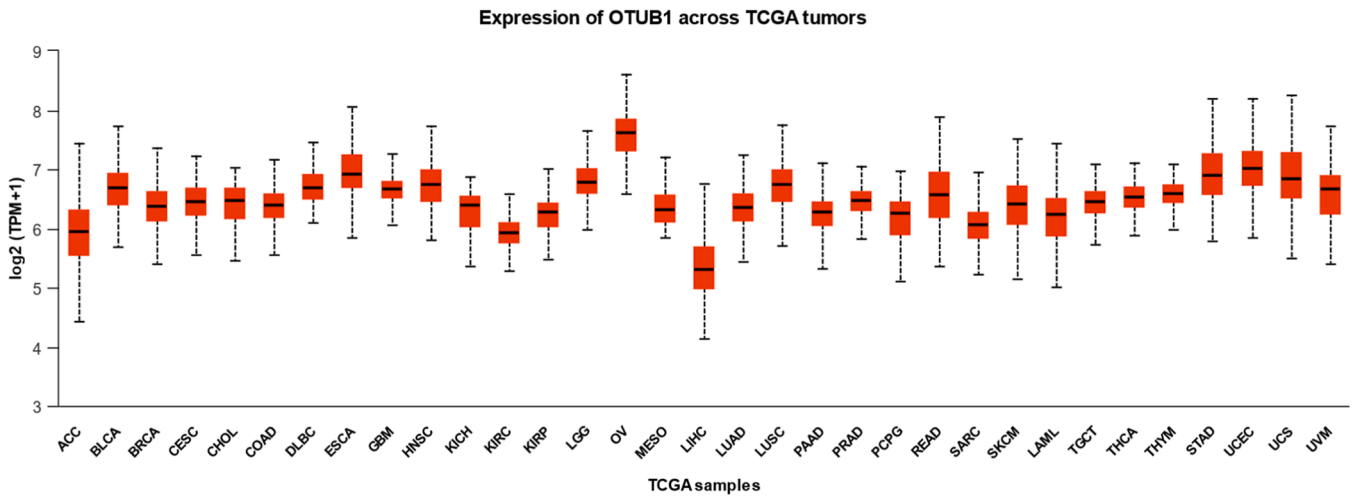

Fig. 4. Expression of OTUB1 in tumors, data from The Cancer Genome Atlas (TCGA), (UALCAN (uab.edu)).

Table 1
Cancers and cancer-associated pathways associated with OTUB1(epithelial-mesenchymal transition (EMT)).

Cancer	Target	Result
Liver cancer	TGF-β	Increased migration, invasion[13]
Breast cancer	FOXM1/ c-MYC	Increased proliferation, epirubicin resistance[69,79]
Ovarian cancer	FOXM1	Increased proliferation, migration, invasion [77]
Esophageal cancer	SNAIL	EMT, increased migration, invasion[73]
Endometrial cancer	ERa	Decreased ERa transcription[84]
Lung cancer	CHK1	Promoted proliferation, radiation resistance [81]
Glioma	SLC7A11	EMT, increased migration[72,82,83]
Thyroid cancer	EYA1	Increased proliferation[71]
Prostate cancer	RhoA	Increased migration, invasion[68]
	Cyclin E1	Increased proliferation, migration[87]
Colon cancer	OTUB1	EMT, increased migration, invasion[28]
Gastric cancer	YAP	EMT, enhanced migration[85]
	GPX4	Enhanced migration, invasion[86]
Renal cell carcinoma	FOXM1	Enhanced proliferation, migration, invasion [75]
Multiple myeloma	C-Maf	Induce cell apoptosis[76]

the ubiquitination of Smad2 by NEDD4L/ UBCH5 in an atypical manner, thereby increasing the activity of TGF-β signaling and promoting cell migration[34].

4.2. *OTUB2 in cancer*

OTUB2 inhibits ubiquitin linkage at K11, K48, and K63[63]. It participates in DNA repair[36], protein homeostasis, and translation[93,94], All of which are important in regulating the physiological activities of cancer cells. OTUB2 is significantly upregulated in liver cancer, endometrial carcinoma (EC), cervical squamous cell carcinoma, and endocervical adenocarcinoma (CESC) (Fig. 5).

In HCC, OTUB2 upregulation correlates with increased cell growth, while its knockdown inhibits this growth[24]. In liver cancer cells, OTUB2 expression is positively correlated with p65 expression, and its down-regulation inhibits NF-κB and p65 activation[23]. Furthermore, in HCC cells, overexpression of OTUB2 greatly diminishes the anti-tumor effect of NF-κB inhibito[23]. In thyroid cancer, OTUB2 upregulation activates the TRAF6/NF-κB signaling pathway, leading to increased cell growth, proliferation, and invasion[95–99]. By deubiquitinating U2AF2, OTUB2 stabilizes it. This activates the AKT/mTOR signaling pathway and the Warburg effect, which promotes the growth of NSCLC tumors[18]. The AKT /mTOR signaling pathway is a central regulator of cell metabolism, growth, proliferation, and survival[18,100], while the

