Advances in the understanding of the role and mechanism of action of PFKFB3-mediated glycolysis in liver fibrosis (Review)

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Abstract. Liver fibrosis is a pathophysiologic manifestation of chronic liver disease and a precursor to cirrhosis and hepatocellular carcinoma. Glycolysis provides intermediate metabolites as well as energy support for cell proliferation and phenotypic transformation in liver fibers. 6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3) is a key activator of glycolysis and plays an important role in the process of glycolysis. The role of PFKFB3-mediated glycolysis in myocardial fibrosis, renal fibrosis and pulmonary fibrosis has been demonstrated, and the role of PFKFB3 in the activation of hepatic stellate cells by aerobic glycolysis has been proven by relevant experiments. The present study reviews the research progress on the role and mechanism of action of PFKFB3-mediated glycolysis in the progression of hepatic fibrosis to discuss the role of PFKFB3-mediated glycolysis in hepatic fibrosis and to provide new ideas for research on PFKFB3 as a target for the treatment of hepatic fibrosis.

Contents

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1. Introduction

The prevalence of chronic liver disease is increasing worldwide and liver disease is responsible for >2 million deaths per year, or 4% of all deaths globally (1). Liver fibrosis is the main pathological manifestation of chronic liver disease and is associated with liver injury caused by alcohol, viral hepatitis, drugs, toxins, nonalcoholic steatohepatitis (NASH) and autoimmune diseases (2,3). It is a structural and functional destruction of the liver caused by excessive deposition of extracellular matrix (ECM) due to persistent chronic injury factors and inflammation (4). Liver fibrosis is the progression of chronic liver disease and the process of liver fibrosis determines the progression of chronic liver disease toward cirrhosis and hepatocellular carcinoma (5). Current studies have confirmed that the reversibility of liver fibrosis and cirrhosis may regress in certain cases (6,7). The mechanism by which liver fibrosis occurs remains largely elusive. Although research on the mechanisms of liver fibrosis has made great progress and certain progress has been made in the study of antifibrotic therapy, there are no effective drugs for the treatment of liver fibrosis, and advanced liver fibrosis can be treated only by liver transplantation (5,8).

6-Phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3 (PFKFB3) is a key glycolysis activator and PFKFB3 catalyzes the synthesis of fructose-2,6-bisphosphate (F-2,6-BP). Phosphofructokinase 1 (PFK-1) is one of the three rate-limiting enzymes of glycolysis and F-2,6-BP is the most potent variant activator of PFK-1. PFKFB3 plays an important role in the progression of fibrosis by triggering aberrant glycolysis (9). In studies of myocardial fibrosis, PFKFB3 is an important driver of endothelial mesenchymal transition (EndoMT) and transforming growth factor-β1 (TGF-β1) specifically increases PFKFB3 protein levels (9). The use of PFKFB3 inhibitors reduces myocardial fibrosis manifestations after myocardial infarction by modulating the TGF-β1/SMAD2/3 pathway (10). PFKFB3-driven glycolysis reduces oxidized pentose phosphate pathway (PPP)-derived NADPH to promote fibrosis progression and is critical for

the development of EndoMT- and EndoMT-associated cardiac fibrosis (9). In diabetic kidney disease (DKD), the early growth response 1 (EGR1) transcription factor interacts with TGF-β to increase ECM production, insulin-like growth factor-binding protein 5 in endothelial cells increases the expression of PFKFB3 through EGR1, and the enhancement of PFKFB3-mediated glycolysis promotes DKD progression (11). Studies on the mechanism of renal fibrosis revealed that the GMP-AMP synthase-interferon gene-stimulating factor signaling pathway promotes the progression of renal fibrosis after hypoxic exposure, and this effect is closely related to PFKFB3-mediated glycolysis (12). In lung fibrosis, lipopolysaccharide (LPS) significantly upregulates PFKFB3 expression, enhances aerobic glycolysis and promotes collagen synthesis in lung fibroblasts by activating the PI3K-Akt-mTOR/PFKFB3 pathway (13).

Fibrosis is a response to the repair of damaged tissue and fibrotic tissue forms mainly due to the abnormal deposition of the ECM secreted by myofibroblasts (14). During the repair of damaged tissue, metabolic reprogramming of mesenchymal cells occurs to provide energy for cell proliferation and raw materials for cell generation (15). In liver fibrosis, the activation and proliferation of hepatic stellate cells (HSCs) is critical (16). HSCs are activated when the liver is stimulated by chronic injury or inflammation, or cytokines released by hepatocytes, lymphocytes, Kupffer cells (KCs) and endothelial cells (8). When HSCs are stimulated, activated quiescent HSCs transdifferentiate into a myofibroblast phenotype with high proliferative and migratory capacity that expresses ECM components such as α-smooth muscle actin (α-SMA) and collagen types I and II (8). The large amount of ECM secreted by myofibroblasts accumulates in large quantities outside the cell, causing remodeling of the liver structure, which is a key factor in the development of liver fibrosis (5). Aerobic glycolysis is an important metabolic pathway for activated HSCs in liver fibrosis, a phenomenon similar to the Warburg effect in cancer cells (17). Although glycolysis produces ATP less efficiently, it can meet the energy and material requirements of highly proliferative HSCs and the inhibition of aerobic glycolysis may reduce the activation of HSCs and attenuate liver fibrosis (3,18).

By reviewing the general properties of PFKFB3, the relationship between PFKFB3 and glycolysis, the role of PFKFB3-mediated glycolysis in liver fibers and the current applications of PFKFB3, this paper discusses the control of the process of liver fibrosis by inhibiting PFKFB3-mediated glycolysis and thus provides a new approach for the treatment of liver fibrosis.

2. Methodology

The studies cited in the present review were published between 2011 and 2024, with the majority published between 2020 and 2024. All of the studies cited in the present review were found in the PubMed database (https://www.ncbi.nlm.nih.gov) using the following key words: 'PFKFB3', 'liver fibrosis', 'glycolysis', 'hepatic stellate cells', 'metabolic reprogramming', 'macrophages', 'lymphocytes', 'hepatic sinusoidal endothelial cell' and 'endothelial mesenchymal transition'.

