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Review Article

Diagnostic and Prognostic Value of Protein Post-translational Modifications in Hepatocellular Carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is a common malignant tumor with high incidence and cancer mortality worldwide. Post-translational modifications (PTMs) of proteins have a great impact on protein function. Almost all proteins can undergo PTMs, including phosphorylation, acetylation, methylation, glycosylation, ubiquitination, and so on. Many studies have shown that PTMs are related to the occurrence and development of cancers. The findings provide novel therapeutic targets for cancers, such as glypican-3 and mucin-1. Other clinical implications are also found in the studies of PTMs. Diagnostic or prognostic value, and response to therapy have been identified. In HCC, it has been shown that glycosylated alpha-fetoprotein (AFP) has a higher detection rate for early liver cancer than conventional AFP. In this review, we mainly focused on the diagnostic and prognostic value of PTM, in order to provide new insights into the clinical implication of PTM in HCC.

Keywords: Diagnosis; Hepatocellular carcinoma; Post-translational modification; Prognosis.

Abbreviations: AFP, alpha-fetoprotein; AFP-L3, N-glycosylated isoform of AFP; AGP, α-1-acid glycoprotein; APOH, β-2-glycoprotein 1; AR, androgen receptor; Asn, asparagine; ATPB, ATP synthase subunit beta; AUROC, area under the curve of receiver operating characteristic; A1AT, Alpha-1-antitrypsin; CCA, cholangiocarcinoma; CE, ceruloplasmin; C3, complement C3; DFS, disease-free survival; Fuc-GP73, fucosylated GP73; Fuc-PON1, fucosylated paraoxonase 1; GPC3, glypican-3; GP73, Golgi phosphoprotein 73; HAT, histone acetyltransferase; HBB, hemoglobin subunit beta; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus, ; HDAC, histone deacetylase; HGF, hepatocyte growth factor; Hp, haptoglobin; HRG, histidine-rich glycoprotein; IHC, immunohistochemistry; MMP2, matrix metalloproteinase-2; MUC 1, mucin 1; M2BPGi, Mac-2-binding protein glycosylation isomer; NAFLD, Nonalcoholic fatty liver disease; ORM, orosomucoid; ORM2, α-1-acid glycoprotein 2; OS, overall survival; PCK1, phosphoenolpyruvate carboxykinase 1; PRMT5, protein arginase methyltransferase 5; PTM, post-translational modification; PVTT, Portal vein tumor thrombosis; SDH, succinate dehydrogenase; TACE, transcatheter arterial chemoembolization; TNFR1, tumor necrosis factor receptor 1; USP, ubiquitin-specific protease; 4E-BP1, 4E-Binding Protein 1.

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Introduction

About 80% of primary liver cancers are hepatocellular carcinomas (HCCs), which are malignant neoplasms of hepatocytes. One of the leading causes of cancer-related mortality around the world is HCC.[1] In developing countries, the incidence of HCC is higher compared with developed countries. HCC occurs mainly in East Asia, Southeast Asia, and Africa.[2] There is an association between chronic hepatitis B virus (HBV) or hepatitis C virus (HCV) infection and approximately 80% of cases of HCC.[3,4] Additionally, alcoholism, aflatoxin infection, smoking, and various metabolic diseases all contribute to HCC risk. However, the causes of liver cancer are gradually changing from viral and alcoholic liver disease to obesity, type 2 diabetes mellitus and nonalcoholic fatty liver disease (NAFLD).[5] The resulting molecular and signal transduction network disturbances, genomic instability, and microenvironmental differences account for HCC heterogeneity. Various therapies have been developed for the treatment of liver cancer, including local area therapy, systemic chemotherapy, hormone therapy, molecular targeted therapy, immune checkpoint therapy, surgical resection, and liver transplantation.[6,7] Although these methods can be used to treat HCC, the side effects of various drugs, high recurrence rate after treatment, and low rate of transplantation all lead to a low HCC cure rate.[8–10]

During translation, proteins undergo a series of chemical modifications. These post-translational modifications (PTMs) have a great impact on protein function, including enzyme activation and inactivation, protein stability, subcellular localization, interaction, and PTM crosstalk.[11] At present, many studies have shown that the development of a wide range of diseases is linked to PTMs, such as tumors, neurological diseases, metabolic diseases, and immune diseases.[12–15] Currently, approximately 400 types of PTMs are known to exist. Almost all proteins can undergo PTMs, including phos-

