



Review Article

Moonlighting Effects of Pyruvate Kinase M2 in Chronic Liver Diseases



Madhav Jadhav, Shailendra Sharma, Vaishnavi Kalmegh, Saumya Kapoor and Amit Shard* 

Department of Medicinal Chemistry, National Institute of Pharmaceutical Education and Research, Ahmedabad, Gujarat, India

Received: July 03, 2023 | Revised: July 27, 2023 | Accepted: September 04, 2023 | Published online: September 28, 2023

Abstract

Hepatic diseases have constituted a significant global problem for over two decades, numerous factors contribute to these diseases, and most eventually result in hepatocellular carcinoma. A particular pivotal factor responsible for hepatic diseases is the abnormal functioning of various metabolic processes. Pyruvate kinase is a crucial regulator of the glycolytic pathway, and overexpression of pyruvate kinase isoform M2 (PKM2) has been observed with various hepatic abnormalities due to genetic malfunctioning and other contributing factors. The present scenario for diagnosing and treating hepatic diseases includes surgery and immunosuppressant therapies. Kinase modulation may also be a potential therapeutic measure for rectifying hepatic diseases, and this can serve as a potential approach. This review summarizes the malfunctions and significance of PKM2 regulation and explores the potential of PKM2 as a target for treating hepatic abnormalities.

Introduction

Liver Diseases

The liver is a large, complex organ essential for maintaining overall body homeostasis. With the primary functionality of carbohydrate, protein, and fat metabolism, it is the site where metabolic

waste products are detoxified via processes such as amino acid deamination, to produce urea. In concert with the spleen, spent red blood cells are degraded and their constituents are reclaimed. Bile synthesis and secretion, lipoprotein and plasma protein synthesis, and the production of clotting factors all take place in the liver. Additionally, blood glucose levels are stabilised via absorbing and storing glucose as glycogen (glycogenesis), breaking this down to glucose when needed (glycogenolysis), and the production of glucose from noncarbohydrate sources such as amino acids (gluconeogenesis).¹ Disruptions to normal liver functions by viral infections, alcohol abuse, metabolic disorders, and genetic predispositions can invariably lead to hepatic diseases.

Hepatic diseases are a significant global health problem, with various factors contributing to their prevalence. Chronic liver diseases (CLDs) refer to a group of diseases with varying degrees of intrahepatic inflammatory necrosis and/or fibrosis caused by various aetiologies and a minimum 6-month history. CLDs usually include cirrhosis, non-cirrhotic chronic liver diseases, chronic hepatitis (chronic hepatitis B and chronic hepatitis C), alcohol-associated liver disease, metabolic-associated fatty liver disease, autoimmune liver disease, genetic metabolic liver disease, and chronic drug-induced liver injury. Acute aggravation is defined as the emergence of new acute inflammatory necrosis in the liver as a result of several inducements (such as hepatitis virus mutation, overlap virus infection, bacterial infection, excessive alcohol intake, drugs, or immune damage), resulting in further aggravation of the original inflammation and/or fibrosis and leading to liver dysfunction, decompensation, or liver damage.² Liver diseases and their most serious complications, such as acute liver failure, cirrhosis, and liver cancer, are among the world's leading and fastest-

Keywords: Pyruvate Kinase M2; Hepatic abnormalities; Hepatocellular carcinoma; Hepatitis; Liver cirrhosis; Fatty liver disease.

Abbreviations: ADP, adenosine diphosphate; AKT, protein kinase B; anti-PD-1, anti-programmed cell death-1; ATP, adenosine triphosphate; Bcl-xL, B-cell lymphoma-extra-large; CK, cytokeratin; CLDs, chronic liver diseases; c-Met, c-mesenchymal epithelial transition factor; EGFR, epidermal growth factor receptor; ER, endoplasmic reticulum; ERK, extracellular signal-regulated kinase; FGFR, fibroblast growth factor receptor; FOXA3, forkhead box protein a3; GWAS, genome-wide association studies; HBV, hepatitis B virus; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; Hep-1, Hepatocyte Parafin 1; HGF, hepatocyte growth factor; HDAC, histone deacetylase; HIF-1 α , hypoxia-inducible factor -1 α ; JNK, c-Jun N-terminal kinase; LKB1, liver kinase B1; LHBS, large viral surface antigens; MAPK, mitogen-activated protein kinase; MEK, mitogen-activated extracellular signal-regulated kinase; NAFLD, non-alcoholic fatty liver disease; miRNA, micro RNA; MiR-122, MicroRNA-122; NASH, nonalcoholic steatohepatitis; PDGFR, platelet-derived growth factor receptor; pERK, phosphorylation extracellular regulated protein kinase; PEP, Phosphoenolpyruvate; PJS, Peutz-Jeghers syndrome; PKM2, pyruvate kinase M2; RAF, rapidly accelerated fibrosarcoma; STK11, Serine/threonine kinase 11; SUOX, sulfite oxidase; TACE, transarterial chemoembolization; TCA, tricarboxylic acid cycle; TRIM35, tripartite motif-containing protein 35; VEGFR, vascular endothelial growth factor receptor; ZFP91, zinc finger protein 91.

*Correspondence to: Amit Shard, Department of Medicinal Chemistry, National Institute of Pharmaceutical Education and Research, Ahmedabad, Opposite Air Force Station, Palaj Gandhinagar-382355, Gujarat, India. ORCID: <http://www.orcid.org/0000-0003-4109-6275>. Tel: +91-79-6674-5555, Fax: +91-79-6674-5560, E-mail: amit@niperahm.res.in

How to cite this article: Jadhav M, Sharma S, Kalmegh V, Kapoor S, Shard A. Moonlighting Effects of Pyruvate Kinase M2 in Chronic Liver Diseases. *Gene Expr* 2023; 22(3):250–257. doi: 10.14218/GE.2023.00038.