3. General characteristics of PFKFB3

The PFKFB3 protein is a homodimer of 520 amino acids with a molecular weight of ~60 kDa (19). The monomeric structure of PFKFB3 is divided into two functional domains within the same polypeptide chain (20). The C-terminal domain of PFKFB3 contains the bisphosphatase activity of the enzyme, which catalyzes the hydrolysis and degradation of F-2,6-BP to F-6-P and inorganic phosphate. The N-terminal domain is responsible for the synthesis of F-2,6-BP from F-6-P and adenosine triphosphate (ATP). The bifunctional PFKFB family controls the steady-state intracellular concentration of F-2,6-BP (21). This family contains four isozymes, PFKFB1-4 (22). Although the sequence homology of the core catalytic domains of these isozymes is high (85%), they have different properties in terms of their tissue expression profiles, ratios of kinase/phosphatase activities, and responses to protein kinase, hormones and growth factor signals (19,23). PFKFB3 is the most important of the PFKFB. One of the bifunctional isozymes encoded by the PFKFB3 gene has the highest kinase:phosphatase activity ratio (710:1), which maintains a high rate of glycolysis and thus provides high-rate energy support (24).

PFKFB3 protein can be expressed ubiquitously in organisms, with elevated expression levels in proliferating tissues, transformed cells, solid tumors and leukemia cells (25). The expression of the PFKFB3 protein is significantly upregulated during the DNA synthesis phase of the mitotic cycle and in tissues stimulated by injurious factors such as inflammation and hypoxia (9). In addition to its involvement in the regulation of glycolysis by modulating the synthesis and catabolism of F-2.6-BP, PFKFB3 is also involved in the regulation of glucose metabolism via the PPP (9). In the presence of excess reactive oxygen species (ROS), PFKFB3 is inactivated, leading to a shift in glucose utilization from glycolysis to the PPP (9). This allows cancer cells to use glucose to synthesize antioxidants, such as NADPH and glutathione, to reduce oxidative stress-induced cellular damage (26).

The regulation of PFKFB3-mediated glycolysis can be achieved by modulating the transcription and translation of the PFKFB3 gene. In a previous study, activation of mammalian target of rapamycin (mTOR) signaling was shown to upregulate PFKFB3 gene transcription in a hypoxia-inducible factor-1α-dependent manner (27). In addition, PFKFB3 mRNA transcription is also directly regulated by the estrogen receptor (ER), and estradiol promotes glucose uptake and glycolysis in cells with ER by inducing PFKFB3 transcription (28). Steroid receptor coactivator-2 and the progesterone receptor have been shown to bind to the progesterone response element within the PFKFB3 promoter, which activates transcription of the PFKFB3 gene in human endometrial stromal cells (29). Stimuli such as NaCl, H_2O_2, UV radiation and anisomycin can regulate PFKFB3 transcription by binding to the serum response element in the PFKFB3 promoter via serum response factor (30). In addition to transcriptional control, during mitosis, AMP-activated protein kinase (AMPK) signaling promotes the translation of PFKFB3 mRNA by engaging the cytoplasmic polyadenylation element in the 3'-untranslated region (3'-UTR) of the PFKFB3 mRNA (31). Multiple posttranslational modifications of PFKFB3 regulate glucose

use, and PFKFB3 protein levels, subcellular localization and activity are largely influenced by posttranslational modifications (32).

PFKFB3 protein and mRNA are highly expressed in patients with prostate, gastric, colon and breast cancers and play important roles in tumor cell proliferation, migration and invasion in gastric, colon and breast cancers (19,33,34). PFKFB3 is also involved in the regulation of neoangiogenesis by promoting glycolysis in vascular endothelial cells, and the upregulation of PFKFB3 mediates glycolytic reprogramming in pulmonary fibrosis (13). Studies have shown that the use of 3-(3-pyridinyl)-1-(4-pyridinyl)-2-propen-1-one (3PO), a PFKFB3 inhibitor, or disruption of the PFKFB3 genome inhibits PFKFB3-mediated glycolysis. Thus, the differentiation of lung fibroblasts into myofibroblasts is suppressed, reducing the profibrotic phenotype in myofibroblasts from patients with idiopathic pulmonary fibrosis (35). In addition, in myocardial infarction studies, PFKFB3 expression was found to be significantly increased in the region of myocardial infarction. Furthermore, the expression of collagen and fibronectin in the heart was reduced and cardiac fibrosis was attenuated after myocardial infarction after treatment with 3PO (10). PFKFB3 also promotes immune activation of vascular endothelial cells, induces an acute inflammatory response and participates in the formation of endothelial immune memory, leading to long-term endothelial inflammatory injury, affecting the blood supply of injured tissues and regulating the pathogenesis of renal fibrosis (36). As a glucose metabolism-regulating enzyme, the structural features of PFKFB3 determine its role in the synthesis and hydrolysis of F-2,6-BP and F-6-P, which are important intermediates of glucose metabolism. Analyzing the role and mechanism of action of PFKFB3 in glucose metabolism can provide new targets for the treatment of glucose metabolism-related diseases.

4. Relationship between PFKFB3 and glycolysis

In normal cells, glycolysis converts glucose to pyruvate and produces ATP, which reduces NAD^+ to NADH (37). The conversion of pyruvate to acetyl coenzyme A in the oxygen-rich state enters the tricarboxylic acid cycle for oxidation to CO_2 and H_2O, the production of ATP and the final reduction of pyruvate to lactate in the anaerobic state (38). The metabolites of glycolysis can enter the anabolic pathway to produce NADPH and the raw materials needed for glycogen, lipid, nucleotide and protein synthesis (38). Under oxygen enrichment, the primary function of glycolysis is to produce intermediate metabolites that provide source materials for biosynthetic pathways, whereas under hypoxia, the primary role of glycolysis is to provide ATP for cell survival (37). Tumor cells, even under oxygen-rich conditions, exhibit high glycolytic activity and produce lactate by activating lactate dehydrogenase and inhibiting pyruvate metabolism in mitochondria (39). This phenomenon was first observed by Otto H. Warburg in the early twentieth century and is known as the Warburg effect or aerobic glycolysis (40). A recent study suggested that under aerobic conditions, both normal proliferating cells and cancer cells increase metabolic flux through glycolysis and related biosynthetic pathways (41). The process of cell proliferation involves the *de novo* synthesis of macromolecules such as lipids and nucleotides, and intermediates of glycolysis are precursors of these anabolic pathways (37).