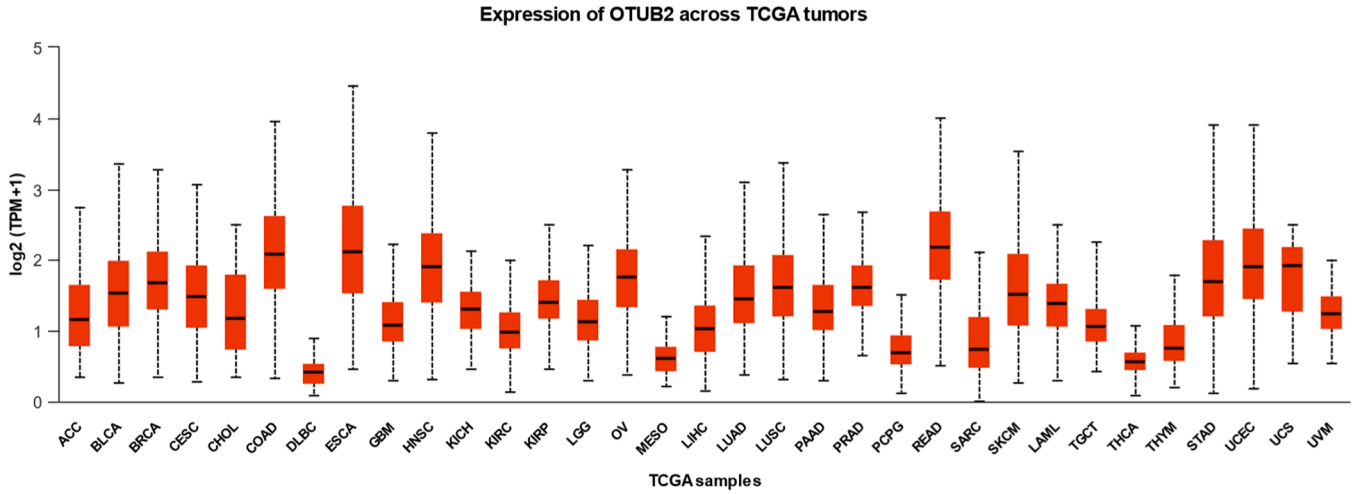

Fig. 5. Expression of OTUB2 in tumors, data from The Cancer Genome Atlas (TCGA), (UALCAN (uab.edu)).

Warburg effect can enhance cell glycolysis to provide energy for cancer cells[101]. In endometrial cancer, OTUB2 regulates proliferation, migration, and invasion of EC cells by regulating PKM2/PI3K/AKT signaling pathway[102]. In cervical cancer, Rbm15-mediated m6 modification leads to the up-regulation of OTUB2, which, via the AKT/mTOR signaling pathway, encourages cervical cancer cells to proliferate and have the capacity to spread[103]. In colorectal cancer (CRC), OTUB2 upregulation inhibits the ubiquitination of PKM2, enhancing pyruvate kinase M2 activity by blocking the interaction between PKM2 and ubiquitin E3 ligase Parkin, thus enhancing the progression of CRC through increased aerobic glycolysis[104]. Overexpression of OTUB2 has the capability to directly stabilize and bind to YAP1/TAZ through SUMO, activate YAP1/TAZ expression, activate downstream target genes such as CYR61 and CTGF, and contribute to tumor cell invasion, growth, and metastasis[105,106]. In ESCC cell lines, where OTUB2 is overexpressed, the proliferation, migration, invasion, and tumor development of ESCC are promoted through post-transcriptional modification of YAP1/TAZ by deubiquitinating enzymes[105](Table 2).

OTUB2 removes the K48 polyubiquitin chain on U2AF2 and prevents its degradation, thereby activating the Akt/mTOR pathway and promoting glycolysis and growth of cancer cells[18,107]. additionally, OTUB2 removes the K48 polyubiquitin chain on Gli2 and prevents its degradation, thereby activating the Hedgehog pathway and promoting osteogenic differentiation and cancer progression[94]. Research has also shown that the inhibition of OTUB2 by siRNA results in an increase in the NF-κB pathway activity in pancreatic β cells and a reduction in insulin secretion, revealing that a significant proportion of pancreatic β cells are apoptotic[108].

4.3. Relationship between the Otubain subfamily and hepatocellular carcinoma

4.3.1. Relationship between OTUB1 and HCC

Numerous studies have consistently shown that OTUB1 is significantly expressed in HCC cells[13,109](Fig. 6A). Ma Qi et al. constructed prognostic prediction model that demonstrated that OTUB1 (HR=1.826, P=0.002) serves as a significant predictor for hepatocellular carcinoma [110]. In addition, the protein expression of OTUB1 serves as an independent prognostic factor and exhibits a significant correlation with the unfavorable prognosis of patients with hepatocellular carcinoma (HCC) [13]. OTUB1 is dramatically elevated in HCC tissues and is linked to a poor prognosis because it encourages the growth and metastasis of the malignancy[109]. Previous studies have found that OTUB2 expression was significantly stronger in HCC tissues and associated with a poor prognosis for patients[24](Fig. 6B).

Table 2
Cancers and cancer-associated pathways associated with OTUB2.

Cancer	Target	Result
Liver cancer	TRAF6	Increased proliferation and migration [23]
Thyroid cancer	TRAF6	Promoted cell growth, proliferation, and invasion[95–99]
Non-small cell lung cancer (NSCLC)	U2AF2	Increased cell growth and glycolysis [18]
Endometrial cancer	PKM2	Increased proliferation and invasion [102]
Cervical cancer	AKT/ mTOR	Promoted cell growth, proliferation [103]
Esophageal squamous cell carcinoma	YAP1/ TAZ	Promoted the proliferation, migration, and invasion[105]
Colorectal cancer	PKM2	Increased proliferation and invasion [104]