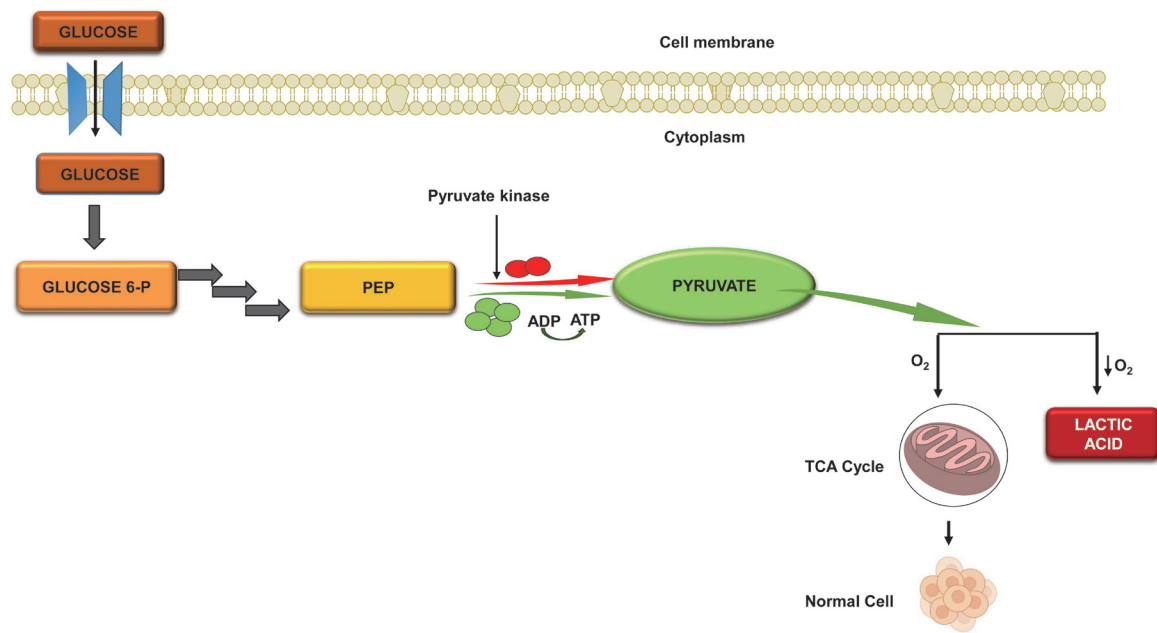


Fig. 1. Illustration depicting the role of pyruvate kinase in glycolysis of normal cells and cancerous cells. The terminal glycolytic step is catalyzed by the pyruvate kinase involved in the conversion of phosphoenolpyruvate to pyruvate. The pyruvate kinase M2 in its dimeric form (exclusively present in cancer cells) results in lactate production whereas its tetrameric form leads to the formation of pyruvate which enters the TCA cycle. ADP, adenosine diphosphate; ATP, adenosine triphosphate; PEP, Phosphoenolpyruvate; TCA, tricarboxylic acid cycle.

growing causes of death. It is estimated that CLDs (cirrhosis, viral hepatitis, and liver cancer) account for over 2 million deaths annually and are responsible for 4% of all deaths worldwide.³ 1 out of 3 liver deaths are of females. It is the 11th foremost cause of death worldwide and the 15th leading cause of disability-associated life-years.⁴ However, there has been no significant development in the treatment of the aforementioned hepatic abnormalities, but various supportive treatments to reduce their incidence are readily available. In this review, we discuss various hepatic abnormalities and their potential therapeutic approaches.

Kinases involved in hepatic diseases

Kinases, a class of enzymes responsible for phosphorylating proteins and modulating signaling pathways, play an influential role in the pathogenesis of hepatic diseases. Several kinases are implicated in hepatic diseases and are crucial for cellular processes inclusive of liver function, metabolism, and inflammation. Some kinases associated with hepatic diseases are c-Jun N-terminal Kinase (JNK), Hepatic Kinase B or Protein Kinase B/Akt, AMP-activated Protein Kinase, Transforming Growth Factor-beta-Activated Kinase 1, Fibroblast Growth Factor Receptor (FGFR), Mitogen-Activated Protein Kinase (MAPK), Protein Kinase C, Extracellular Signal-Regulated Kinase (ERK), Phosphoinositide 3-Kinase, Glycogen Synthase Kinase-3, and Pyruvate kinase M2 (PKM2).⁵

Stress-activated protein kinases, MAPKs, are a type of serine/threonine kinase that can regulate cellular processes by transducing extracellular stimuli into intracellular responses. In the liver, MAPKs play a significant role in adaptable processes that regulate inflammation.⁶ MAPK/ERK signaling has prognostic significance because increased expression of renin-angiotensin system effectors is strongly associated with a poor survival rate in hepatocellular carcinoma (HCC) patients.⁷ Rapidly accelerated fibrosarcoma kinase (RAF), mitogen-activated extracellular signal-regulated kinase

(MEK), and ERK mRNA were overexpressed in 33, 40, and 50% of HCC patients, respectively, and MEK1/2 phosphorylation was seven-fold higher in HCC tissues compared to adjacent benign tissues.⁸

Specific oncogenic kinase targets include v-RAF murine sarcoma viral oncogene homolog B1, epidermal growth factor receptor (EGFR), vascular endothelial growth factor receptor (VEGFR), human epidermal growth factor receptor 2, Kit, platelet-derived growth factor receptor (PDGFR), mammalian target of rapamycin, hepatocyte growth factor (HGF)/c-mesenchymal epithelial transition factor (c-Met), FGFR, and others. These proteins are linked to hallmarks of carcinogenesis such as maintaining proliferative signaling, inducing angiogenesis, anti-apoptotic mechanisms, and activating invasion and metastasis.⁹ Intracellular pathways, with EGFR, PDGFR, VEGFR, HGF/c-Met, and FGFR, are all deregulated in HCC. The activation of these receptors initiates additional intracellular renin-angiotensin system /RAF/ERK/MEK protein kinase signaling cascades. In experimental models, activating these mediators can cause liver tumors. Knockdown experiments revealed that deactivating these kinases may have anticancer effects, demonstrating that pathway blockade is a credible target for controlling HCC.¹⁰

PKM2 is an enzyme that plays an important role in cellular metabolism. It is expressed specifically in proliferating cells, including cancer cells, and in certain tissues such as the liver. Although PKM2 is most commonly associated with cancer, new research suggests that it may also be involved in hepatic diseases. PKM2 has been linked to several processes that contribute to liver pathologies in the context of hepatic diseases.

PKM2 is overexpressed in the most common type of liver cancer, hepatocellular carcinoma. It promotes tumor growth and survival by increasing glucose metabolism and producing biosynthetic precursors required for rapid cell proliferation (Fig. 1). PKM2 also regulates the Warburg effect, which is characterized by an increase in metabolic rate.¹¹ PKM2 expression is barely

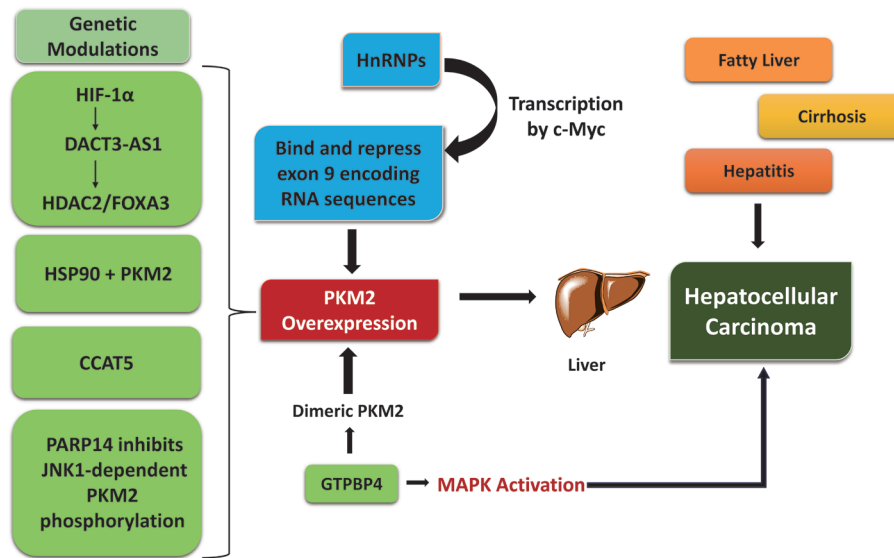


Fig. 2. Schematic representation provides insight into the intricate molecular factors contributing to liver diseases and genetic modulations of markers like HDAC2/FOXA3, HSP90, CCAT5, PARP14 along with GTPBP4 which eventually results in pyruvate kinase M2 overexpression, finally leading to alterations causing hepatic abnormalities. FOXA3, forkhead box protein a3; HDAC2, histone deacetylase 2; JNK, c-Jun N-terminal kinase; MAPK, mitogen-activated protein kinase; PKM2, pyruvate kinase M2.