Hexokinase 2, phosphofructokinase 1 (PFK1), and M2-type pyruvate kinase are three key enzymes of glycolysis (42). Control of the glycolytic process can be achieved through the regulation of key enzymes of glycolysis, and the F-2,6-BP produced by PFKFB3 is the most potent metabolic activator of PFK-1. Numerous studies have shown that the regulation of cellular glycolytic flux can be achieved through the intervention of transcriptional and translational processes, as well as protein modification of PFKFB3 (22). Glycolysis occurs mainly in the cytoplasm and the PFKFB3 protein in the cytoplasm can be phosphorylated by different protein kinases. Phosphorylated PFKFB3 activates PFK-1 and promotes an increase in cellular glycolytic flux by facilitating the F-2,6-BP transition (13,43). PFKFB3 in the cytoplasm contains a nuclear localization signal (NLS) that transports PFKFB3 to the nucleus, where it regulates cell cycle progression and DNA repair under specific conditions (44). However, when the Lys472/473 site of the PFKFB3 protein is acetylated, NLS recognition is blocked and PFKFB3 is unable to move to the nucleus, thus accumulating in the cytoplasm and promoting an increase in cellular glycolysis (45). Similar to acetylation, when the Arg131/134 sites of PFKFB3 are methylated, the stability of the PFKFB3 protein increases and degradation decreases, resulting in an increase in the cytoplasmic content of PFKFB3 and thus promoting an increase in cellular glycolytic flux (46,47). Therefore, by blocking the NLS of the PFKFB3 protein, the intranuclear translocation of PFKFB3 can be prevented, thereby increasing its intracytoplasmic content and enhancing cellular glycolysis (45). In addition, AMPK can control glycolytic flux through the mitotic-specific promotion of PFKFB3 translation and the phosphorylation of PFKFB3 at the Ser461 site (32). PFKFB3 is a key factor in the control of glycolytic flux. Reducing PFKFB3 levels inhibits glucose consumption, inhibits lactate production and promotes premature death of mitotic cells (31). PPP is a glucose metabolic pathway that parallels glycolysis, converting glucose-6-phosphate (G-6-P) to ribulose 5-phosphate and producing NADPH and raw materials for the synthesis of ribulose 5-phosphate, and the inhibition of PFKFB3 shifts glucose metabolism from the glycolytic flux to PPP (26). ROS induce inactivation of PFKFB3, shifting metabolism from glycolysis to the PPP and promoting NAPDH production and ROS detoxification (48,49). A study has shown that in HSCs, the cytoplasmic polyadenylation element (CPE) of the 3'-UTR of PFKFB3 mRNA recruits CPE-binding protein (CPEB), which promotes the translation of PFKFB3 mRNA and that the upregulation of PFKFB3 through the CPEB4-PFKFB3 pathway can promote glycolysis in HSCs (22). In addition, the Fascin (a prometastatic actin-bundling protein) Yes1-associated transcriptional regulator-PFKFB3 signaling pathway can also increase glycolytic flux in tumor cells and promote tumor invasion and metastasis by promoting PFKFB3 transcription (Figs. 1 and 2) (50,51).

5. Relationships between PFKFB3-mediated glycolysis and liver fibrosis

Occurrence and regulation of liver fibrosis. Liver fibrosis is a dynamic developmental process. This process mainly involves

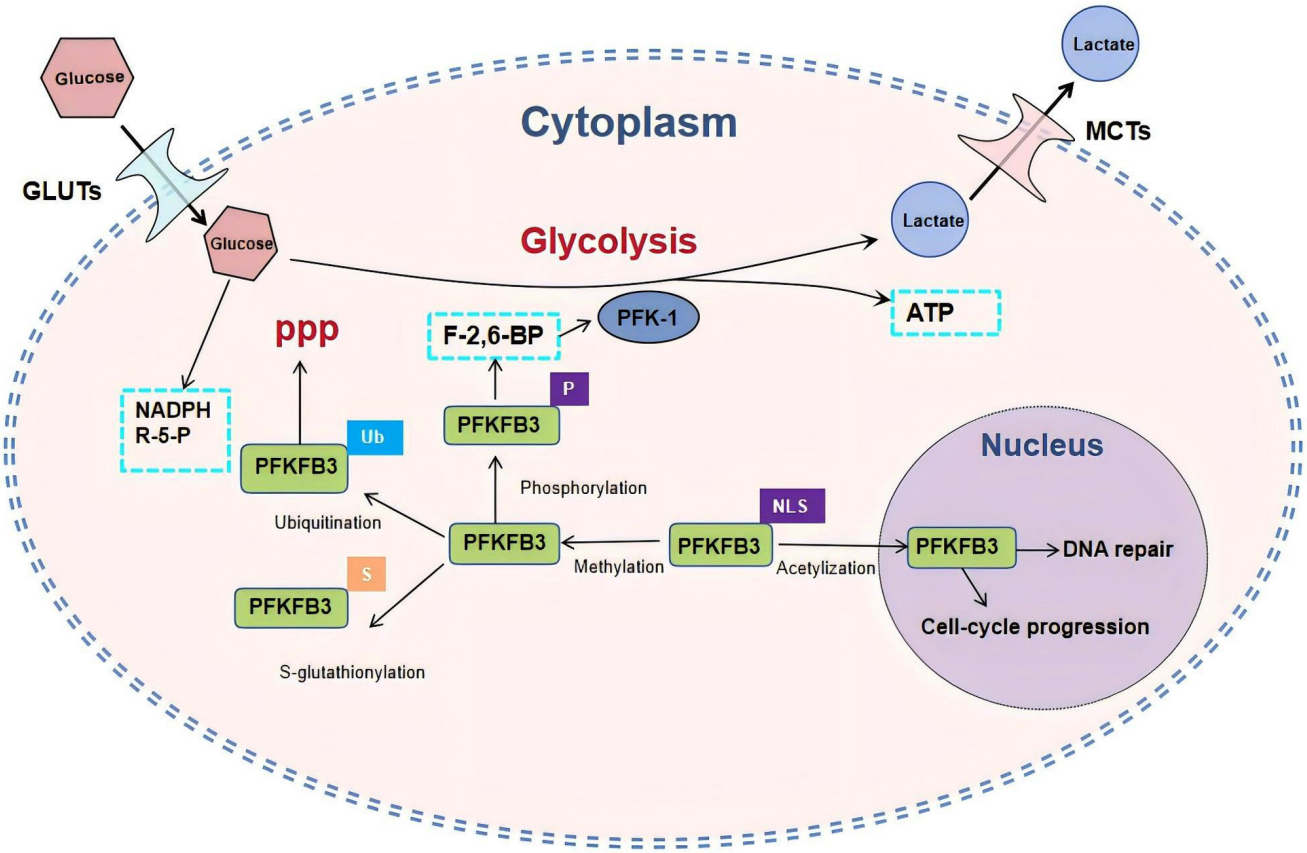

Figure 1. Functions of PFKFB3 in cells. The PFKFB3 protein is mainly located in the cytoplasm. In the cytoplasm, PFKFB3 is phosphorylated by a series of kinase proteins and promotes glycolysis by modulating and maintaining the production of F-2,6-BP to allosterically activate the glycolytic enzyme PFK-1. Cytoplasmic PFKFB3 contains an NLS, allowing it to traffic to the nucleus, where it participates in controlling cell cycle progression and DNA repair in specific contexts. However, acetylation prevents NLS recognition and promotes PFKFB3 cytoplasmic retention. PFKFB3 S-glutathionylation and ubiquitination are also important factors in the mechanism of PFKFB3. Glutathionylation and ubiquitination inactivate PFKFB3 and lead to a shift in glucose utilization from glycolysis to the PPP. PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3; NLS, nuclear localization signal; PPP, pentose phosphate pathway; MCTs, monocarboxylate transporters; GLUTs, glucose transporters; F-2,6-BP, fructose-2,6-bisphosphate; PFK-1, phosphofructokinase 1; ATP, adenosine triphosphate; NADPH, nicotinamide adenine dinucleotide; R-5-P, ribose 5-phosphate.