phorylation, acetylation, methylation, glycosylation, ubiquitination, and so on.[16,17] Phosphorylation is one of the most common and well-studied PTMs. Protein phosphorylation is a reversible process. Protein kinase can covalently transfer a phosphate group to an amino acid residue, while protein phosphatase removes the phosphate group.[18] Phosphorylation of proteins can further lead to activation of some signaling pathways, which then affect downstream molecules and promote the occurrence, proliferation, migration, and invasion of tumors.[19] Acetylation modification of proteins mainly includes histone and nonhistone acetylation modification. At present, histone acetylation is more studied in liver cancer than nonhistone acetylation.[20] A balance exists between histone acetyltransferases (HATs) and histone deacetylase (HDAC) activities that regulate histone acetylation. Gene silencing occurs through histone deacetylation induced by HDAC, while histone acetylation induced by HAT is linked to gene transcription.[21] Protein acetylations have been reported to be abnormally expressed in HCC tumor tissues and have shown a connection with clinical stage, prognosis, and survival. Tumor cells with altered glycosylation are common in humans and the heterogeneity and functional diversity of tumor molecules are mainly derived from the diversity of glycosylation.[22] At least nine basic amino acid residues can have their structure changed by sugar groups. There are two main protein glycosylation pathways, namely N-linked and O-linked. The most common glycosidic bond in nature is N-glycosylation and the part of the asparagine (Asn) part of the protein that is linked to the carbohydrate is the N-glycosidic bond.[23] O-glycosylation usually refers to the ligation of O-glycans to Ser or Thr residues on proteins by enzymatic reactions.[24] Altered glycosylation profiles have been proved to associated with many cancers including liver, breast, and lung.

PTM studies provide new insights into the diagnosis, prognosis, and therapy of cancers. The clinical implication of PTMs in HCC have been identified in a number of articles. The glycoprotein, alpha-fetoprotein (AFP), has been used widely as a biomarker for HCC. The clinical utility of AFP has been recently reviewed.[25–28] Glypican-3 (GPC3), as a member of the proteoglycan family, is a promising therapeutic target and biomarker for the diagnosis and prognosis of HCC. Further discussion of the clinical value of GPC3 in HCC can be found in several reviews.[29–32] This review provides an overview of the diagnostic and prognostic value of major types of PTM in HCC.

Diagnostic value of PTM in HCC

A set of glycoproteins, including AFP, AFP-L3, Golgi phosphoprotein 73 (GP73), GPC3, Fuc-GP73 and fucosylated paraoxonase 1 (Fuc-PON1), are considered to be diagnostic markers for HCC.

AFP and its fucosylation

AFP, as a glycoprotein, is the most widely used serum biomarker for the diagnosis of HCC worldwide. However, the elevation of AFP is not detected in many HCC patients, and AFP may be elevated in cirrhosis or hepatitis cases.[33] Many studies aim to search for meaningful diagnostic markers in patients with liver cancer whose AFP is negative.[34] The N-glycosylated isoform of AFP (AFP-L3), which contains core fucosylation on its N-linked glycans, shows potential ability for diagnosing AFP-negative HCC. Zhang *et al*.[35] detected AFP-L3 in the serum of 50% patients with liver cancer, only 3.33% of patients with other liver diseases, and 2.00% of healthy participants. Studies shown that AFP-L3 promoted the proliferation of cancer cells by activating the Wnt-β-catenin pathway and promoted the invasion and metastasis of liver cancer cells by activating the downstream TGF-β and VEGF pathways.[36]

GP73 and its fucosylation

GP73, a transmembrane glycoprotein, was detected in 66% of AFP-negative HCC, 10% of non-HCC participants, and 0% of healthy participants.[35] Hu *et al*.[37] measured serum levels of GP73 in a hepatitis B-endemic Asian population and showed the area under the curve of the receiver operating characteristic (AUROC) was 0.89. GP73 downregulation led to the accumulation of matrix metalloproteinase-2 (MMP2), which inhibited the activation of AAPK/JNK and P53-P21 pathways, and attenuated cell invasion. The two pathways also regulated MMP2 activity by a negative feedback mechanism.[38] Fucosylation is a type of glycosylation. Zhao *et al*.[39] found that the AUROC of fucosylated GP73 (Fuc-GP73) for diagnosis of HCC was 0.885, with a specificity of 95% and a sensitivity of 82%.

APOH, ORM2, and C3

Cao *et al*.[40] designed a straightforward and highly efficient scheme to identify glycoprotein biomarkers using a nonglycopeptide-based mass spectrometry pipeline. The diagnostic sensitivities were 0.901 for APOH, 0.945 for ORM2, 0.944 for C3, while the diagnostic sensitivity of AFP was only 0.633. The results showed that three glycoproteins, β-2-glycoprotein 1 (APOH), α-1-acid glycoprotein 2 (ORM2), and complement C3 (C3) could be used as biomarkers to distinguish HCC patients from healthy individuals. APOH interacted with hepatitis B surface antigen (HBsAg) to activate the NF-κB pathway, thus promoting tumor cell proliferation.[41] Fang *et al*.[42] reported that ORM2 was regulated by CCAAT/enhancer-binding protein β and inhibited the progression of liver cancer. Glycosylation of C3 affected various biological functions of C3. C3 activated the P38-MAPK signaling pathway, thus inhibiting the secretion of cytotoxic T cells and leading to tumor cell proliferation.[43]

Glycosylation of α-1-acid glycoprotein (AGP)