detectable in healthy livers but is markedly upregulated in liver cancer. Interestingly, despite the fact that a switch from PKM1 to PKM2 is observed in many types of tumors, PKM2 is the dominant isoform of PKM in both normal livers and HCC.¹² The level of PKM2 in plasma ectosome gradually increases prior to tumor formation during N-nitroso diethylamine/carbon tetrachloride or streptozotocin/high-fat diet induced murine hepatocarcinogenesis. Furthermore, PKM2 is significantly more abundant in ectosomes from HCC patients than in healthy donors, indicating that PKM2 may function as an early diagnostic marker for HCC.¹³ PKM2 is significantly upregulated in tumor tissues in HCC patients and is associated with a poor prognosis. Furthermore, PKM2 overexpression in HCC is associated with a high tumor, node, metastasis stage and level of vascular invasion. Patients who express PKM2 typically have an increased risk of postsurgical HCC recurrence.¹⁴

Non-alcoholic fatty liver disease (NAFLD) is a liver condition characterized by fat accumulation. PKM2 has been linked to the development and progression of NAFLD. It has the ability to influence lipid metabolism by controlling the expression of genes elaborated in fatty acid synthesis, oxidation, and storage. PKM2 activity changes may contribute to the abnormal lipid accumulation and hepatic steatosis seen in NAFLD.¹⁵ Liver fibrosis is a wound-healing response to chronic liver injury characterized by an overabundance of extracellular matrix components. PKM2 has been linked to hepatic stellate cell activation, which contributes significantly to liver fibrosis. PKM2 stimulates the proliferation and activation of hepatic stellate cells, increasing the production of collagen and other fibrotic proteins.¹⁶

Correlation between various liver diseases and PKM2

Genetic regulation of hepatocellular carcinoma by PKM gene

As PKM2 is pivotal in the metastasizing mechanism of carcinogenic cells (termed the Warburg effect), studies have shown that HCC is linked with the PKM gene by numerous mechanisms (Fig.

2). A common feature of carcinogenic cells is the malfunction of biotransformation, for example, their ability to cause alterations in metabolic processes like glycolysis. In 2012, Wang *et al.* studied the genetic cross-walk of HCC alongside the oncogenic pathway protein kinase B (AKT) activation and lowering of the sprout 2 (*spry2*) gene. They reported that AKT-induced HCC development increased extensively through the overexpression of *Spry2Y55F*. This overexpression gave rise to increased cell proliferation, increasing glycolysis, and resulting in significantly increased frequency of PKM2 and MAPK pathway activity.¹⁷

Overexpression of PKM2 in HCC patients and its role in the enhancement of cell proliferation and ultimate progression of HCC occurs through various critical pathways including PRMT6-ERK-PKM2 regulatory axis, circMAT2B/miR-338-3p/PKM2 axis, and PARP14-JNK1-PKM2 regulatory axis.^{18,19} There are certain oncogenic factors like heat shock protein 90 (HSP 90) that bring about Thr-328 phosphorylation, or c-Myc, which drives the expression of MNX1-AS1 to consequently induce the upregulation of PKM2.^{20,21}

Cellular metabolism is regulated by MicroRNA-122 (MiR-122), and is abundantly expressed in normally functioning cells, however, metabolic alterations that occur in HCC result in the reduction of MiR-122. Studies reveal that genes demonstrating the interaction of MiR-122 at 3'UTR areas are *PK*, *ALDOA*, *GNPDA1*, *PFKFB2*, and *AKR1B10*, among which *PK* gene has maximum anticorrelation with MiR-122. In HCC cells, expression of PKM2 is comparably higher in the metastasizing state. Overexpression of MiR-122, therefore, is indicative of reduced lactate production, inhibited cell proliferation and increased chemosensitivity.²² Additionally, MiR-122-5p has been investigated for its assistance in the function of lncRNA-SOX2OT, and modulating the metabolism and metastasis of HCC cells. lncRNA-SOX2OT is a PKM2 regulator and fosters PKM2-facilitated glucose metabolism. As such, PKM2 inhibition hinders lncRNA-SOX2OT in promoting the metastasis of HCC.²³

When PKM2 expressions in HCC patients were studied by immunohistochemistry analysis, overexpression of PKM2 was observed throughout, and was seemingly associated with the worse

clinicopathologic characteristics in the patients. PKM2 overexpression was also correlated with endoplasmic reticulum (ER) stress. ER stress downregulates the expression of MiR-188-5p in the HCC cells, its target gene hnRNP2B1, when downregulated, decreases PKM2 expression and enhances apoptosis.²⁴

Wang *et al.* suggest lncRNA DACT3-AS1 is associated with the progression of HCC through the upregulation of PKM2, and this upregulation is brought about by the histone deacetylase 2/ forkhead box protein a3 (HDAC2/FOXA3) pathway.²⁵ It promotes interaction with HDAC and FOXA3 and elicits the removal of an acetyl group from FOXA3, lowering FOXA3 protein levels.²⁵ Another study by Zhang *et al.* suggests HDAC8 carries out the deacetylation of K62 residue of PKM2, which subsequently alters its regulation. This process is activated by hypoxia-inducible factor-1 α , which transcriptionally activates DACT3 antisense RNA 1 (DACT3-AS1) expression under hypoxic conditions.^{18,25}

Activation of the transcription of hypoxia-inducible factor-1 α (HIF-1 α) by STAT3 phosphorylation, at tyrosine705, is a potential mechanism for the proliferation of HCC cells. Further studies demonstrated that up-regulation of HIF-1 α and Bcl-xL (B-cell lymphoma-extra-large) by PKM2 enhances cell proliferation in HCC.²⁶

Guanosine triphosphate binding protein 4, one of the pivotal regulators of MAPK and cell cycle progression, encourages HCC progression by PKM2-regulated glucose metabolism.²⁷

A study by Chen *et al.* identified E3 ligase zinc finger protein 91 (ZFP91) to have a suppressing effect on the reprogramming of metabolic processes in HCC and cell propagation and metastasis through *in vitro* and *in vivo* studies.²⁸ According to this report, Lys48-linked ubiquitination of the oncoprotein hnRNP A1 at lysine 8 by ZFP91, and inhibiting hnRNP A1-dependent PKM splicing by proteasomal degradation results in greater production of PKM1 isoform than PKM2, leading to a suppressing effect on hepatocellular carcinogenesis.²⁸

Tripartite motif-containing protein 35 (TRIM35) was identified as a novel tumor suppressor, particularly in HCC. It interacts with PKM2 and inhibits the phosphorylation of tyrosine residue 105 (Y105), which impedes glycolysis and suppresses cell proliferation.^{29,30}

Genetic regulation of hepatitis by PKM gene

HCC is a highly aggressive malignancy, its onset is related to hepatitis virus infection, alcoholism, and other metabolic disorders.³¹ A recent study by Wu *et al.* screening the interactions of large viral surface antigens (LHBS) with the host factors set by affinity purification screen illustrated the role of aerobic glycolysis played in supporting the hepatitis B virus.³² PKM2 oligomerization in hepatocytes was affected by viral LHBS, increasing the lactate production in the cells.³²