chronic hepatocellular injury, damage to the epithelial or endothelial barrier, the release of inflammatory cytokines, the recruitment of bone marrow-derived inflammatory cells, the production of TGF-β by macrophages, the activation of hepatic myofibroblasts, the overproduction of ECM [mainly collagen type I (COL1A1), and the formation of fibrous scarring (7,15). Current research on liver fibrosis focuses on the reversal of liver fibrosis. Numerous clinical studies have shown that liver fibrosis is reversible in human patients and animal models (52,53). After removal of chronic liver injury factors, regression of liver fibrosis is associated with decreased production of proinflammatory or profibrotic cytokines, increased collagenolytic activity, disappearance of hepatic myofibroblasts, inhibition of ECM production and dissolution of fibrous scarring (7,54). During liver injury, HSCs are activated and transdifferentiate into collagen-producing myofibroblasts (16). In chronic liver disease, profibrotic fibroblasts are present, and an imbalance between profibrotic and antifibrotic mechanisms leads to overactivation of HSCs and accumulation of ECM (55,56). The liver enters an antifibrotic phase, or profibrotic phase dependent on the regulation mainly by nonparenchymal cells, such as KCs and other immune cells (57). Hepatocyte apoptosis and damage-associated molecular patterns (DAMPs) not only directly activate HSCs but also induce the recruitment and activation of lymphocytes and macrophages (58). These cells produce proinflammatory and profibrotic cytokines that promote the activation of HSCs to transdifferentiate into myofibroblasts (54). Matrix metalloproteinases (MMPs) are antifibrotic factors and certain macrophages can produce MMPs involved in the regression of liver fibrosis (59,60). Different cytokines are involved in the regulation of liver fibrosis through different signaling pathways. Current studies have shown that the TGF-β, platelet-derived growth factor (PDGF) and inflammatory vesicle (NLR family pyrin domain containing 3)-Caspase1 pathways and WNT/β-linker protein signaling are the key signaling pathways associated with the activation of HSCs and the progression of liver fibrosis (61-64). TGF-β1 plays a key role in the activation of HSCs and is secreted by a variety of cells, including hepatocytes, KCs, hepatic sinusoidal endothelial cells, bile duct epithelial cells and HSCs (65). TGF-β1 promotes the synthesis of ECM by HSCs while also inhibiting MMP synthesis and thus ECM degradation (66). PDGF is the strongest mitogen in HSCs and TGF-β1 can stimulate the expression of the PDGF receptor in HSCs, thereby enhancing the role of PDGF and promoting HSC proliferation (67). TGF-β can also drive