AGP, with an official name of orosomucoid (ORM), is also known as AAP. Tanabe *et al*.[44] assessed glycopeptides obtained from serum proteins of 42 HCC patients and 80 controls by liquid chromatography time-of-flight mass spectrometry and revealed that AGP with multifucosylated tetra-antennary N-glycans was higher in HCC patients. High levels of sialylated and fucosylated peptides from AGP were reported in HCC patients compared to controls. The diagnostic potential of these glycopeptides was reported to differentiate HCC patients from cirrhosis participants with AUCs greater than 0.9.[45]

Fucosylated paraoxonase 1 (Fuc-PON1)

Zhang *et al*.[46] used an ELISA index to assess the fucosylation level of PON1. The utility of Fuc-PON1 in distinguishing HCC from liver cirrhosis was indicated by an AUC of 0.803, sensitivity of 80% and specificity of 64.4%. In addition, the data showed a better AUROC curve and higher sensitivity and specificity in AFP-negative patients.

Fucosylation of alpha-1-antitrypsin (A1AT)

Glycosylation of A1AT increases with the development of HCC, and there are five major isoforms. A patient cohort of 458 patients was used to evaluate the lever of fucosylated A1AT

compared with 375 patients with other liver diseases and 20 with no evidence of liver disease. Core fucosylation was observed only in patients with HCC. The AUROC of fucosylated A1AT was 0.871, suggesting core fucosylation of A1AT had the ability to be used as a diagnostic biomarker for HCC.[47]

Glycosylation of haptoglobin (Hp)

Hp is an acute-phase response protein secreted by the liver. Various types of glycosylation of Hp were reported to be higher in HCC. Ang *et al*.[48] performed a systematic analysis of serum concentrations of Hp and its glycosylation in HCC patients and chronic liver diseases. Using Hp for HCC diagnosis, the sensitivity could be 79% with the specificity of 95%. Serum Hp concentrations of hypersialylated fucosylated and hyposialylated fucosylated species were significantly increased in patients with advanced HCC. Five N-glycopeptides at sites N184 and N241 of Hp were found to be significantly increased during the progression of cirrhosis to HCC. The glycopeptides had a diagnostic potential in detection of HCC, with an AUC greater than AFP.[49] In addition, a total of 26 complete O-glyopeptides that could be used to distinguish HCC from liver cirrhosis were discovered on Hp using mass spectrometry.[50]

C3, CE, HRG, CD14, HGF

Ceruloplasmin (CE), a glycoprotein, played an important role in iron homeostasis. In liver cancer cells, the absence of CE promoted the accumulation of lipid reactive oxygen species, which led to ferroptosis.[51] In the presence of tumor necrosis factor receptor 1 (TNFR1), HRG bound to TNFR1 to promote apoptosis and inhibit the activation of NF-κB signaling pathway and the expression of survival-promoting genes.[52] In addition, it was reported that HRG inhibited cell proliferation by inhibiting FGF-ERK1/2 phosphorylation.[53] Liu *et al*.[54] developed an integrated platform to discover glycoprotein biomarkers in early HCC. The data indicated C3, CE, histidine-rich glycoprotein (HRG), CD14, and hepatocyte growth factor (HGF) were biomarker candidates for distinguishing early-stage HCC from cirrhosis. The combination of the five proteins had an AUROC of 0.811 and the AUROC curve of each glycoprotein were better than AFP of 0.661.

Combination of biomarkers

Meta-analyses and studies of combinations of biomarkers or patient characteristics were applied to investigate the diagnosis of HCC. AFP-L3 % is the ratio of AFP-L3 to total AFP in serum. Marrero *et al*.[55] performed a phase 2 biomarker case-control study with 836 patients at seven academic medical centers. The results showed that the AUC of AFP was better than that of AFP-L3% in ROC analysis for the diagnosis of early-stage HCC.[55] However, Leerapun *et al*.[56] found that when AFP-L3% was greater than 35%, the specificity reached 100% for HCC patients with AFP values of 10–200 ng/ml.[56] In a meta-analysis that included 2,447 patients, Zhou *et al*.[57] reported that AFP-L3% had a high pooled specificity (92%), low pooled sensitivity (34%), and moderate AUC of the summary ROC curve (0.755) for HCC diagnosis. The results suggested that AFP-L3% could be used as an adjunct biomarker for HCC diagnosis. In a recent prospective phase III biomarker study of 534 patients. Tayob *et al*.[58] showed that GALAD (a combination of Gender, Age, AFP-L3, AFP, and DCP) significantly improved the sensitivity of HCC detection, but with an increasement in the false-positive rate.[58] The results of two meta-analyses indicate that a combination of AFP and GP73 had the best AUCs in the diagnosis of HCC among AFP, AFP-L3, GP73, and DCP alone or combined.[59,60] The combination of AGP and AFP had an AUC of 0.943, whereas AFP and AAG had AUCs of 0.750 and 0.907 respectively, to differentiate HCC from chronic liver disease. The data indicate that combining of AAG and AFP improved the diagnostic potential of HCC.[61]