4.4. Role of Otubain subfamily in HCC-related pathways

4.4.1. Otubains and TGF-β pathway in HCC

OTUB1 plays a key role in transfiguring growth factor-β (TGF-β)-mediated gene transcription and cell migration by stabilizing SMAD 2/3[111]. According to Gungor MZ et al., blocking TGF-β signaling can stop HCC from growing and may even be a treatment option for advanced HCC[112,113]. Blanca Herrera et al. by experiments found that TGF-β affects fetal hepatocytes, where c-IAP-1 is a substrate of caspases during TGF-β-induced apoptosis in fetal hepatocytes[113]. After treatment using TGF-β, c-IAP-1 is almost completely adhered [113], and hepatocytes produce inactivation of the NF-κb pathway [114]. In addition, Goncharov et al. identified OTUB1 as a c-IAP interacting protein that selectively removes K48-linked polyubiquitin chains from c-IAP-1 and regulates c-IAP-1 proteasome degradation stimulated by IAP antagonists[115]. Sanchez et al. suggested that TGF-β secreted at high concentrations during hepatocarcinogenesis may cause the death of normal cells[116], and severe degradation of c-IAP-1 proteasome[113]. HCC has much higher levels of PJA1 mRNA and protein expression than normal liver (Same as the results of ONCOMINE database)[117,118]. PJA1 has recently been shown to increase cell proliferation in HCC through TGF-β/SMAD3/SPTBN1 signaling[118,119].

Hu et al. showed that OTUB2 may increase the malignant proliferation and migration of HCC cells by enhancing the stability of PJA1 by deubiquitylation[24]. Kazufumi Ohshiro et al. reported that PJA1 could potentially be a new target in HCC defective TGF-β signaling[119]. The OTUB2/PJA1 axis may play a role in HCC via the TGF-β/NF-κB signaling pathway[24]. Furthermore, it was observed that OTUB2 expression was significantly upregulated in HCC tissues and correlated with poor prognosis in patients. Functionally, OTUB2 overexpression promoted malignant proliferation and metastasis of HCC cells, while OTUB2 knockdown exerted opposite effects. Through two bioinformatics approaches, PJA1 was identified as a candidate gene regulated by OTUB2. Immunoprecipitation (IP) and cycloheximide (CHX) experiments confirmed that OTUB2 enhanced PJA1 stability by deubiquitylation. Additionally, overexpression of PJA1 counteracted the inhibitory effects of OTUB2 depletion on the malignant phenotypes of HCC cells[24].

These findings suggested that OTUB1 and OTUB2 play important roles in modulating the TGF-β pathway, potentially either promoting HCC proliferation or inducing apoptosis in normal cells by regulating c-IAP stability. However, underlying mechanisms need further investigation to elucidate the correlation of otubains in TGF-β pathway.

4.4.2. Otubains and NF-κB/TNF pathway in HCC

OTUB1 is an inhibitor of TNF-dependent necroptosis[120], and TNF is a critical cytokine that activates the NF-κB signaling pathway [121–123].

NF-κB is the central molecule of the pathway, and was previously proven to promote tumor growth, and have a certain linkage with inflammation[123]. In fact, Czauderna et al. demonstrated that the activation of NF-κB signaling was closely related to the development of primary liver cancer[124]. However, although inflammation-induced NF-κB expression in the liver is associated with the carcinogenesis of HCC, inhibiting NF-κB has shown promise in counteracting this process, as proposed by Pikarsky et al.[125]. Accordingly, the expression of inflammation-induced NF-κB was significantly adjusted in HCC, and thus blocking such a pathway might prevent carcinogenesis in the liver.

Knocking down OTUB1 enhances c-IAP1 degradation, thereby inhibiting TNFR-mediated NF-κB and MAPK pathways[88]. The use of sorafenib, in combination with NF-κB inhibition has shown effectiveness in reducing MAPK signaling and decreasing the levels of the anti-apoptotic protein Mcl1, which ultimately slows down the growth of tumor[126].

Besides OTUB1, Gu et al. found that the expression of OTUB2 and p65 expression were significantly correlated, in a positive manner, suggesting that OTUB2 knockdown could possibly reduce the