Chronic hepatitis B is a major risk factor for the development and progression of HCC. Hence the pharmacological targets that are involved in the HCC progression were investigated, including Hep-1 (Hepatocyte Parafn 1), CK18 (cytokeratin-18), CK19 (Cytokeratin 19), SUOX (sulfite oxidase), pERK (phosphorylation extracellular regulated protein kinases), TRIM35 (tripartite motif containing 35), and PKM2.³³

Hepatitis C infections occur when the hepatitis C virus (HCV) core protein reduces the activity of (PPAR)- α/γ in hepatocytes.³⁴ This core protein surrounds the lipid vesicle membrane of the liver and causes lipid deposition in the cells via the activation of sterol regulatory-element-binding protein-1c.³⁵ In the glycolytic pathway, HCV down-regulates glucose transporter 2, resulting in less

intracellular glucose transport. HCV also up-regulates the genes for phosphoenolpyruvate carboxykinase and glucose 6-phosphatase, which are pivotal enzymes for hepatic gluconeogenesis.³⁶ Studies carried out by Wu *et al.* reported on the specific interaction of PKM2 isoforms through the hepatitis C virus NS5B. The group studied both virus-encoded proteins and cellular factors involved in the pathogenesis and replication of HCV, ascertaining the interactions of NS5B with PKM2. They also showed that shRNA downregulated the expression of PKM2 which subsequently inhibited the replication of HCV in 9B replicon HCV cell lines.³⁷

By binding to and down regulating HIF-1 α activity, PKM2 is also a potential mediator and therapeutic target for regulating sterile inflammation of the liver, alcoholic, and non-alcoholic steatohepatitis (NASH).^{38,39}

Genetic regulation of Liver cirrhosis by PKM gene

Hepatitis B and NASH are both related to liver cirrhosis. One of the most serious implications of NASH is the subsequent development of cirrhosis and HCC, which can also occur in patients with no history of alcohol consumption.⁴⁰ Lee *et al.* proposed increased gene expression of various glycolytic enzymes like hexokinase 2, aldolase A, and PKM2 as an indication for precancerous cirrhotic livers, which is predominantly associated with the development of HCC.⁴¹ In liver cirrhosis, hepatocytes develop chronic inflammation, fibrosis, scarring, and hepatocellular regeneration. This leads to the accumulation of clusters of cells with genetic mutations causally associated with liver malignancy and HCC. The implication of PKM2 in both cirrhotic and non-cirrhotic liver disease has been extensively studied and over-expression of PKM2 correlates to the development of HCC. This observation suggests that PKM2 is a potential early stage biomarker for HCC.⁴² PKM2 in peripheral blood is also considered a crucial marker of hepatitis B-related cirrhosis, where increased concentrations of PKM2 cause an increase in the rate of liver cirrhosis.⁴⁰

Genetic regulation of fatty liver disease by PKM gene

PKM2 overexpression is an indication of fatty liver disease, NAFLD, one of the most common reasons for the development of chronic liver diseases. Numerous genetic risk factors are associated with this disease as determined by genome-wide association studies (GWAS). A study highlights the genes PNPLA3 and TM6SF2 as correlating with steatosis and HCC. This connection then purports NAFLD as a primary or early risk factor for HCC as end stage chronic liver disease.⁴³ PKM2-governed progression of fatty liver disease is the result of changes in metabolic processes linked to miR-122-5p downregulation.⁴⁴ In steatosis, this imbalance causes lipid and mitochondrial ROS accumulation in hepatocytes.⁴⁵ Additionally, because PKM2 controls the metabolic skewing of Th17 cells, and cell-specific PKM2 deletion dramatically reduces NAFLD and hepatic inflammatory disease, PKM2 was considered to be a potential new target for the diagnosis and treatment of NAFLD.^{15,46}

Therapeutics available for hepatic diseases

Hepatic treatment approaches may vary depending on the specific liver condition. Several therapies are highly accessible, and surgical procedures for treating severe liver illnesses and extending patient survival have increased markedly in recent years. All chronic viral hepatitis types have a common pathophysiology and clinical course. Lamivudine, tenofovir-disoproxil, adefovir-dipivoxil, telbivudine and entecavir are available drugs used to treat HBV orally.⁴⁷ For

NASH, a category of pharmaceuticals known as farnesoid X receptor agonists is being studied to assess their suitability for treatment. In clinical trials, these medications, which target bile acid metabolism, have demonstrated promise in lowering liver fat and inflammation. Additionally, antifibrotic medications, such as obeticholic acid and cenicriviroc, are being evaluated for their capacity to lessen liver fibrosis in NASH patients.⁴⁸ Amino acids can be used successfully to treat liver disease since they are connected to a variety of biological processes. These advantages include using aspartate to treat fatty liver disease caused by cholesterol consumption and glycine to treat alcohol-induced liver damage. Recent studies have employed a variety of amino acids, including peptides and branched-chain amino acids, to treat various liver conditions.⁴⁹ New pharmacological treatments that prevent hepatic steatosis, the development of NASH, and even more serious liver disease are clearly needed to address unmet medical needs.⁵⁰ Hepatitis B therapy's current objectives are to lower the risk of cirrhosis progression, treat extra-hepatic side effects, slow the growth of hepatocellular cancer, and stop ongoing transmission. Reduction of HBV-DNA typically happens when using pegylated interferon alpha or nucleos(t)ide analogs, with the viral load being undetectable in a significant number of treated patients. The normalization of liver enzymes and a decreased risk of cirrhosis and liver cancer are all associated with undetectable serum HBV-DNA, which also stops the transmission.⁵¹

Available treatments and modalities

Although healthcare professionals may attempt to treat liver injury directly, treatment frequently entails managing problems and giving the liver time to heal. Specific consequences of decompensated cirrhosis, such as ascites, variceal hemorrhage, and hepatic encephalopathy, have a wide range of proven therapies.⁵² Some liver complications can be treated with lifestyle changes, such as limiting alcohol consumption or weight loss, typically as part of a wider medical program that includes careful monitoring of liver function. Other liver complications may be treated with medication or may require surgery. Additional methods, such as the use of immunosuppressants, have been investigated, but these studies have insufficient output to support its use in clinical practice. Bile acid approaches and therapy remain the mainstays of primary biliary cholangitis treatment.⁴⁸ Along with medication to treat cholesterol or insulin resistance for liver disease, weight loss is the primary objective of treatment. It has been shown that bariatric surgery works in the case of overweight patients. Even though no drug treatment has been authorized, recent research on thiazolidinediones has shown improvements in both liver enzymes and histology. Statins are hepatoprotective in various liver diseases, although these studies are not many. Histologic research has not supported the initial enthusiasm for ursodeoxycholic acid. There is no agreed-upon definition of hepatoprotective pharmaceuticals, which are medications that can accelerate liver detoxification, promote liver cell regeneration, and/or improve liver function. It is loosely classified as detoxifying drugs (like NAC and GSH), anti-inflammatory pharmaceuticals (like Glycyrrhizic acid preparation), hepatocyte membrane protectors (like PPC), and antioxidant drugs (like Bicyclol and silymarin) based on the various mechanisms of action.⁵³

Is kinase modulation an option?