H2A.Z acetylation and ALDOA phosphorylation

In addition to glycoproteins, other types of posttranslational modifications of protein are involved in HCC development and be potential biomarkers. Histone variant H2A.Z is involved in the proliferation, cell cycle, apoptosis, and metastasis of HCC cells. Acetylated HA2.Z inhibited the transcription of downstream target genes and thus affected various tumor behaviors. Yuan *et al*.[62] measured the acetylation level of H2A.Z and found that it was elevated in HCC cells and tissue samples. The results indicate that H2A.Z and its acetylation had diagnostic potential for HCC. Gao *et al*.[63] conducted a proteogenomic study of HBV-associated HCC. The results showed that in CTNNB1-mutated tumors, glucose metabolism was regulated by Ser36 phosphorylation of ALDOA and further affected cell proliferation. Increased Ser36 phosphorylation of ALDOA was found in CTNNB1-mutated tumors and was used as a potential diagnostic biomarker for such tumors.[63]

Prognostic value of PTM in HCC

Phosphorylation of p53 at serine 15 (p53 Ser15-P)

Phosphorylation of p53 at serine 15 (p53 Ser15-P) may be a prognostic marker of HCC. P53 Ser15-P was found to inhibit tumor progression by binding with p21 to stop cell cycle progression. Yang *et al*.[64] performed an immunohistochemistry analysis to determine the prognostic value of PCNA, p53, p53 phosphorylation at serine 15 (p53 Ser15-P) and Ser392 (p53 Ser392-P) in 199 patients with HCC. The results showed that the levels of p53 Ser15-P, but not p53 or p53 Ser392-P, were correlated with 5-year survival of HCC. Moreover, patients with positive PCNA and negative p53 Ser15-P had worse survival outcomes than those with positive PCNA and positive p53 Ser15-P. The results indicate that p53 Ser15-P was a prognosis marker not only of overall HCC but also of patients with positive PCNA.[64] Identification of poor survival groups by monitoring p53 Ser15-P in PCNA-positive patients may contribute to the treatment of liver cancer, especially in PCNA-positive patients.

Phosphorylation of PCK1, INSIG1, and INSIG2

Phosphorylated PCK1 was shown to reduce binding of INSIG1/2 to SCAP, leading to translocation of the SCAP/SREBP complex to the Golgi. Activation of SREBP proteins was related to transcription of downstream lipid-related genes, tumor cell proliferation, and tumorigenesis in mice. Xu *et al*.[65] reported the phosphorylation at Ser90 of phosphoenolpyruvate carboxykinase 1(PCK1), Ser207 of INSIG1, and Ser151 of INSIG2 was positively correlated with nuclear accumulation of SREBP1 in HCC samples. IHC analysis showed that increased levels of phosphorylation of PCK1 Ser90 and INSIG1 Ser207/INSIG2 Ser151 were associated with reduced overall survival in 90 HCC patients.

Androgen receptor (AR) phosphorylation at Ser96

Ren *et al*.[66] performed IHC staining on human steatosis liver tissue and HCC samples using anti-ARpS96 antibodies. High

AR S96 phosphorylation was found in human liver adipose tissue and HCC tissue. Survival analysis showed that p-AR S96 expression was associated with HCC survival and was an independent risk factor for OS in HCC patients. MTOR signaling stimulated AR phosphorylation. Phosphorylation at Ser96 increased the stability and transcriptional activity of AR and activated downstream SREBP signaling, which enhanced liver steatosis and hepatocarcinogenesis in mice.[66]

Recombinant Human Eukaryotic Translation Initiation Factor 4E-Binding Protein 1 (4E-BP1) phosphorylation at Thr46

HCC with early formation of portal vein tumor thrombosis (PVTT) had a higher risk of metastasis. Lin *et al*.[67] performed a phosphorylated proteomic analysis and identified a total of 1,745 phosphoproteins in HCC tissues, normal tissues, and PVTT tissues. The results showed that HCC and PVTT tissues had higher phosphorylation levels of 4E-BP1 than surrounding noncancerous tissues. The expression of phosphorylated 4E-BP1 in patients who relapsed within 1 year was significantly higher than that in patients who relapsed after 3 years. The reduction of mTOR signal stimulated phosphorylation of 4EBP1. Phosphorylation of 4E-BP1 weaken its binding to eukaryotic translation initiation factor 4E, thereby promoting the initiation of protein translation and promoting tumor cell proliferation. Therefore, the prediction of early recurrence of HCC may be assisted by 4E-BP1 with phosphorylation at Thr46 as a reliable biomarker.[67]