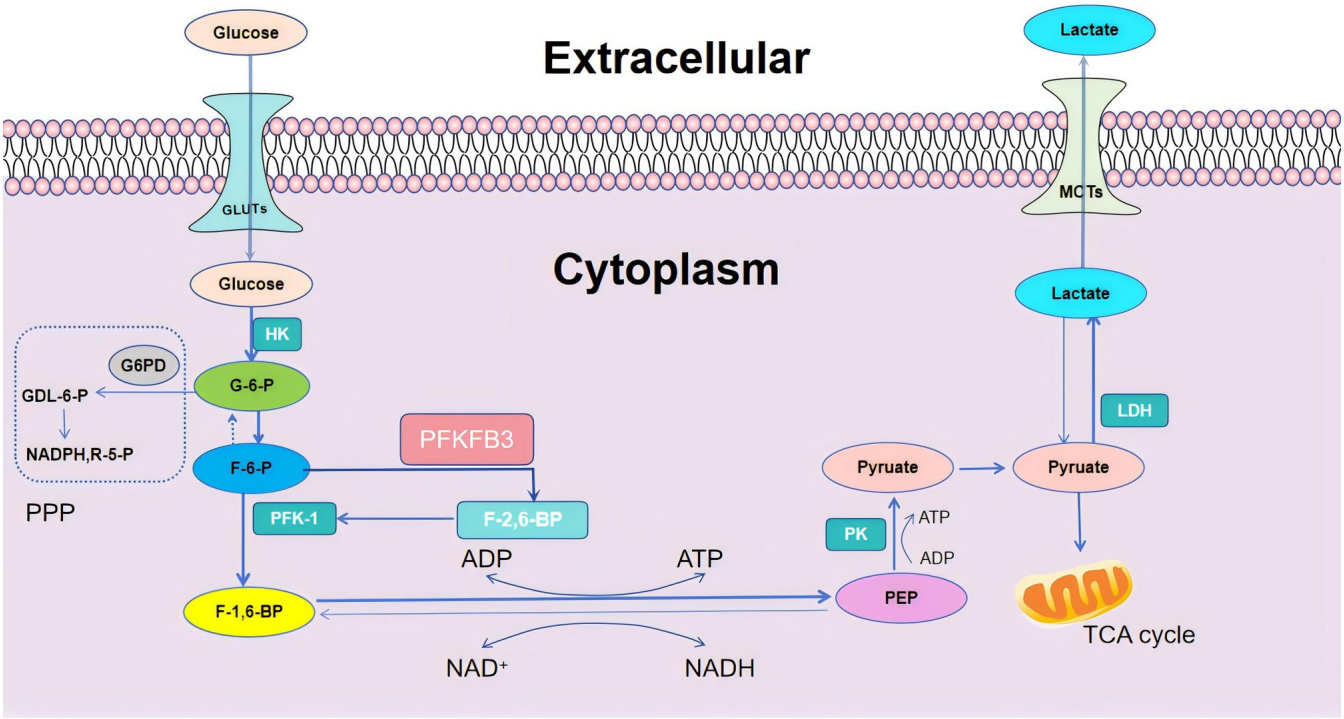

Figure 2. Glycolytic cycle. Glycolysis is an ancient metabolic pathway that converts glucose into pyruvate. GLUTs transfer proteins located on the cell membrane and uptake extracellular glucose. Cytoplasmic glucose is phosphorylated by HK, the first rate-limiting enzyme of glycolysis, to form G-6-P. G-6-P is then rearranged into F-6-P by glucose phosphate isomerase. F-6-P is then irreversibly converted to F-1,6-BP under the catalysis of PFK-1, the main rate-limiting enzyme of glycolysis. F-2,6-BP, a product of the reaction catalyzed by PFKFB3, is the most potent positive allosteric effector. Then, following a series of reversible enzymatic reactions, F-1,6-BP is converted to PFKFB3, which is the most potent positive allosteric effector of PFK-1. In addition, NADH and ATP are generated in these reactions. Finally, PEP is phosphorylated by PK, the third rate-limiting enzyme of glycolysis, to form pyruvate and a molecule of ATP. In the absence of oxygen, pyruvate is converted to lactate under the catalysis of LDH. Lactate is then transported extracellularly through MCTs. In the presence of oxygen, pyruvate enters the mitochondria for the TCA cycle. GLUTS, glucose transporters; HK, hexokinase; G-6-P, glucose 6-phosphate; PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3; PEP, phosphoenolpyruvate; PK, pyruvate kinase; LDH, lactate dehydrogenase; MCTs, monocarboxylate transporters; TCA, tricarboxylic acid; GDL-6-P, 6-phosphogluconolactone; G6PD, glucose-6-phosphate dehydrogenase; NADPH, nicotinamide adenine dinucleotide; R-5-P, ribose-5-phosphate; PPP, pentose phosphate pathway; HK, hexokinase 2; F-6-P, fructose-6-phosphate; PFK-1, phosphofructokinase 1; F-1,6-BP, fructose-1,6-bisphosphate; F-2,6-BP, fructose-2,6-bisphosphate; ADP, adenosine diphosphate; ATP, adenosine triphosphate.

the activation of HSCs in a Smad2- or Smad3-dependent manner (68). In addition, connective tissue growth factor and IL-13 promote COL1A1 expression in activated HSCs through a TGF-β1-independent pathway (69). During the progression of hepatic fibrosis, the control of hepatic fibrotic processes can be achieved by regulating the cells and cytokines involved in hepatic fibrosis-related processes. Cellular glycolysis provides energy and raw materials for cell proliferation and cytokine release (3). By controlling glycolysis in cells involved in liver fibrosis, glycolysis may regulate the liver fibrosis process and provide a new way of treating liver fibrosis. During the progression of hepatic fibrosis, the control of hepatic fibrotic processes can be achieved by regulating the cells and cytokines involved in hepatic fibrosis-related processes. Cellular glycolysis provides energy and raw materials for cell proliferation and cytokine release (3). By controlling glycolysis in cells involved in liver fibrosis, glycolysis may regulate the liver fibrosis process and provide a new way of treating liver fibrosis (Fig. 3).

Role of PFKFB3-mediated glycolysis in liver fibrosis cells

PFKFB3-mediated regulation of glycolysis in HSCs in liver fibrosis. HSCs in the normal liver are quiescent, nonproliferative, periportal, hepatic sinusoidal cells (70). During liver injury, HSCs are activated and transdifferentiate into myofibroblasts with contractile and proliferative properties (16). Activated HSCs produce many myofibroblasts and large amounts of ECM components, such as collagen types I, III and IV, fibronectin, laminin, proteoglycans and proinflammatory factors (70). Activated HSCs express high levels of α-SMA and tissue inhibitor of metalloproteinase 1, which promotes phenotypic switching of HSCs and facilitates the progression of fibrosis (56). In advanced fibrosis, the contraction of numerous activated HSCs and myofibroblasts promotes hepatic sinusoidal contraction, which affects the blood flow and nutrient exchange while accelerating hepatic dysfunction (70,71). Fibrosis regression is associated with the inactivation and apoptosis of HSCs and myofibroblasts (72,73). The regulation of HSC death is an important mechanism to resolve hepatic fibrosis, and tumor necrosis factor (TNF)-related apoptosis-inducing ligand-mediated apoptosis of HSCs has been associated with the improvement of hepatic fibrosis (74). The activation of HSCs is characterized by accelerated cell proliferation and increased ECM secretion, with aerobic glycolysis providing the substances and energy required for cell proliferation and phenotypic transformation (64,75).

A study by Mejias *et al* (22) suggested that PFKFB3 protein expression is higher in hepatic fibrotic tissues than in normal