Serine/threonine kinase 11 (STK11, Par-4 in *C. elegans*), also known as liver kinase B1 (LKB1), was initially discovered in its mutant form with loss of function in Peutz-Jeghers syndrome (PJS), a rare and predominantly inherited autosomal disorder. Germline-inactivating mutations of LKB1 are linked to the patho-

physiology of PJS, which is marked by a number of benign gastrointestinal polyps and metastatic cancers.⁵⁴ Jun N-terminal kinase (JNK), activated in the fibrotic liver of both human and mouse models in myofibroblasts, is essential for HSC activation and liver fibrosis progression.⁵⁵

The MAPK pathway, as a regulator of hepatic metabolism, also plays an important role in driving liver metabolic control mechanisms.⁵⁶

PKM2 modulation: a fruitful way to depreciate hepatic disease?

The Warburg effect, a metabolic change in cancer cells that encourages tumor growth and survival, applies to PKM2. Preclinical studies inhibiting PKM2 activation decreased the proliferation of HCC cells, triggered apoptosis, and inhibited tumor growth. As such, PKM2 may be a predictor of HCC and a potential therapeutic target for immune checkpoint inhibitors. PKM2 regulation has also been investigated as a method to make HCC cells more susceptible to chemotherapy or other targeted treatments.¹¹

PKM2 modulation effects on the different hepatic ailments

Propagation, migration, and incursion of HCC cells in vitro, along with the development of tumors in vivo, were all decreased by PKM2 knockdown. Surprisingly, PKM2 demonstrated a significant connection with immune inhibitory cytokine production and lymphocyte infiltration in HCC. A study showed that covalently modified PKM2 reduces lipid build-up, inflammation, and fibrosis in the liver. It was also noted that livers from patients with NAFLD and NASH, particularly their hepatic macrophages, had high expression of PKM2.⁵⁷

Future of therapeutics to curb hepatic disorders

Non-alcoholic fatty liver diseases

The current standard treatment protocol for non-alcoholic fatty liver disease is lifestyle management, including decreased caloric intake and a low-fat diet.⁵⁸ For obese patients, bariatric surgery has been proven helpful. Although no FDA-approved drug has come to the market for NAFLD, certain potential therapies like vitamin E, pioglitazone and liraglutide have shown promising results against NAFLD in randomized clinical trials.^{59,60} Due to the failure of a number of drugs against NAFLD in clinical trials, many pharmaceutical companies are exploring combination therapies to treat NAFLD. Therefore, the future of NAFLD treatment may well be with a combination of different classes of drugs.⁶¹

Hepatitis B and C

Antivirals are the main therapy for treating HBV. There are several therapeutics available targeting specific stages of the HBV lifecycle. However, treatment with current antivirals is not very effective and relapses after the discontinuation of nucleos(t)ide analogs-based antivirals are a chance occurrence.⁶² Future treatments should be based on a combination of current antivirals with new anti-HBV agents. The nucleoside analogue could be given with HBV entry inhibitors or some antivirals with immunomodulatory vaccines.

In HCV, treatment with antivirals has achieved a cure rate close to 100%. Despite this, the high cost of treatment, poor diagnosis, and chances of re-infection are still challenges to be met, creating a need for the development of a vaccine against HCV. Developing an effective vaccination against HCV has been hampered by the virus's genetic diversity and a dearth of relevant animal models.⁶³

Hepatocellular carcinoma

HCC is the third leading cause of cancer-related mortality, with only a 3% five-year survival rate. Liver resection and liver transplantation are the two most common surgical treatment strategies to limit HCC. Non-surgical treatments include transarterial radioembolization, or transarterial chemoembolization (TACE). TACE refers to the hepatic artery's catheterization followed by chemotherapy administration. Drug-eluting bead TACE is a novel combination method for delivering embolising particles that have been loaded with a chemotherapeutic drug. The comparatively slower release of chemotherapeutic medication causes patients undergoing drug-eluting bead TACE to experience fewer side effects than those treated with TACE.⁶⁴

For late stage HCC, systemic therapies are the first choice of treatment. Molecular-targeted therapies against various tyrosine kinases have been developed. Sorafenib, a tyrosine kinase inhibitor was the first drug approved for the treatment of HCC.⁶⁵ Following this, a number of tyrosine kinases became commercially available. Various immune checkpoint inhibitors and anti-programmed cell death-1 (anti-PD-1) antibodies have also been approved for the treatment of HCC.⁶⁶ Future treatment options may arise from combining a variety of such approaches. Recently, the combination of atezolizumab (targeting PD-L1) and bevacizumab (targeting VEGF) proved beneficial in the treatment of HCC.⁶⁷

Micro RNAs (miRNAs) based therapy could also be an alternative approach as a future therapy for liver diseases. Both acute hepatitis and NASH mice models benefited from miR-223 3p treatment.⁶⁸ Liver fibrosis was improved in long-term CCl₄-treated animals due to miR-221-3p suppression.⁶⁹ Multiple microRNAs have been shown to target ATP-binding cassette transporters that cause resistance in HCC.⁷⁰ This indicates that miRNA-based therapy could be helpful in overcoming resistance in HCC. However, miRNA-based therapy is still at an early stage with numerous shortcomings like difficulty in drug delivery.

Conclusion

Given the rising incidences of hepatic abnormalities, the present review gives a basic introduction to liver disease followed by a discussion about enzymes involved in hepatic disorders. The role of various kinases involved in the pathophysiology of hepatic diseases was discussed with special emphasis on PKM2. The PKM gene is intricately linked to the genetic regulation of HCC, hepatitis, NAFLD, and liver cirrhosis. In HCC, PKM2 is overexpressed, resulting in enhanced proliferation of tumorigenic cells. Expression of PKM2 was also affected in the presence of hepatitis viral proteins. Overexpression of PKM2 acts as a biomarker for HCC which is the end stage for most hepatic disorders. Current therapies available for liver diseases were also discussed, highlighting PKM2 as a potential therapeutic target. Future treatment strategy is likely to garner the most success through combination therapies. A synergistic effect could be achieved by targeting the disease through a multi-target approach. PKM2 could be recognized as one such target and the development of novel therapeutic modalities against PKM2.

Acknowledgments

The authors gratefully acknowledge the Director of NIPER Ahmedabad and the Department of Pharmaceuticals, Government of India for all the support and encouragement.

Funding

The authors gratefully acknowledge the Indian Council of Medical Research for their financial support (Project ID: 5/4/8-29/CD/AS/2022-NCD-II). The communication number for this manuscript is NIPER-A/907/06/2023.

Conflict of interest

All authors have approved the final version of the manuscript. The authors declare no conflict of interests.

Author contributions

AMS conceptualized and designed the manuscript. MJ had done the data curation and was engaged in compilation and preparation of the manuscript. SS wrote the therapeutics available for hepatic diseases. VK and SMK wrote the manuscript and helped with figure preparation.