Acetylation of AFP

Acetylation at lysines 194, 211, and 242 of AFP were reported to enhance the protein stability of AFP and strengthen its oncogenic function by inhibition of binding to the phosphatase PTEN and the pro-apoptotic protein caspase-3. Results of the immunostaining of 70 HCC liver specimens showed that patients with higher levels of AcK194-AFP, AcK211-AFP, and AcK242-AFP had poorer progression-free and overall survival. Moreover, elevated acetylation levels of AFP were correlated with metastasis and HBV infection. The data suggest that acetylation of AFP played a vital role in HCC development and could serve as a novel potential marker for the prognosis of HCC.[68]

Acetylation and methylation of histone

Histone-related modifications also have an important role in tumors. Acetylation of lysine 120 on histone H2B (H2B-K120ac), lysine 18 on histone H3.3 (H3.3K18ac), and lysine 77 on histone H4 (H4K77ac) was upregulated in HCCs compared with paracancerous or normal liver tissues. Patients with high acetylation levels of all three histones had obviously worse OS than patients with low acetylation levels.[69] He *et al*.[70] performed immunohistochemical experiments and statistical analysis to assess the expression and clinicopathologic association of methylation of lysine 4 in histone H3 (H3K4me3) in two cohorts of HCC patients. The results revealed that high expression of H3K4me3 was associated with worse survival in both the testing cohort and validation cohort. However, high level of H3K4me3 discriminated differences in OS for the subset of patients with TNM stage III/IV only in the testing cohort. Multivariate analysis was carried out to indicate that the expression of H3K4me3 was a significant independent prognosis factor for poor overall survival in both the testing cohort and validation cohort.

Mac-2-binding protein glycosylation isomer (M2BPGi)

Serum Mac-2-binding protein glycosylation isomer (M2BPGi) is a novel glycoprotein biomarker for liver fibrosis or cirrhosis. M2BPGi enhanced the migration and invasion of HCC via the mTOR signaling pathway.[71] Tak *et al*.[72] studied the serum M2BPGi levels of 226 HCC patients received transcatheter arterial chemoembolization (TACE). The study demonstrated that patients with low M2BPGi levels had significantly better OS and PFS than those with high M2BPGi levels. The hepatoma arterial embolization prognostic (HAP) score is a prognostic tool based on albumin, bilirubin, AFP, levels, and tumor size.[73] Combination of serum M2BPGi and the HAP score increased the prognostic ability. Serum M2BPGi level is a useful prognostic indicator for survival of HCC patients treated with TACE.

Human C3

Human C3 in HCC plasma was reported to have high-mannose and hybrid glycoforms at Asn85 with the utility of post-proteomic site-specific N-glycan analysis. The level of plasma mannose-5 or mannose-6 glycoform at Asn85 of C3 was significantly associated with the HCC tumor grade. Low tumor recurrence and mortality rates were found in the plasma of HCC patients with C3 with a hybrid glycoform at Asn85. The results suggest that specific plasma N-glycoproteins are potential noninvasive markers of HCC prognosis.[74]

Conclusions and perspectives

PTM of proteins affects the biology of almost all normal cells and the pathogenesis of various diseases. Differences in PTMs provide novel insights into the clinical of HCC. In this review, various PTMs, mainly including phosphorylation, glycosylation, acetylation, and methylation of proteins that had diagnostic and prognostic value for HCC were summarized (Fig. 1, Tables 1 and 2[35,37,39,40,44-50,54,57,59,60,64-70,72,74]). Some PTMs had clinical value for the therapy of HCC. GPC3, as a member of the proteoglycan family, was a promising therapeutic target of HCC. A total of 33 GPC3-targeted CAR-T trials were registered at ClinicalTrials.gov (https://clinicaltrials.gov/) as of December 21, 2022. The initial safety of CAR-GPC3 T cell therapy has been demonstrated in phase I Trials.[75] Phase II clinical trials for two GPC3-targeted therapies for HCC are presently underway. One patient with advanced HCC had complete tumor resolution 30 days after intratumoral injection of anti-GPC3-7 × 19 CAR-T (a CAR-T cell expressing IL7 and CCL19).[76] Wu *et al*.[77] constructed a mouse model to demonstrate the potential of sorafenib in combination with GPC3-targeted CAR-T cells for the treatment of HCC. Meanwhile, because of the shedding of GPC3 from the cell surface, the content of GPC3 in the serum of HCC patients is high. Sun *et al*.[78] found that shed GPC3 competed with GPC3 on the cell membrane for CAR-T binding, thus contributing to immune escape of HCC cells. Further discussion on the clinical value of GPC3 in HCC can be found in r reecent views.[29,30] Mucin 1 (MUC 1), as a tumor-associated antigen with high glycosylation, is highly expressed in HCC. Currently, a few promising clinical trials of immunotherapies targeting MUC 1 are ongoing.[79] A phase I clinical trial of MUC1-targeting TILs/CAR-TILs cells treatment for HCC was initiated in 2021. In addition, some targets have clinical therapeutic potential, but have not yet entered the clinical trial stage. Animal studies have found that the interaction between phosphorylated p62 and Keap1 can inhibit tumor development, and thus inhibitors of this process have the potential to be used as therapeutic drugs for human HCC.[80] Inhibition of NF-κBp65 phosphorylation might inhibit the occurrence of HCC, suggesting that NF-κBp65 phosphorylation as a new therapeutic target for