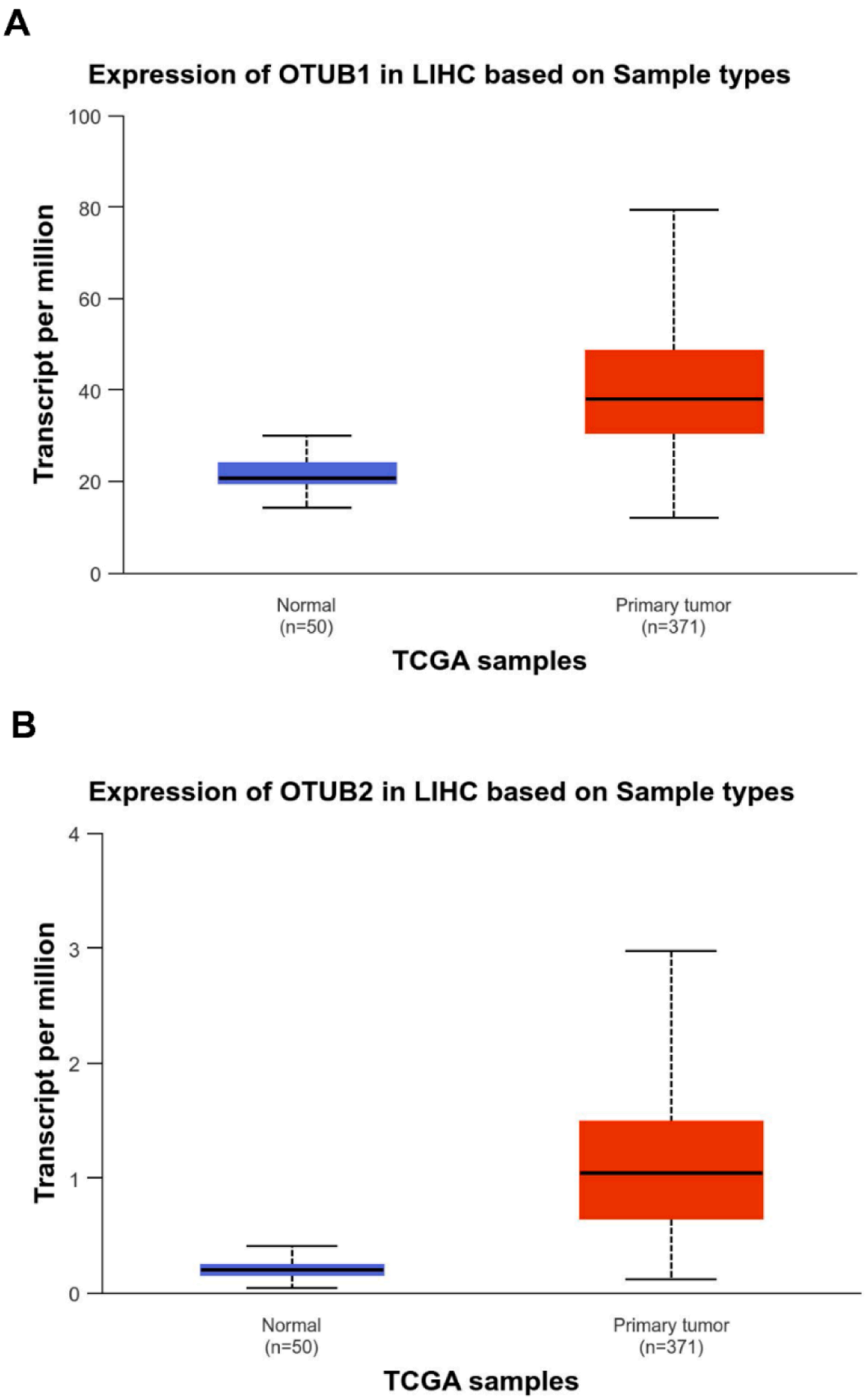

Fig. 6. Expression of OTUB1(Fig A) and OTUB2(Fig B) in Liver hepatocellular carcinoma(LIHC), data from The Cancer Genome Atlas (TCGA), (UALCAN (uab.edu): Increased expression of OTUBs in HCC.

phosphorylation and activation of p65[23]. It was also found that the over-expression of OTUB2 strengthened HepG2 cells' resistance to NF-κB inhibitors, highlighting the potential of the otubains subfamily in inhibiting HCC progression and improving patient prognosis by regulating the NF-κB/TNF pathway.

4.4.3. Otubains and FOXM1 pathway in HCC

Forkhead box M1 (FOXM1) is a transcription factor that plays a significant role in the regulation of cell proliferation, metastasis, apoptosis, and DNA damage repair[127].

Karunarathna et al. have demonstrated that targeting FOXM1 can enhance proliferation rates and epirubicin resistance[77]. By controlling the cell cycle, FOXM1 overexpression enhanced the expression of cyclin B1 and cyclin D1, promoting HCC cell proliferations[128].

Yu et al. discussed the clinical significance of FOXM1 in HCC[128]. Immunohistochemistry (IHC) analysis showed that FOXM1 expression was higher in HCC tissues than that in adjacent non-tumorous tissues as well as normal liver tissues. Furthermore, correlations were found between FOXM1 expression and several clinicopathological parameters of HCC patients, including tumor stage, size, number, encapsulation integrity, tumor thrombus, and AFP level. These results align with earlier research connecting FOXM1 expression to the proliferation marker PCNA[129]. Wei J et al. reported that sorafenib inhibits FOXM1 via p53, thereby suppressing HCC cell proliferation and invasion [130].

However, conflicting data from another study discovered no association between FOXM1 expression and clinicopathological parameters in Western HCC patients[131].

OTUB1 is a potent activator of p53 and can act as an tumor suppressor[37,132]. It promotes apoptosis by enhancing the stability of MDMX[90] and modulating P53 levels and activity[133]. Using both loss-of-function and gain-of-function assays, Bian et al. showed that elevated flap endonuclease 1(FEN1) expression stimulates HCC cell proliferation, migration, and epithelial-mesenchymal transition (EMT) [134]. FEN1 was found to promote HCC progression by up-regulating USP7/MDM2-mediated p53 inactivation[134]. These studies suggest that OTUB1 may promote the proliferation and progression of HCC by regulating p53 inactivation.

Jing Xiao et al. demonstrated that OTUB2 facilitates cervical cancer progression by deubiquitinating and stabilizing FOXM1[135,136]. However, the role of OTUB2 in HCC progression through FOXM1 pathway remains unexplored.

4.4.4. Otubains and PD-L1/ERAD pathway in HCC

Targeting immune checkpoints is essential for cancer immunotherapy, and programmed death ligand 1 (PD-L1), along with its receptor PD-1 are critical targets [136]. The PD-1/PD-L1 axis is a significant immune checkpoint mechanism that suppresses T-lymphocyte activities, including cytotoxicity and cytokine release, through PD-1 and PD-L1 interaction[137,138]. This interaction induces apoptosis of tumor-specific T-cells[139] and enhances tumor cell resistance[140, 141]. The stability of PD-L1 protein is regulated by multiple mechanisms, including specific enzymes like E3 ligases, DUBs, proteases, glycosylases, and regulators of the proteasome and lysosomes. Previous research identified DUBs like USP7, USP9X, and USP22 as stabilizers of PD-L1 in different cancers, such as gastric cancer, oral squamous cell carcinoma, and liver cancer[142–144].Disrupting this pathway is therefore a promising strategy for enhancing antitumor immunity.