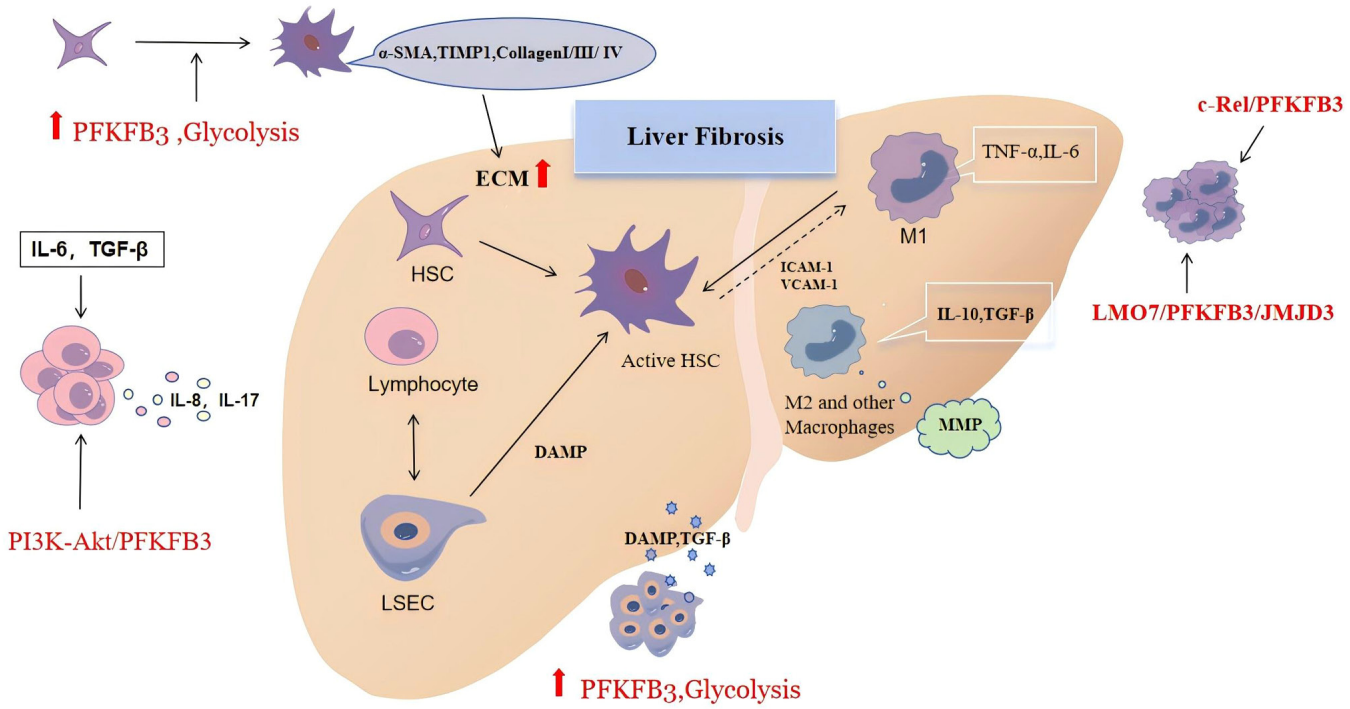

Figure 3. PFKFB3-mediated glycolysis regulates liver fibrosis. In liver fibrosis, substances such as collagen I/III/IV and α-SMA are produced after HSCs are activated, which promotes the accumulation of ECM in the liver and promotes the formation of liver fibrosis. HSCs can also secrete ICAM-1 and VCAM-1 to interact with immune cells and promote the formation of fibrosis. M1 macrophages can promote the progression of inflammation in the liver, promote the formation of liver fibrosis, and some macrophages can also show anti-inflammatory properties and secrete IL10, TGF-β and MMP to promote the degradation of ECM and the regression of liver fibrosis. Hepatic sinusoidal endothelial cells can undergo capillary vascularization to promote the formation of liver fibrosis, and can also secrete proinflammatory factors to promote the activation of HSCs and promote the formation of liver fibrosis. PFKFB3-mediated glycolysis regulates liver fibrosis by regulating the biosynthesis and proliferation activation of LSECs, HSCs, lymphocytes and macrophages in liver fibrosis. PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3; SMA, smooth muscle actin; HSC, hepatic stellate cell; ECM, extracellular matrix; ICAM, intracellular adhesion molecule; VCAM, vascular cell adhesion molecule; LSEC, liver sinusoidal endothelial cells; IL-6, interleukin 6; TGF-β, transforming growth factor β; IL-17, interleukin 17; PI3K-Akt, phosphoinositide 3-kinase-Akt; TIMPl, tissue inhibitor of metal protease 1; TNF-α, tumor necrosis factor α ; M1, proinflammatory macrophages; TGF-β, transforming growth factor β; LMO7, LIM domain only 7; JMJD3, jumonji structural domain-containing protein 3; M2, anti-inflammatory macrophages; MMP, matrix metalloproteinases; DAMP, damage-associated patterns of hepatocyte release.

livers and that PFKFB3-positive cells accumulate mainly in oxygen-rich fibrotic liver regions, supporting the abnormally high rate of glycolysis in activated HSCs. 3PO significantly reduces F-2,6-BP in LX2 human HSCs, markedly attenuates LX2 glycolytic flux and inhibits LX2 activation (22). Of note, this phenomenon suggests that PFKFB3 overexpression in liver fibrosis causes activated HSCs to undergo a high rate of glycolysis without the induction of a hypoxic environment. Mechanistically, PFKFB3 promotes the translation of PFKFB3 mRNA through the binding of the RNA-binding protein CPEB4 to the CPE within the 3'-UTR of the PFKFB3 mRNA, increasing the PFKFB3 content and thus enhancing glycolysis in HSCs (22). This study revealed that the increase in PFKFB3 protein-mediated glycolytic flux is an important feature of HSC activation in liver fibrosis and that the upregulation of PFKFB3 accelerates the onset of glycolysis in HSCs, provides biosynthetic raw materials and fuels for the proliferation, activation and phenotypic transformation of HSCs, and accelerates the process of liver fibrosis. In liver regeneration (LR) studies, after partial hepatectomy (PHx), HSCs exhibited high glycolytic metabolic flux and increased PFKFB3 expression in the liver and cells, and LR after PHx involved the activation of PFKFB3 in HSCs, as well as an increase in PFKFB3 to promote the production of the glycolysis product lactic acid, which promotes the proliferation of HSCs through the p38/ERK MAPK signaling pathway (76). In addition, the use of PDGF increased the protein levels of PFKFB3, the extracellular acidification rate (ECAR) and lactate in LX2 cells and decreased PFKFB3 expression. It significantly reduced the PDG-induced ECAR and lactate production, which contrasts with the findings of previous studies on pulmonary artery smooth muscle cells. Smooth muscle cells treated with PDGF exhibited a significant increase in PFKFB3 expression (Table I) (76,77).

PFKFB3-mediated regulation of glycolysis in hepatic macrophages in liver fibrosis. Hepatic macrophages play important roles in hepatic inflammation and fibrosis and consist mainly of liver-resident KCs and bone marrow-derived monocytes (78). Control of inflammatory progression and macrophage activation in liver injury is critical for regulating the development of liver fibrosis (56). Macrophages can be categorized as proinflammatory macrophages. Macrophages can be divided into proinflammatory macrophages (M1) and immunomodulatory cells (M2). M1 macrophages are characterized by the expression of proinflammatory cytokines (TNF-α, IL-1β and IL-6), whereas M2 macrophages express anti-inflammatory mediators (IL-4) (79). M1 and M2 macrophages coexist and play different roles at different stages of liver fibrosis. Proinflammatory factors stimulate KCs to polarize to the M1 phenotype, secrete proinflammatory factors

Table I. Role of PFKFB3-mediated glycolysis in liver fibrosis cells.

Cell type	Role of cells in liver fibrosis	Mechanism of PFKFB3-mediated regulation of glycolysis	(Refs.)
HSC	Produces extracellular matrix components as well as pro-inflammatory factors that promote hepatic sinusoidal contraction.	Promotes lactate production, HSC activation and the p38/ERK MAPK/PFKFB3 signaling pathway for cell proliferation.	(22,70,71,76)
Macrophage	M1 is pro-inflammatory and promotes the activation of HSCs. M2 is anti-inflammatory and produces MMP, which is involved in the regression of fibrosis.	c-Rel/PFKFB3 and miR-193a-3p/PFKFB3 are involved in the regulation of inflammation and fibrosis. The LMO7/PFKFB3/JMJD3 axis regulates M1. Regulates lactate levels in the environment in which M2 is located.	(78,79,81, 84,85,87,88)
Lymphocyte	Secretion of pro-fibrotic factors and amplification of tissue damage.	PI3K/Akt/PFKFB3 regulates lymphocyte proliferation. Involved in the regulation of $CD4^+$ T metabolism; a decrease in the PFKFB3:G6PD ratio pushes cells toward the pentose phosphate pathway.	(91-98,100)
Endothelial cells of the liver sinusoids	Secretion of vasoactive substances, modulation of immunity and activation of HSCs; maintenance of HSC quiescent phenotype in healthy liver.	Promotes CXCL1 expression and pro-vascular signaling. Increases endothelial-to-mesenchymal transition-related vascular remodeling. Inhibition of PFKFB3 quiesces proliferating endothelial cells.	(115-118)
Myofibroblast	Not present in normal liver, activated in liver injury.	Downregulation of PFKFB3 inhibits TGF-β-induced activation of myofibroblast cells. PFKFB3 expression is regulated by fibroblast positive feedback.	(119-122)