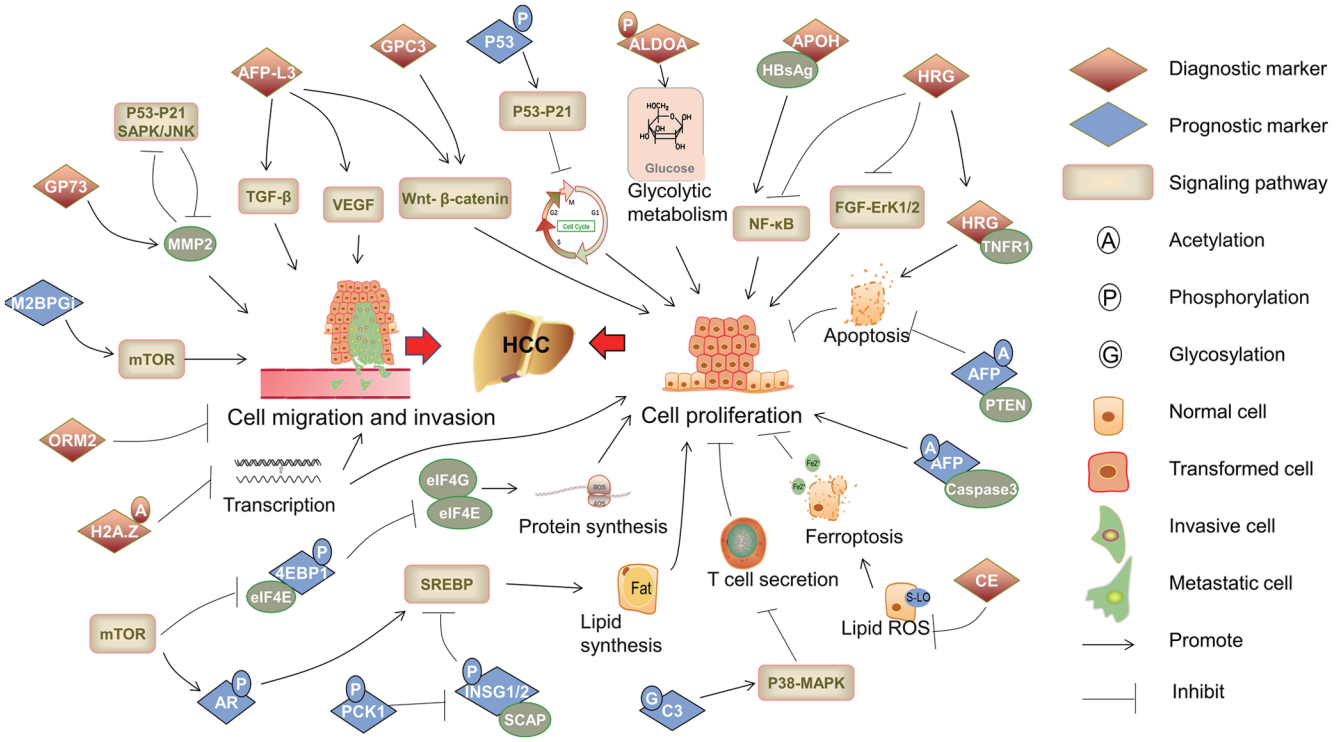

Fig. 1. The mechanisms affecting tumor behavior by diagnostic and prognostic PTMs in HCC. PTM, post-translational modification; HCC, hepatocellular carcinoma.

HCC.[81] In a study by Li *et al*.,[82] computer-aided screening and inhibition assays were used to identify inhibitors of CD147 glycosylation, and finally, compound 72 (methyl 3′-(4-chlorophenyl)-4′,5′-dihydro-[3,5′-biisoxazole]-5-carboxylate), was the best candidate for CD147 inhibition, which provided new ideas for the design of CD147 glycosylation targeting drugs in HCC treatment.[82]