Research has shown that when OTUB1 is knocked down, PD-L1 undergoes ubiquitination and is degradation through the proteasome-dependent endoplasmic reticulum-associated protein degradation (ERAD) pathway [145]. Deficiency in OTUB1 renders cancer cells more susceptible to T cell-mediated cytotoxicity. Zhu, D. et al. used Western blotting to reveal that knockdown of OTUB1 also attenuated PD-L1 protein levels in HepG2 cells, thereby enhancing human cellular immunity[145]. This finding highlights the potential of targeting OTUB1 to improve antitumor immune responses by modulating PD-L1 levels.

Experimental findings from Ren et al. demonstrated that OTUB2 serves as a negative regulator of T-lymphocyte-mediated antitumor immune responses in both controlled laboratory environments and living organisms[146]. This regulatory role is mediated by stabilization of PD-L1, a crucial protein involved in tumor immune evasion. Maintaining the appropriate levels of PD-L1 protein is crucial for various biological functions, and disturbances in this balance can lead to immune-related disorders[147]. The deubiquitinase OTUB2 plays a crucial role in tightly regulating PD-L1 protein levels. OTUB2 interacts with PD-L1, stabilizing it by removing ubiquitin molecules, thus interfering with the ERAD pathway. Notably, the amino acid Cys51, vital for OTUB2's enzymatic function, is necessary for its interaction with PD-L1, indicating the impact of OTUB2 on PD-L1 beyond its enzymatic activity to direct protein interaction. This aligns with previous research demonstrating that mutation of Cys51 in OTUB2 abolishes its ability to bind to STAT1 and perform deubiquitinate[148].

However, various reports have highlighted different mechanisms through which DUBs regulate their substrates, suggesting that the key residues responsible for the enzymatic activity may not always be essential for substrate interaction[149]. It was indicated that OTUB2 likely regulates the physiological PD-L1 degradation level by reducing polyubiquitination, affecting both premature and mature forms of the protein. Importantly, OTUB2 was indicated as a crucial DUB that prevents PD-L1 in various cancers, independent of interferon-gamma (IFN-γ) stimulation[150]. Furthermore, targeting OTUB2 with drugs to decrease PD-L1 levels increases tumor susceptibility to cytotoxic T cells [146].

4.4.5. Otubains and VEGF pathway in HCC

A range of factors during cancer development can lead to hypoxia, resulting in VEGF release[151,152]. VEGF plays a key role in angiogenesis by acting as an autocrine or paracrine growth factor to stimulate blood vessel formation[153]. Hypoxia-inducible factor 1 alpha is the main molecular element involved in the initiation of hypoxia-induced angiogenesis (HIF-1α)[151]. HIF-1 stimulates the VEGF gene expression by attaching to the hypoxia response element (HRE) in the VEGF promoter region[154]. HIF-1 expression is increased in several human malignancies and is linked to tumorigenesis and angiogenesis[114,155]. HIF-1 consists of HIF-1α and HIF-1β subunits[156,157]. All targeted agents approved by the FDA for systemic treatment of liver cancer block angiogenesis by interfering with VEGF signaling[158].

VEGF overexpression is associated with poor prognosis in HCC patients[159], and high levels of HIF-1α are linked to HCC progression and worse outcomes[160]. This suggested both VEGF and HIF-1α play important roles in the pathology of HCC.The atypical ubiquitination inhibitory activity of OTUB1 allows it to bind to HIF-1α and prevent its K48-linked ubiquitination, thereby stabilizing the HIF-1α protein[161]. This stabilization of HIF-1α by OTUB1 may contribute to cancer development and progression[161]. OTUB1 wasimplicated in hypoxia adaptation and in regulating the stability of vascular structures[88,162], suggested that OTUB1 may contribute to angiogenesis. Overexpression of OTUB1 promotes angiogenesis, migration, and proliferation in human hemangioma endothelial cells[163]. On the other hand, OTUB2 promotes the Warburg effect by regulating HIF-1α and c-Myc[18]. HIF-1α dependent signaling can be triggered by c-Myc[164]. Li et al. showed that OTUB2 enhances the production of HIF-1α and c-Myc proteins and triggers activation of the AKT/mTOR pathway[18]. It's worth noting that c-Myc and HIF1α, kidnown regulators of the Warburg effect, target glycolytic enzymes[165–168].

The Warburg effect can provide energy to cancer cells. The Warburg effect, characterized by increased aerobic glycolysis, is a common phenomenon in cancer cells. This metabolic alteration provides energy to tumor cells, contributing to their aggressive features such as heightened growth capacity, enhanced metastatic potential, and resistance to chemotherapy[169]. c-Myc and HIF-1α are two key factors that modulate the Warburg effect by targeting glycolytic enzymes[18,165–168]. According to the above studies, Otubains may affect the VEGF pathway by regulating HIF-1α to treat HCC. Therefore, more relevant clinical and basic research is needed to support it.

4.4.6. Otubains and RACK1 pathway in HCC

RACK1 is a conserved intracellular adapter protein that shares considerable similarities with Gβ[170,171]. OTUB1 has been shown to reduce the K48-linked ubiquitination of RACK1 and directly interact with oncoprotein RACK1 to increase its expression[109]. Through stabilizing the oncoprotein RACK1, it regulates HCC proliferation, metastasis, and progression[109]. RACK1 is also significantly expressed by activated hepatic stellate cells and is up-regulated in hepatocellular cancer patients[171]. OTUB2 promotes the Warburg effect by regulating HIF-1α and c-Myc[18]. PI3K/Akt/Rac1 pathway activation is triggered by RACK1[172], controling HIF-1 transcriptional activity[173,174], and is increased during angiogenesis[175]. The Warburg effect enables efficient proliferation of hepatocellular carcinoma (HCC) cells in a hypoxic environment, thereby promoting HCC cell proliferation[176, 177]. Moreover, c-Myc and K-Ras gene mutations enhance the expression of glucose transporters on the surface of HCC cells, leading to increased glucose uptake and further promotion of the Warburg effect [178,179]. Conversely, inhibition of hypoxia-inducible factor-1α expression significantly reduces GLUT1 expression and glucose uptake in HCC cells[180].Taken together, OTUB1 can regulate RACK1 to affect

HCC. However, whether OTUB2 can indirectly affect angiogenesis and promote the occurrence of HCC by regulating RACK1 remains to be studied.