References

- [1] Giannini EG, Testa R, Savarino V. Liver enzyme alteration: a guide for clinicians. *CMAJ* 2005;172(3):367–379. doi:10.1503/cmaj.1040752, PMID:15684121.
- [2] Zhang YY, Meng ZJ. Definition and classification of acute-on-chronic liver diseases. *World J Clin Cases* 2022;10(15):4717–4725. doi:10.12998/wjcc.v10.i15.4717, PMID:35801045.
- [3] Devarbhavi H, Asrani SK, Arab JP, Nartey YA, Pose E, Kamath PS. Global burden of liver disease: 2023 update. *J Hepatol* 2023;79(2):516–537. doi:10.1016/j.jhep.2023.03.017, PMID:36990226.
- [4] Rungay H, Arnold M, Ferlay J, Lesi O, Cabasag CJ, Vignat J, *et al*. Global burden of primary liver cancer in 2020 and predictions to 2040. *J Hepatol* 2022;77(6):1598–1606. doi:10.1016/j.jhep.2022.08.021, PMID:36208844.
- [5] Alshehade S, Alshawsh MA, Murugaiyah V, Asif M, Alshehade O, Almoustafa H, *et al*. The role of protein kinases as key drivers of metabolic dysfunction-associated fatty liver disease progression: New insights and future directions. *Life Sci* 2022;305:120732. doi:10.1016/j.lfs.2022.120732, PMID:35760093.
- [6] Min L, He B, Hui L. Mitogen-activated protein kinases in hepatocellular carcinoma development. *Semin Cancer Biol* 2011;21(1):10–20. doi:10.1016/j.semcancer.2010.10.011, PMID:20969960.
- [7] Moon H, Ro SW. MAPK/ERK Signaling Pathway in Hepatocellular Carcinoma. *Cancers (Basel)* 2021;13(12):3026. doi:10.3390/cancers13123026, PMID:34204242.
- [8] Chen L, Shi Y, Jiang CY, Wei LX, Wang YL, Dai GH. Expression and prognostic role of pan-Ras, Raf-1, pMEK1 and pERK1/2 in patients with hepatocellular carcinoma. *Eur J Surg Oncol* 2011;37(6):513–520. doi:10.1016/j.ejso.2011.01.023, PMID:21324414.
- [9] Muntané J, De la Rosa AJ, Docobo F, García-Carbonero R, Padillo FJ. Targeting tyrosine kinase receptors in hepatocellular carcinoma. *Curr Cancer Drug Targets* 2013;13(3):300–312. doi:10.2174/15680096113139990075, PMID:23016985.
- [10] da Fonseca LG, Reig M, Bruix J. Tyrosine Kinase Inhibitors and Hepatocellular Carcinoma. *Clin Liver Dis* 2020;24(4):719–737. doi:10.1016/j.cld.2020.07.012, PMID:33012455.
- [11] Li TE, Wang S, Shen XT, Zhang Z, Chen M, Wang H, *et al*. PKM2 Drives Hepatocellular Carcinoma Progression by Inducing Immunosuppressive Microenvironment. *Front Immunol* 2020;11:589997. doi:10.3389/fimmu.2020.589997, PMID:33193421.
- [12] Bluemlein K, Grüning NM, Feichtinger RG, Lehrach H, Kofler B, Ralser M. No evidence for a shift in pyruvate kinase PKM1 to PKM2 expression during tumorigenesis. *Oncotarget* 2011;2(5):393–400. doi:10.18632/oncotarget.278, PMID:21789790.
- [13] Hou PP, Luo LJ, Chen HZ, Chen QT, Bian XL, Wu SF, *et al*. Ectosomal PKM2 Promotes HCC by Inducing Macrophage Differentiation and Remodeling the Tumor Microenvironment. *Mol Cell* 2020;78(6):1192–