In addition, many enzymes that regulate PTMs are promising targets for HCC treatment. Protein arginase methyltransferase 5 (PRMT5), a protein methyltransferase, had an important role in carcinogenesis. A novel PRMT5 inhibitor, DW14800, suppressed tumor growth *in vitro* and *in vivo* by promoting the transcription of HNF4α.[83] Luo *et al*.[84] found that another inhibitor of PRMT5, GSK3326595, increased the infiltration of immune cells in tumors of mouse models and improved therapeutic effects in HCC. In the treatment of cancer, drug resistance is a major problem. Therefore, improving the drug sensitivity of tumors is an important research direction. HDAC was found to deacetylate histones and to have an important role in chromosome structural modification and gene expression regulation. Bi *et al*.[85] found that patients treated with sorafenib therapy with low levels of HDAC11 had an increased OS. In addition, various cytological and mouse experiments have demonstrated that HDAC11 could protect HCC cells from sorafenib-induced cytotoxicity, providing a new target for addressing sorafenib resistance during HCC treatment. 5-FU is a substrate of organic anion transporter 2 (OAT2). HDAC inhibitor SAHA reversed the histone deacetylation status of OAT2 and enhanced its interaction with 5-FU, thereby increasing the sensitivity of liver cancer cells to 5-FU.[86] The ubiquitin-specific protease (USP)family is the largest class of deubiquitination enzymes and is related to a variety of signaling pathways in biological processes. USP7 inhibitor P22077 could inhibit tumor growth in nude mice.[87] Ubiquitin-conjugating enzyme UBE2S was found to promote the development of HCC by accelerating the cell cycle. Zhang *et al*.[88] demonstrated that the small-molecule cephalomannine attenuate the malignant progression of HCC by inhibiting the expression of UBE2S both *in vitro* and in vivo.

There are also some post-translational modification protein-targeting drugs in clinical trials in cancer types other than HCC. CD52 is an anchor glycoprotein mainly distributed on lymphocytes and lymphoid tumor cells. Anti-CD52 monoclonal antibodies (Campath and Lemtreda) have been approved for the treatment of chronic B-cell leukemias and multiple sclerosis in the USA and the European Union. CD47 is an important tumor antigen that is involved in the occurrence and development of various cancers. The anti-CD47 monoclonal antibody CC-90002 was investigated in a phase I study in patients with relapsed/refractory acute myeloid leukemia and high-risk myelodysplastic syndromes.[89] SRF231, a fully human IgG4 anti-CD47 antibody, has completed phase I clinical trials in advanced solid and hematologic cancers (NCT03512340). Currently, there are a total of 32 CD70-targeting agents in clinical trials, mainly for renal cell carcinoma and hematological tumors. Although these targets were not reported in HCC, these therapeutics might also provide insight into HCC.

In addition to the PTMs described in this review, there are some modification types with certain clinical implications in HCC. S-nitrosylation of endothelial proteins may regulate angiogenesis, adhesion of tumor cells to the endothelium, intra- and extravasation of tumor cells, and contribute to metastasis.[90] Khan *et al*.[91] used anti-SNO-cysteine for immunoblotting and identified a novel and biologically relevant post-translational modification of CYB5A thiol only in HCC specimens. Two other nuclear envelope proteins, ATP syn-

Table 1. Diagnostic value of post-translational modification of proteins in hepatocellular carcinoma

Protein	Modified type	No. of sample	Sensitivity	Specificity	AUC	Reference
AFP-L3	Glycosylation	130	50.0%	97.5%	–	35
GP73	Glycoprotein	130	66.0%	96.2%	–	35
Fuc-GP73	Fucosylation	124	77.4%	83.9%	0.89	37
Fuc-GP73	Fucosylation	150	82%	95%	0.885	39
APOH	Glycoprotein	62	90.1%	–	Combination of the three glycoproteins and AFP was 0.978	40
ORM2	Glycoprotein	62	94.5%	–		
C3	Glycoprotein	62	94.4%	–		
AGP	Glycosylation	122	93%	86%	0.98	44
AGP	Sialylation and fucosylation	259	–	–	Over than 0.9	45
Fuc-PON1	Fucosylation	180	80%	64.4%	0.803	46
A1AT	Fucosylation	853	70%	86%	0.871	47
Hp	Fucosylation	96	79%	95%	0.733	48
Hp	Glycosylation at sites N184 and N24	70	73%	70%	0.733, 0.775	49
Hp	Glycosylation	158	–	–	–	50
C3, CE, HRG, CD14, HGF	Glycoprotein	74	72%	79%	Combination of five proteins was 0.811	54
AFP-L3%	Glycoprotein	2,447	34%	92%	0.755	57
AFP+GP73	Glycoprotein	28 articles	The sum of sensitivity and specificity was 1.76		0.93	59
AFP+GP73	Glycoprotein	40 articles	–	–	0.943	60

AFP, Alpha-fetoprotein; AFP-L3, N-glycosylated isoform of AFP; AGP, α-1-acid glycoprotein; APOH: β-2-glycoprotein 1; AUROC, area under the curve of receiver operating characteristic; A1AT, Alpha-1-antitrypsin; CE, ceruloplasmin; C3, complement C3; Fuc-GP73, fucosylated GP73; Fuc-PON1, fucosylated paraoxonase 1; GP73, Golgi phosphoprotein 73; HCC, hepatocellular carcinoma; HGF, hepatocyte growth factor; Hp, haptoglobin; HRG, histidine-rich glycoprotein; ORM2, α-1-acid glycoprotein 2.