4.4.7. Otubains and Hippo/TAZ pathway in HCC

The Hippo pathway exerts its crucial functions through its primary downstream effectors, Yes-associated protein (YAP)and Transcriptional coactivator with PDZ-binding motif (TAZ)[181]. This pathway stimulates apoptosis, restricts cell growth, and effectively inhibits tumor proliferation by phosphorylating and inhibiting YAP and TAZ transcriptional coactivator through kinase cascade[181]. The Hippo pathway thus acts as a tumor suppressor[182].

In the otubains subfamily, downregulation of OTUB1 inhibited the Hippo pathway signal transduction by regulating YAP protein levels, thereby inhibiting the proliferation of gastric cancer cells[85]. Surprisingly, despite its role as a tumor suppressor, YAP has been identified as a driver oncogene in human HCC[183].

In addition, overexpression of OTUB2 has the capability to directly stabilize and bind to YAP1/TAZ through small ubiquitin-like modifier (SUMO), activating YAP1/TAZ expression along with downstream effectors such as CYR61 and CTGF, contributing to tumor cell growth, invasion, and metastasis[105,106]. Zhang C et al. found that CYR61 can inhibit the migration, invasion, growth and proliferation of liver cancer by reducing the tumor-promoting effect of YAP[184]. The increased expression of CTGF is associated with the clinicopathological malignancy of HCC[185]. Interestingly, mutations in RAS oncogene implicated in SUMO pathway are likely the cause[106]. Lately, it was discovered that SUMO-1 was extremely low in liver tissues that were not cancerous, but it was significantly expressed in HCC cell lines and histological HCC samples[186]. These findings suggest that OTUB2 may regulate YAP1/TAZ expression, thereby influencing cellular behaviors related to tumorigenesis, invasion, and metastasis.

Therefore, OTUB1 and OTUB2 may inhibit the proliferation and invasion of HCC by regulating the Hippo/TAZ pathway. Further research is needed.

4.4.8. Otubains and non-coding RNA pathway in HCC

Non-protein-coding RNAs closely associated with cancer are long non-coding RNAs (lncRNAs) and short hairpin RNAs (shRNAs)[187,188]. Through the use of short hairpin RNA (shRNA), the endogenous expression of OTUB1 inhibits the migration, invasion, and proliferation of HCC cells[13]. In addition, lncRNA GAS6-AS2 uses 3′UTR to target miR-493–5p in order to control the expression of downstream OTUB1 [22]. Cell quiescence is reduced by activation of PI3K/Akt signaling, thereby inhibiting the growth of HCC cells[189,190]. According to research by Liang, C. et al., the down-regulation of the LncRNA GAS6-AS2 stimulates the invasion, growth, metastasis, and apoptosis of HCC cells by controlling the miR-493–5p/OTUB1 axis and triggering the PI3K/AKT/FoxO3a pathway[22]. In the study of OTUB2 and non-coding RNA, miR-29a-3p has been shown to affect thyroid cancer by targeting OTUB2[99], but not in hepatocellular carcinoma. The above studies suggest that OTUB1 can regulate HCC disease progression through