- 1206.e10. doi:10.1016/j.molcel.2020.05.004, PMID:32470318.
- [14] Zhao R, Li L, Yang J, Niu Q, Wang H, Qin X, *et al*. Overexpression of Pyruvate Kinase M2 in Tumor Tissues Is Associated with Poor Prognosis in Patients with Hepatocellular Carcinoma. *Pathol Oncol Res* 2020;26(2):853–860. doi:10.1007/s12253-019-00630-3, PMID:30852741.
- [15] Meoli L, Gupta NK, Saeidi N, Panciotti CA, Biddinger SB, Corey KE, *et al*. Nonalcoholic fatty liver disease and gastric bypass surgery regulate serum and hepatic levels of pyruvate kinase isoenzyme M2. *Am J Physiol Endocrinol Metab* 2018;315(4):E613–E621. doi:10.1152/ajpendo.00296.2017, PMID:29462566.
- [16] Delgado ME, Cárdenas BI, Farran N, Fernandez M. Metabolic Reprogramming of Liver Fibrosis. *Cells* 2021;10(12):3604. doi:10.3390/cells10123604, PMID:34944111.
- [17] Wang C, Delogu S, Ho C, Lee SA, Gui B, Jiang L, *et al*. Inactivation of Spry2 accelerates AKT-driven hepatocarcinogenesis via activation of MAPK and PKM2 pathways. *J Hepatol* 2012;57(3):577–583. doi:10.1016/j.jhep.2012.04.026, PMID:22617155.
- [18] Zhang R, Shen M, Wu C, Chen Y, Lu J, Li J, *et al*. HDAC8-dependent deacetylation of PKM2 directs nuclear localization and glycolysis to promote proliferation in hepatocellular carcinoma. *Cell Death Dis* 2020;11(12):1036. doi:10.1038/s41419-020-03212-3, PMID:33279948.
- [19] Iansante V, Choy PM, Fung SW, Liu Y, Chai JG, Dyson J, *et al*. PARP14 promotes the Warburg effect in hepatocellular carcinoma by inhibiting JNK1-dependent PKM2 phosphorylation and activation. *Nat Commun* 2015;6:7882. doi:10.1038/ncomms8882, PMID:26258887.
- [20] Xu Q, Tu J, Dou C, Zhang J, Yang L, Liu X, *et al*. HSP90 promotes cell glycolysis, proliferation and inhibits apoptosis by regulating PKM2 abundance via Thr-328 phosphorylation in hepatocellular carcinoma. *Mol Cancer* 2017;16(1):178. doi:10.1186/s12943-017-0748-y, PMID:29262861.
- [21] Wu Y, Wang Y, Yao H, Li H, Meng F, Li Q, *et al*. MNX1-AS1, a c-Myc induced lncRNA, promotes the Warburg effect by regulating PKM2 nuclear translocation. *J Exp Clin Cancer Res* 2022;41(1):337. doi:10.1186/s13046-022-02547-3, PMID:36476366.
- [22] Liu AM, Xu Z, Shek FH, Wong KF, Lee NP, Poon RT, *et al*. miR-122 targets pyruvate kinase M2 and affects metabolism of hepatocellular carcinoma. *PLoS One* 2014;9(1):e86872. doi:10.1371/journal.pone.0086872, PMID:24466275.
- [23] Liang Y, Zhang D, Zheng T, Yang G, Wang J, Meng F, *et al*. lncRNA-SOX2OT promotes hepatocellular carcinoma invasion and metastasis through miR-122-5p-mediated activation of PKM2. *Oncogenesis* 2020;9(5):54. doi:10.1038/s41389-020-0242-z, PMID:32467565.
- [24] Zhou B, Lu D, Wang A, Cui J, Zhang L, Li J, *et al*. Endoplasmic reticulum stress promotes sorafenib resistance via miR-188-5p/hnRNPA2B1-mediated upregulation of PKM2 in hepatocellular carcinoma. *Mol Ther Nucleic Acids* 2021;26:1051–1065. doi:10.1016/j.omtn.2021.09.014, PMID:34786210.
- [25] Wang L, Li B, Bo X, Yi X, Xiao X, Zheng Q. Hypoxia-induced lncRNA DACT3-AS1 upregulates PKM2 to promote metastasis in hepatocellular carcinoma through the HDAC2/FOXO3 pathway. *Exp Mol Med* 2022;54(6):848–860. doi:10.1038/s12276-022-00767-3, PMID:35764883.
- [26] Dong T, Yan Y, Chai H, Chen S, Xiong X, Sun D, *et al*. Pyruvate kinase M2 affects liver cancer cell behavior through up-regulation of HIF-1 α and Bcl-xL in culture. *Biomed Pharmacother* 2015;69:277–284. doi:10.1016/j.biopha.2014.12.010, PMID:25661370.
- [27] Zhou Q, Yin Y, Yu M, Gao D, Sun J, Yang Z, *et al*. GTPBP4 promotes hepatocellular carcinoma progression and metastasis via the PKM2 dependent glucose metabolism. *Redox Biol* 2022;56:102458. doi:10.1016/j.redox.2022.102458, PMID:36116159.
- [28] Chen D, Wang Y, Lu R, Jiang X, Chen X, Meng N, *et al*. E3 ligase ZFP91 inhibits Hepatocellular Carcinoma Metabolism Reprogramming by regulating PKM2 splicing. *Theranostics* 2020;10(19):8558–8572. doi:10.7150/thno.44873, PMID:32754263.
- [29] Chen Z, Lu X, Wang Z, Jin G, Wang Q, Chen D, *et al*. Co-expression of PKM2 and TRIM35 predicts survival and recurrence in hepatocellular carcinoma. *Oncotarget* 2015;6(4):2538–2548. doi:10.18632/oncotarget.2991, PMID:25576919.
- [30] Chen Z, Wang Z, Guo W, Zhang Z, Zhao F, Zhao Y, *et al*. TRIM35 Interacts with pyruvate kinase isoform M2 to suppress the Warburg effect and tumorigenicity in hepatocellular carcinoma. *Oncogene* 2015;34(30):3946–3956. doi:10.1038/nc.2014.325, PMID:25263439.
- [31] El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. *Gastroenterology* 2007;132(7):2557–2576. doi:10.1053/j.gastro.2007.04.061, PMID:17570226.
- [32] Wu YH, Yang Y, Chen CH, Hsiao CJ, Li TN, Liao KJ, *et al*. Aerobic glycolysis supports hepatitis B virus protein synthesis through interaction between viral surface antigen and pyruvate kinase isoform M2. *PLoS Pathog* 2021;17(3):e1008866. doi:10.1371/journal.ppat.1008866, PMID:33720996.
- [33] Liu K, Duan J, Liu H, Yang X, Yang J, Wu M, *et al*. Precancer antiviral treatment reduces microvascular invasion of early-stage Hepatitis B-related hepatocellular carcinoma. *Sci Rep* 2019;9(1):2220. doi:10.1038/s41598-019-39440-7, PMID:30778112.
- [34] Dharancy S, Lemoine M, Mathurin P, Serfaty L, Dubuquoy L. Peroxisome proliferator-activated receptors in HCV-related infection. *PPAR Res* 2009;2009:357204. doi:10.1155/2009/357204, PMID:19343188.
- [35] McPherson S, Jonsson JR, Barrie HD, O'Rourke P, Clouston AD, Powell EE. Investigation of the role of SREBP-1c in the pathogenesis of HCV-related steatosis. *J Hepatol* 2008;49(6):1046–1054. doi:10.1016/j.jhep.2008.06.022, PMID:18752865.
- [36] Shlomai A, Rechtman MM, Burdelova EO, Zilberberg A, Hoffman S, Solar I, *et al*. The metabolic regulator PGC-1 α links hepatitis C virus infection to hepatic insulin resistance. *J Hepatol* 2012;57(4):867–873. doi:10.1016/j.jhep.2012.06.021, PMID:22732512.
- [37] Wu X, Zhou Y, Zhang K, Liu Q, Guo D. Isoform-specific interaction of pyruvate kinase with hepatitis C virus NS5B. *FEBS Lett* 2008;582(15):2155–2160. doi:10.1016/j.febslet.2008.05.033, PMID:18519040.
- [38] Ouyang X, Han SN, Zhang JY, Dioletis E, Nemeth BT, Pacher P, *et al*. Digoxin Suppresses Pyruvate Kinase M2-Promoted HIF-1 α Transactivation in Steatohepatitis. *Cell Metab* 2018;27(2):339–350.e3. doi:10.1016/j.cmet.2018.01.007, PMID:29414684.
- [39] Patel S, Das A, Meshram P, Sharma A, Chowdhury A, Jariyal H, *et al*. Pyruvate kinase M2 in chronic inflammations: a potpourri of crucial protein-protein interactions. *Cell Biol Toxicol* 2021;37(5):653–678. doi:10.1007/s10565-021-09605-0, PMID:33864549.
- [40] Xia XH, Huang D. Diagnostic value of alpha-fetoprotein, pyruvate kinase M2 and albumin mRNA expression in peripheral blood in hepatitis B virus-related cirrhosis. *World Chinese J Digestology* 2014;22(13):1876–1880. doi:10.11569/wcjd.v22.i13.1876.
- [41] Lee NCW, Carella MA, Papa S, Bubic C. High expression of glycolytic genes in cirrhosis correlates with the risk of developing liver cancer. *Front Cell Dev Biol* 2018;6:138. doi:10.3389/fcell.2018.00138, PMID:30430110.
- [42] Liu Y, Wu H, Mei Y, Ding X, Yang X, Li C, *et al*. Clinicopathological and prognostic significance of PKM2 protein expression in cirrhotic hepatocellular carcinoma and non-cirrhotic hepatocellular carcinoma. *Sci Rep* 2017;7(1):15294. doi:10.1038/s41598-017-14813-y, PMID:29127353.
- [43] Wang J, Conti DV, Bogumil D, Sheng X, Nouredin M, Wilkens LR, *et al*. Association of Genetic Risk Score With NAFLD in An Ethnically Diverse Cohort. *Hepatol Commun* 2021;5(10):1689–1703. doi:10.1002/hep4.1751, PMID:34558842.
- [44] Inomata Y, Oh JW, Taniguchi K, Sugito N, Kawaguchi N, Hirokawa F, *et al*. Downregulation of miR-122-5p Activates Glycolysis via PKM2 in Kupffer Cells of Rat and Mouse Models of Non-Alcoholic Steatohepatitis. *Int J Mol Sci* 2022;23(9):5230. doi:10.3390/ijms23095230, PMID:35563621.
- [45] Zhao P, Han SN, Arumugam S, Yousaf MN, Qin Y, Jiang JX, *et al*. Digoxin improves steatohepatitis with differential involvement of liver cell subsets in mice through inhibition of PKM2 transactivation. *Am J Physiol Gastrointest Liver Physiol* 2019;317(4):G387–G397. doi:10.1152/ajpgi.00054.2019, PMID:31411894.
- [46] Moreno-Fernandez ME, Giles DA, Oates JR, Chan CC, Damen MSMA, Doll JR, *et al*. PKM2-dependent metabolic skewing of hepatic Th17 cells regulates pathogenesis of non-alcoholic fatty liver disease. *Cell Metab* 2021;33(6):1187–1204.e9. doi:10.1016/j.cmet.2021.04.018, PMID:34004162.
- [47] Uhl P, Fricker G, Haberkorn U, Mier W. Current status in the therapy of liver diseases. *Int J Mol Sci* 2014;15(5):7500–7512. doi:10.3390/