PFKFB3, 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3; HSC, hepatic stellate cell; miR, microRNA; CXCL1, C-X-C motif chemokine ligand 1; ECM, extracellular matrix; ERK, extracellular regulated protein kinases; MAPK, mitogen-activated protein kinases; M1, proinflammatory macrophages; M2, anti-inflammatory macrophages; MMP, matrix metalloproteinases; LMO7, LIM domain only 7; JMJD3, jumonji structural domain-containing protein 3; G6PD, glucose-6-phosphate dehydrogenase; TGF-β, TGF-β, transforming growth factor β.

and ROS, and promote the recruitment of other immune cells to induce an inflammatory cascade response that further accelerates hepatic injury, whereas M2 KCs mediate inflammation abatement and participate in tissue remodeling through the secretion of immunomodulatory mediators (80). In the early stages of liver injury, proinflammatory macrophages play an important role, the recruitment of proinflammatory cells drives liver inflammation and the reciprocal stimulation of inflammatory cells and HCSs promotes the progression of liver fibrosis (79,81,82). Activated macrophages secrete cytokines to promote the activation of HSCs, which subsequently produce IL-6 and other cytokines that perpetuate profibrotic macrophage activity (81,82). During liver injury, certain macrophages exhibit anti-inflammatory features; these macrophages respond to IL-10, IL-4 and IL-13 and secrete anti-inflammatory mediators such as IL-10 and TGF-β. Certain macrophages exhibit a pro-wound healing phenotype characterized by the production of MMPs, which are involved in matrix degradation and regression of fibrosis (79,81).

In recent years, metabolic studies have revealed that specific metabolic pathways of macrophages are closely related to their phenotype and function and that in the physiological state, macrophages use oxidative phosphorylation as their main metabolic pathway (83). In physiological states, macrophages use oxidative phosphorylation as their main metabolic pathway (83). In the early stage of liver injury, when macrophages are stimulated by inflammation, the metabolism of proinflammatory macrophages (M1) is dominated by glycolysis, which meets the energy demands of the rapid inflammatory response, whereas anti-inflammatory macrophages (M2) are more dependent on mitochondrial oxidative phosphorylation (OXPHOS) (84). PFKFB3, a key regulator of macrophage glycolysis, is upregulated in macrophages after LPS stimulation and promotes proinflammatory cytokine production (85). A study by Leslie *et al* (86) revealed that damage to c-Rel, an NF-κB activator, promotes the expression of PFKFB3 in macrophages and that c-Rel/PFKFB3 is involved in the regulation of metabolism required for inflammatory and fibrotic activities in macrophages. Fuhrman and Brüne (87) reported that microRNA-193a-3p promotes Akt phosphorylation and that upregulation in human macrophages during hypoxia-induced glycolysis, PFKFB3 activation promotes glycolysis. Zhang *et al* (88) suggested that endothelial cell deletion of PFKFB3 reduces lactate, a product of glycolysis, and that the environment in which macrophages live is reduced in terms of lactate and fails to maintain the M2-like macrophage phenotype, which in turn inhibits tissue repair. Duan *et al* (85) suggested that LIM domain only 7 (LMO7) binds to and induces the ubiquitination and degradation of PFKFB3 and that LMO7 is downregulated in M1

macrophages. PFKFB3 is required for the expression of the histone demethylase Jumonji structural domain-containing protein 3 (JMJD3), and the LMO7/PFKFB3/JMJD3 axis plays a key role in regulating macrophage function (85). The progression of M1-mediated hepatic fibrosis is further regulated via the regulation of PFKFB3-mediated glycolysis in M1 macrophages (84,85). In addition, although OXPHOS is the main mode of action in the metabolism of M2 cells, lactate production by PFKFB3-mediated glycolysis in endothelial cells can regulate the lactate level of the environment in which M2 cells are located by shuttling lactate between cells, modulating the role of M2 cells in the process of liver fibrosis (84,88).

PFKFB3-mediated regulation of glycolysis in lymphocytes in liver fibrosis. Chronic liver injury induces the production of proinflammatory factors and leukocyte infiltration in the subendothelial space (16). Inhibition of hepatic lymphocyte recruitment reduces the fibrogenic response (89,90). In fibrosis, myofibroblasts secrete cytokines such as IL-6, hepatocyte growth factor and TGF-β to promote lymphocyte migration (89). Type 2 T-helper cells participate in fibrosis development by stimulating profibrotic gene expression in myofibroblasts and the synthesis of immunomodulatory mediators in macrophages (91). IL-17-producing CD4+ T cells and regulatory T cells (Tregs) have also been identified as effector cells in hepatic fibrogenesis (89). IL-17 expression is upregulated in hepatic fibrotic tissues and promotes proinflammatory cytokine expression, liver injury and fibrosis (92). Increased Treg populations are observed in patients with liver fibrosis, which may further promote fibrosis through the secretion of IL-8 and EGFR (89,93,94). Lymphocytes may promote fibrosis by secreting profibrotic cytokines or amplifying tissue damage (95).