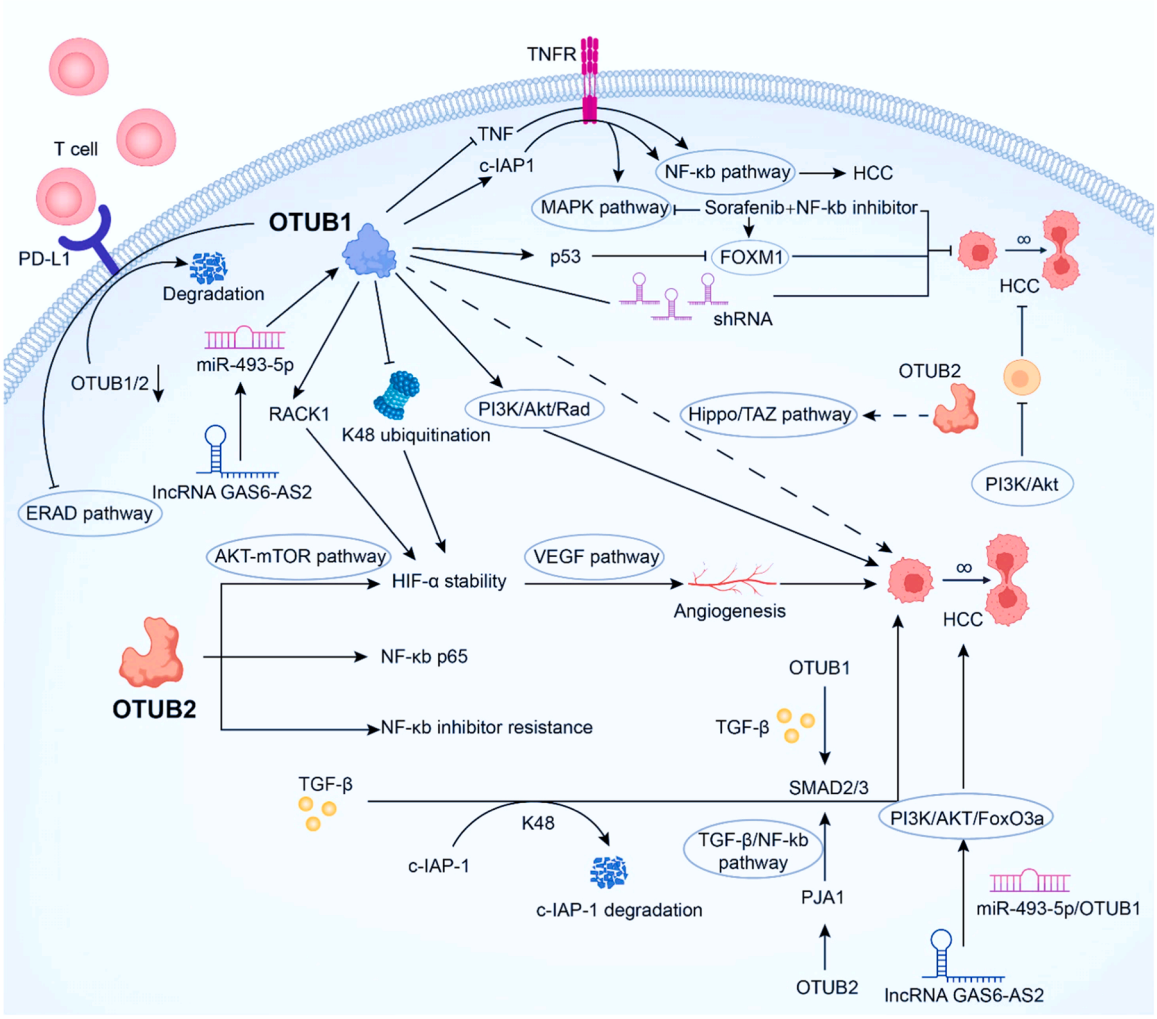

Figure 7. Otubains subfamily in HCC-related pathways (Dashed arrows indicate: potential; Solid arrows indicate: proven theories.).

non-coding RNA, while OTUB2 remains to be investigated in this regard (Fig. 7).

5. Otubains as potential therapeutic targets and prognostic indicators

Early-stage patients classified by BCLC exhibit asymptomatic conditions, well-preserved liver function, and a single tumor less than 2 cm in size[191]. Although alpha-fetoprotein (AFP) levels can aid in the diagnosis of HCC at early stages[192], their effectiveness is minimal [193]. Pain in the liver is often the primary symptom leading to a diagnosis of liver cancer. However, advanced HCC patients have poor prognoses after treatment. Therefore, early detection and treatment are crucial for managing HCC. Studies showed that OTUB1 and OTUB2 are highly expressed in liver tumor tissues[13,24,109], suggested the utility of otubains as biomarkers for HCC diagnosis.

For early-stage hepatocellular carcinoma (HCC), radical treatment strategies such as surgical resection and liver transplantation are the primary therapeutic options, while ablation may be considered in select cases[194]. However, the presence of a macrofocal or multifocal tumor combined with an underlying diseased liver state can sometimes preclude safe resection. In such instances, alternative treatment modalities like chemoembolization or radioembolization can be utilized[195]. Systemic therapy with sorafenib or best supportive care should be considered for patients with advanced hepatocellular carcinoma[196]. However, each of these treatments has its own side effects, and in severe cases, it is easy to cause liver failure[197]. Although the therapeutic effect of sorafenib has been remarkable, it is also of great significance to study its combination drugs. The current study demonstrates that by downregulating the expression level of SLC7A11, inactivation of OTUB1 can promote ferroptosis and inhibit cancer cell growth[83]. Furthermore, by blocking the transduction of NF-κB signals, down-regulation of otubain 2 expression can prevent liver cancer cells from proliferating [23]. Therefore, both OTUB1 and OTUB2 may be drug targets for HCC. It is of great significance to develop inhibitors of Otubains. Soon, it may also be combined with sorafenib to improve the quality of life of HCC patients.

During sorafenib treatment, elevated AFP levels are correlated with poorer clinical outcomes in patients experiencing tumor progression [198]. In cases of surgically removed hepatocellular carcinoma, overexpression of osteopontin has been linked to intrahepatic metastases, early recurrence, and a worse prognosis[199]. The expression of Otubains is significantly correlated with the prognosis of hepatocellular carcinoma (HCC) patients, wherein high expression levels may be indicative of a poorer prognosis. Otubains have been implicated in the promotion of cancer in liver cancer, primarily through their regulation of various signaling pathways, including NF-κB/TNF, FOXM1, VEGF, and TGF-β, which in turn enhances the survival, invasion, and drug resistance of tumor cells. By investigating the Otubain expression in HCC, we can enhance our ability to accurately predict patient survival and disease progression.

6. Challenges and opportunities

There are common protein interactions between OTUB1 and OTUB2 proteins. However, the specific pathways underlying their interactions are poorly understood. Cancer is a heterogeneous disease composed of various cell types. It remains unclear what functions OTUB1 and OTUB2 play and how they interact within the tumor and its microenvironment. For example, what the function looks like inside the immune cells surrounding the tumor cells, and what it looks like inside the mesenchymal cells surrounding the tumor cells. OTUB1 is a protein that exhibits deubiquitinase activity, enabling it to regulate a diverse range of signaling pathways and biological processes directly or indirectly[34]. These processes include cell proliferation, migration, apoptosis, DNA damage repair, immune response, and others. The expression and functionality of OTUB1 in liver cancer have garnered considerable attention in recent studies. It has been observed that OTUB1 can exert either promotional or inhibitory effects on the initiation and progression of liver cancer through distinct mechanisms. These mechanisms involve modulation of cell cycle dynamics, metabolism regulation, angiogenesis control, tumor microenvironment modulation, as well as drug resistance mediation.