- ijms15057500, PMID:24786290.
- [48] Hasegawa S, Yoneda M, Kurita Y, Nogami A, Honda Y, Hosono K, *et al*. Cholestatic Liver Disease: Current Treatment Strategies and New Therapeutic Agents. *Drugs* 2021;81(10):1181–1192. doi:10.1007/s40265-021-01545-7, PMID:34142342.
- [49] Lee DY, Kim EH. Therapeutic Effects of Amino Acids in Liver Diseases: Current Studies and Future Perspectives. *J Cancer Prev* 2019;24(2):72–78. doi:10.15430/JCP.2019.24.2.72, PMID:31360687.
- [50] Griffett K, Solt LA, El-Gendy Bel-D, Kamenecka TM, Burris TP. A liver-selective LXR inverse agonist that suppresses hepatic steatosis. *ACS Chem Biol* 2013;8(3):559–567. doi:10.1021/cb300541g, PMID:23237488.
- [51] Soriano V, Barreiro P, Cachay E, Kottitil S, Fernandez-Montero JV, de Mendoza C. Advances in hepatitis B therapeutics. *Ther Adv Infect Dis* 2020;7:2049936120965027. doi:10.1177/2049936120965027, PMID:33117536.
- [52] Fallowfield JA, Jimenez-Ramos M, Robertson A. Emerging synthetic drugs for the treatment of liver cirrhosis. *Expert Opin Emerg Drugs* 2021;26(2):149–163. doi:10.1080/14728214.2021.1918099, PMID:33856246.
- [53] Li M, Luo Q, Tao Y, Sun X, Liu C. Pharmacotherapies for Drug-Induced Liver Injury: A Current Literature Review. *Front Pharmacol* 2021;12:806249. doi:10.3389/fphar.2021.806249, PMID:35069218.
- [54] Huang E, Li S. Liver Kinase B1 Functions as a Regulator for Neural Development and a Therapeutic Target for Neural Repair. *Cells* 2022;11(18):2861. doi:10.3390/cells11182861, PMID:36139438.
- [55] Kluwe J, Pradere JP, Gwak GY, Mencin A, De Minicis S, Osterreicher CH, *et al*. Modulation of hepatic fibrosis by c-Jun-N-terminal kinase inhibition. *Gastroenterology* 2010;138(1):347–359. doi:10.1053/j.gastro.2009.09.015, PMID:19782079.
- [56] Lawan A, Zhang L, Gatzke F, Min K, Jurczak MJ, Al-Mutairi M, *et al*. Hepatic mitogen-activated protein kinase phosphatase 1 selectively regulates glucose metabolism and energy homeostasis. *Mol Cell Biol* 2015;35(1):26–40. doi:10.1128/MCB.00503-14, PMID:25312648.
- [57] Fan N, Zhang X, Zhao W, Zhao J, Luo D, Sun Y, *et al*. Covalent Inhibition of Pyruvate Kinase M2 Reprograms Metabolic and Inflammatory Pathways in Hepatic Macrophages against Non-alcoholic Fatty Liver Disease. *Int J Biol Sci* 2022;18(14):5260–5275. doi:10.7150/ijbs.73890, PMID:36147457.
- [58] Vilar-Gomez E, Martinez-Perez Y, Calzadilla-Bertot L, Torres-Gonzalez A, Gra-Oramas B, Gonzalez-Fabian L, *et al*. Weight Loss Through Lifestyle Modification Significantly Reduces Features of Nonalcoholic Steatohepatitis. *Gastroenterology* 2015;149(2):367–378.e5. doi:10.1053/j.gastro.2015.04.005, PMID:25865049.
- [59] Belfort R, Harrison SA, Brown K, Darland C, Finch J, Hardies J, *et al*. A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. *N Engl J Med* 2006;355(22):2297–2307. doi:10.1056/NEJMoa060326, PMID:17135584.
- [60] Armstrong MJ, Gaunt P, Aithal GP, Barton D, Hull D, Parker R, *et al*. Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): a multicentre, double-blind, randomised, placebo-controlled phase 2 study. *Lancet* 2016;387(10019):679–690. doi:10.1016/S0140-6736(15)00803-X, PMID:26608256.
- [61] Rau M, Geier A. An update on drug development for the treatment of nonalcoholic fatty liver disease - from ongoing clinical trials to future therapy. *Expert Rev Clin Pharmacol* 2021;14(3):333–340. doi:10.1080/17512433.2021.1884068, PMID:33535836.
- [62] Lee HW, Lee JS, Ahn SH. Hepatitis B Virus Cure: Targets and Future Therapies. *Int J Mol Sci* 2020;22(1):213. doi:10.3390/ijms22010213, PMID:33379331.
- [63] Massoud O. Hepatitis C: looking into the future. *Expert Rev Gastroenterol Hepatol* 2020;14(5):367–374. doi:10.1080/17474124.2020.1746641, PMID:32216467.
- [64] Ranieri G, Laface C. Loco-Regional and Systemic Chemotherapies for Hepato-Pancreatic Tumors: Integrated Treatments. *Cancers (Basel)* 2020;12(10):2737. doi:10.3390/cancers12102737, PMID:32987630.
- [65] Ranieri G, Gadaleta-Caldarola G, Goffredo V, Patruno R, Mangia A, Rizzo A, *et al*. Sorafenib (BAY 43-9006) in hepatocellular carcinoma patients: from discovery to clinical development. *Curr Med Chem* 2012;19(7):938–944. doi:10.2174/092986712799320736, PMID:22214462.
- [66] Fulgenzi CAM, Talbot T, Murray SM, Silletta M, Vincenzi B, Cortellini A, *et al*. Immunotherapy in Hepatocellular Carcinoma. *Curr Treat Options Oncol* 2021;22(10):87. doi:10.1007/s11864-021-00886-5, PMID:34424422.
- [67] Liu X, Lu Y, Qin S. Atezolizumab and bevacizumab for hepatocellular carcinoma: mechanism, pharmacokinetics and future treatment strategies. *Future Oncol* 2021;17(17):2243–2256. doi:10.2217/fon-2020-1290, PMID:33663220.
- [68] Jimenez Calvente C, Del Pilar H, Tameda M, Johnson CD, Feldstein AE. MicroRNA 223 3p Negatively Regulates the NLRP3 Inflammasome in Acute and Chronic Liver Injury. *Mol Ther* 2020;28(2):653–663. doi:10.1016/j.ymthe.2019.09.013, PMID:31585800.
- [69] Tsay HC, Yuan Q, Balakrishnan A, Kaiser M, Möbus S, Kozdrowska E, *et al*. Hepatocyte-specific suppression of microRNA-221-3p mitigates liver fibrosis. *J Hepatol* 2019;70(4):722–734. doi:10.1016/j.jhep.2018.12.016, PMID:30582979.
- [70] Borel F, Han R, Visser A, Petry H, van Deventer SJ, Jansen PL, *et al*. Adenosine triphosphate-binding cassette transporter genes up-regulation in untreated hepatocellular carcinoma is mediated by cellular microRNAs. *Hepatology* 2012;55(3):821–832. doi:10.1002/hep.24682, PMID:21932399.