thase subunit beta (ATPB) and hemoglobin subunit beta (HBB) were found to be nitrosylated in HCC. S-nitrosylation of mitochondrial chaperone TRAP1 led to loss of S-nitrosoglutathione reductase (GSNOR) and increased succinate dehydrogenase (SDH) levels and activity. Thus, S-nitrosylation of mitochondrial chaperone TRAP1 enhanced the sensitivity of HCC cells to succinate dehydrogenase inhibitors.[92] S-palmitoylation is is the attachment of fatty acids (lipidylation), such as palmitic acid, to cysteine of proteins. Oncoproteins such as RAS-family GTPases require palmitoylation to promote tumor formation.[93] Sun *et al*.[94] designed a peptide containing a mutant site to compete for S-palmitoylation of PCSK9 *in vivo*, and confirmed that the inhibitor enhanced the inhibitory effect of sorafenib on hepatoma cells by both *in vivo* and *in vitro* experiments.[94] The data suggest that some uncommon PTMs may also have clinical significance in HCC.

Because of the diversity and dynamics of the immune system and the heterogeneity between and within tumors, to receive a sustained response for cancer therapy in all patients is challenging.[95] Single-cell technologies, including single-cell proteomics, make it possible to assess the heterogeneity of tumor, microenvironmental cell type composition, and cell state transitions that influence therapeutic response.[96] Krieg *et al*.[97] determined subsets of immune cells in peripheral blood samples from patients with metastatic melanoma before and after 12 weeks of immunotherapy against PD-1 using high-dimensional single-cell mass cytometry. With this single-cell proteomic profiling, a class of monocyte was identified that was associated with better treatment response and patient survival prior to anti-PD-1 therapy. With the advancement of single-cell proteomics, the clinical value of single-cell PTM is achievable.

There have been many studies on post-translational modification of liver cancer, but there are still few biomarkers or drugs available for clinical use. It is important to identify novel PTM biomarkers of diagnosis or prognosis and to conduct clinical trials to test the clinical value of the present studies. Finding abnormal PTM of molecular targets in cancer and understanding the mechanisms of the modification after translation is helpful to reveal the process of tumor progression and provide novel target of therapy.

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Table 2. Prognostic value of post-translational modification of proteins in hepatocellular carcinoma

Protein	Modified type	No. of sample	*P* values for OS	HR(95%CI)	Other significance	Reference
P53	Phosphorylation at Ser15	199	0.016	0.614 (0.418–0.902)	Correlation with 5-year survival of patients (p=0.013)	64
PCK1; INSIG1; INSIG2	Phosphorylation at Ser90; Ser207; Ser151	90	0.0064; 0.0069	–	–	65
AR	Phosphorylation at Ser96	140	0.038	2.385 (1.162–4.893)	Correlated with tumor size (p=0.045) and tumor pathological grade (p=0.028). An independent risk factors for OS in patients with HCC (p=0.018)	66
4E-BP1	Phosphorylation at Thr46	20	–	–	Higher in patients with late recurrence than that in early recurrence	67
AFP	Acetylation at Lys194, 211, 242	70	0.0007; 0.0004; 0.0002	–	Correlated with high T classification, high TNM classification, metastasis, and HBV infection	68
Histone H2B Histone H3.3 Histone H4	Acetylation at Lys120; Acetylation at Lys18; Acetylation at Lys77	5	0.007; 0.029; 0.034	–	Poor differentiation (p=0.002). Microvascular invasion (p=0.031). Elevated alpha-fetoprotein (p=0.035), larger tumors (p=0.017) and microvascular invasion (p=0.047).	69
Histone H3	Methylation at Lys4	168	<0.0001	3.592 (2.302–5.605)	An independent prognostic factor for poor OS (p < 0.001)	70
M2BPGi	Glycosylation	226	0.011	1.858 (1.144–3.018)	An independent predictor of PFS in patients undergoing TACE. An independent factor of OS and recurrence.	72
C3	Glycosylation at Asn85	315	C3-Man5 p=0.004; C3-Man6 p=0.007; C3-hybrid$p$=0.002	1.499 (1.030–2.183); 1.473 (0.998–2.174); 0.579 (0.371–0.902)	C3-Man5 and C3-hybrid were independent factors for the recurrent HCC (p=0.046, 0.034)	74

4E-BP1, 4E-binding protein 1; AFP, alpha-fetoprotein; AR, androgen receptor; CCA, cholangiocarcinoma; C3, complement C3; DFS, disease-free survival; HCC, hepatocellular carcinoma; IHC, immunohistochemistry; INSIG1, insulin induced gene 1; INSIG2, Insulin induced gene 2; M2BPGi, mac-2-binding protein glycosylation isomer; OS, overall survival; PCK1, phosphoenolpyruvate carboxykinase 1; PVTT, portal vein tumor thrombosis; TACE, transcatheter arterial chemoembolization.

Conflict of interest

The authors have no conflict of interests related to this publication.

Author contributions

Writing of the manuscript (JW), revision of the manuscript (FFW, NW and MYZ), and developing the idea for the article and critically reviewing it (HYW and GLH). All authors read and approved the final version of the manuscript.

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