OTUB1 and OTUB2 are potential therapeutic targets for hepatocellular carcinoma (HCC). Some studies have indicated that OTUB1 and OTUB2 can serve as biomarkers for diagnosing and treating HCC[13,23, 109]. However, there is a concern that chemotherapeutic drugs developed to target them might become resistant. The mechanisms of resistance to these drugs are not yet well understood. Clinical trials are necessary to test the effectiveness of these targets. Preclinical studies are also needed to verify their efficacy as diagnostic and prognostic biomarkers.

Downregulating the expression level or activity of OTUB1 and OTUB2 can impact HCC cells. Techniques such as RNA interference, gene knockout, and chemical inhibitors can be used. These interventions have the potential to inhibit cell growth, invasion, metastasis, and anti-apoptotic capabilities. However, they may also induce adaptive changes and resistance in these cells. Extensive preclinical and clinical trials are necessary to evaluate the therapeutic efficacy and safety of drugs targeting OTUB1 and OTUB2 expression in HCC. Some studies suggest that OTUB1 and OTUB2 can serve as biomarkers for diagnosing HCC. Developing targeted drugs for OTUB1 remains challenging. Comprehensive research is essential to understand the synergistic effects of OTUB1 and OTUB2 inhibitors with other treatments. Factors like molecular subtypes of liver cancer, tumor heterogeneity, signaling pathways, cellular stress responses, and immune evasion mechanisms need thorough investigation to enhance our understanding in this field.

7. Discussion and conclusions

The Otubain subfamily, including OTUB1 and OTUB2, plays a key role in tumor growth and immunologic escape of hepatocellular carcinoma (HCC) by regulating protein ubiquitination and deubiquitination. Specifically, Wenfeng Ren et al. discovered that OTUB2 plays a crucial role in maintaining the stability of PD-L1 protein by selectively cleaving the K48-linked polyubiquitin chain from PD-L1 while exerting no influence on K63-linked polyubiquitination[146]. However, it is not clear whether there is a non-canonical pathway for OTUB2. It is possible to use OTUB1 and OTUB2 as novel biomarkers and therapeutic targets because they are highly expressed in HCC cells and are linked to a poor prognosis. Furthermore, by means of their impact on diverse transcription factors and tumor-related signaling pathways like FOXM1, PD-L1/ERAD pathways, NF-κB/TNF, and TGF-β pathways. These pathways play a role in cell proliferation, migration, invasion, and immunologic escape in HCC cells. Additionally, they form a complex regulatory network by interacting with other deubiquitinating enzymes or ubiquitinating enzymes to impact the occurrence and development of HCC.

Due to their role in HCC, OTUB1 and OTUB2 may potentially be targets for future therapeutic strategies. Small molecule inhibitors or antibodies against these proteins may help to inhibit tumor growth and metastasis. The specific target proteins and genes of OTUB1 and OTUB2 should be fully elucidated, and the type and location of their deubiquitination modification need to be further elucidated. The expression and function of OTUB1 and OTUB2 are subject to regulation by various factors, including gene mutation, methylation, splicing, and post-translational modification. The role and mechanism of these factors in liver cancer need to be investigated. Furthermore, comprehensive clinical data and experimental verification are required to ascertain the differences in specificity between OTUB1 and OTUB2 across different subtypes, stages, and prognoses of liver cancer.

Although OTUB1 and OTUB2 show promise as molecular targets,

more clinical research is required to confirm their effectiveness and potential applications, as their precise mechanism of action in HCC is still unknown. To develop and screen OTUB1 and OTUB2 specific inhibitors or activators as novel therapeutic drugs or adjuvant therapy for hepatocellular carcinoma (HCC), CRISPR/Cas9 and other gene editing technologies were used to construct OTUB1 and OTUB2 gene knockout or overexpression HCC cell or animal models. These models were also used to investigate the roles and processes of OTUB1 and OTUB2 in HCC. By integrating transcriptome, proteome, ubiquitin, and other omics analyses, the comprehensive regulatory network and crucial nodes associated with OTUB1 and OTUB2 in liver cancer are elucidated. Additionally, new molecular markers for early diagnosis and prognosis evaluation of liver cancer are identified.

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CRediT authorship contribution statement

Yanming Wu: Writing – review & editing, Writing – original draft, Software, Resources, Conceptualization. **Sa'udah Badriah Mohd Sani:** Writing – original draft, Software, Resources. **Ke Peng:** Writing – review & editing, Resources. **Tao Lin:** Writing – review & editing. **Chenghao Tan:** Writing – review & editing. **Xufeng Huang:** Writing – original draft, Supervision, Software, Resources, Conceptualization. **Zhengrui Li:** Writing – review & editing, Supervision, Formal analysis, Funding acquisition,Conceptualization.

Declaration of Competing Interest

The authors declare that Copilot and QuillBot tools were used for translation and language polishing in the writing of this manuscript. This tool was used only to improve the linguistic quality of the articles and was used under the supervision of a human author. The authors take full responsibility for the accuracy and completeness of the content of the article and confirm that the tool was not used for any activity that might give rise to a conflict of interest other than the use described above. The views expressed in this article are solely those of the authors and have not been influenced by any funding agency or commercial entity.

Data availability

Availability of data and material not applicable.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2024.117348.

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