The process of lymphocyte proliferation activation requires the coordination of cell signaling cascades and metabolic enzymes to express and secrete inflammatory factors, a process that involves aerobic glycolysis in lymphocytes (96). In proliferating T lymphocytes, the energy and raw materials for cell proliferation are derived mainly from glycolysis (96,97). Lymphocyte activation is associated with an increase in the level of intracellular F-2,6-BP, which is the most potent metabotropic activator of PFK-1, and PFKFB3 regulates F-2,6-BP levels (98). A study by Simon-Molas *et al* (99) revealed that the regulation of glycolysis during lymphocyte proliferation and activation could be achieved via PFKFB3 regulation through the PI3K-Akt signaling pathway in activated T lymphocytes. In activated $CD4^+$ T cells, which were fed mainly by glycolysis and oxidative phosphorylation, PFKFB3 is involved in regulating the metabolic pattern of activated $CD4^+$ T cells (100). A decrease in the ratio of PFKFB3 to G6PD pushes the cells toward the pentose phosphate pathway (100). Lymphocytes play a profibrotic role in hepatic fibrosis by secreting proinflammatory factors and interacting with endothelial cells to promote tissue damage (92). PFKFB3-mediated glycolysis regulates lymphocyte activation and modulates the pattern of cellular metabolic reprogramming, which in turn regulates the secretion of inflammatory factors by lymphocytes and is involved in the regulation of fibrosis-associated diseases (100). Previous studies have shown that PFKFB3-mediated glycolysis has an important role in liver fibrosis (22). However, whether the involvement of PFKFB3 in the regulation of fibrosis is related to its involvement in the regulation of lymphocyte glycolysis in the liver has not been addressed. PFKFB3-mediated glycolysis regulates lymphocyte function (101), which, in combination with the role of lymphocytes in liver fibrosis, may provide new insights for further exploration of whether PFKFB3-mediated glycolysis is involved in the regulation of liver fibrosis by modulating the metabolism of lymphocytes (Table I).

PFKFB3 mediates the regulation of hepatic sinusoidal endothelial cell glycolysis in liver fibrosis. Liver sinusoidal endothelial cells (LSECs) are the gateway to the liver and play an important role in the etiopathogenesis of liver fibrosis by generating pathologic blood vessels involved in hepatic tissue repair and inflammation (102). LSECs secrete vasoactive substances involved in the regulation of vascular tone and act as apoptotic cells to regulate immune dynamic homeostasis (103). During liver injury, LSECs go through phenotypic and functional changes and undergo capillarization into hepatic sinusoids, which have provasoconstrictive, proinflammatory and prothrombinogenic functions (104). During liver fibrosis, hepatic fibrosis enhances angiogenesis and hepatic angiogenesis exacerbates hepatic fibrosis (105). As the capillarization of LSECs allows the proper oxidation of hepatocytes to be dysregulated, apoptosis and necrosis occur, leading to the secretion of DAMPs (106). DAMP- and LSEC-derived factors activate HSCs, and activated HSCs produce excess ECM and promote fibrosis (107). During chronic hepatitis C virus infection, LSECs maintain their phenotype and capillary action is induced only in the initial stages of fibrosis (108). The main features of NASH are inflammation, steatosis, hepatocellular injury and fibrosis (109). LSECs also undergo capillarization in the early stages of nonalcoholic fatty liver disease (NAFLD) (110). Dysfunctional LSECs affect the state of intrahepatic microcirculation, resulting in increased hepatic vascular resistance, which leads to portal hypertension and steatosis, and contributes to the progression of NAFLD to NASH (111). Dysfunctional LSECs also produce profibrotic molecules, such as TGF-β, which promote the activation of HSCs, thereby increasing ECM production and endothelial vasoconstriction in the hepatic sinusoids and accelerating the development of liver fibrosis (112). LSECs in healthy livers maintain a quiescent HSC phenotype, which is lost in capillarized LSECs (105). A recent study has shown that myofibroblasts in fibrotic diseases can originate from endothelial cells and acquire a mesenchymal phenotype through EndoMT (113). The TGF-β-Smad signaling pathway is the most important transduction pathway regulated by EndoMT (114). The TGF-β-Smad signaling pathway is the most important pathway for EndoMT regulation (114). EndoMT also occurs in patients with cirrhosis and in experimental mice with liver fibrosis. Prevention of the TGF-β-Smad signaling pathway reduces EndoMT and attenuates the development of liver fibrosis (113).

In a study by Greuter *et al* (115), liver fibrosis was attenuated in a mouse model of carbon tetrachloride-induced liver fibrosis after treatment with the PFKFB3 inhibitor 3PO. Mechanistically, during liver fibrosis, glycolysis promotes C-X-C motif chemokine ligand 1 expression by altering nuclear pores and increasing NF-κB translocation. Furthermore, glycolytic enzymes promote inflammation, fibrosis and portal hypertension by secreting vascular signals in stiffness-induced vessels (115). Edema and inflammation

lead to increased stiffness of hepatic tissue and promote hepatic fibrosis (116). Liver stiffness induces the recruitment of glycolytic enzymes, particularly PFK-1, to focal adhesions, and glycolysis is increased in cells with endothelial cell migration and angiogenesis (115). Glycolytic enzymes are involved in vascular secretory signaling induced by vascular stiffness in hepatic sinusoidal endothelial cells and may serve as drug targets in early liver disease (115). In studies of renal fibrosis, PFKFB3-mediated increases in glycolysis can drive EndoMT in renal capillary endothelial secretions (117). The glycolysis-mediated TGF-β-Smad pathway has also been shown to induce EndoMT in a study on vascular remodeling, where inhibition of PFKFB3 activity was observed to affect EndoMT-related arterial remodeling (114). PFKFB3 plays an important role in manipulating endothelial phenotypic changes and PFKFB3-driven glycolysis is involved in pathologic angiogenesis by affecting vascular sprouting as well as endothelial proliferation and migration (9). Wei *et al* (113) also suggested that high mobility group box 1 induces EndoMT in LSECs but only in the ECM of umbilical vein endothelial cells, but there is no study on whether LSECs can also differentiate into myofibroblasts, to the best of our knowledge. The inhibition of PFKFB3 was shown to quench proliferating endothelial cells to maintain phenotypic homeostasis (118). Although most findings concerning PFKFB3 have been reported in the context of vascular sprouting manipulations, these events also suggest a potential role for PFKFB3 in the control of EndoMT, considering that endothelial cells are largely dependent on glycolysis to support cell growth and differentiation (9). By inhibiting PFKFB3-mediated glycolysis, the capillarization of LSECs, generation of vascular secretory signals and EndoMT, and activation of HSCs can be reduced, thereby reducing hepatic inflammation, fibrosis and portal hypertension (Table I).

PFKFB3 regulates glycolysis in myofibroblasts in liver fibrosis. Myofibroblasts are not present in the normal liver; however, myofibroblast activation occurs in chronic liver injury (119). The activation of myofibroblasts is an important part of the hepatic fibrosis mechanism and resident hepatic mesenchymal stromal cells, activated HSCs and activated portal fibroblasts are the main contributors of myofibroblasts in fibrotic livers (119,120). TGF-β is a key factor in myofibroblast activation, and in a recent study on fibrosis, it was shown that glycolysis can be inhibited by downregulating the expression of PFKFB3, thereby ameliorating TGF-β-induced myofibroblast activation (121). Mechanistically, studies in both pulmonary and renal fibrosis suggest that the inhibition of PFKFB3, which reduces the level of the glycolytic product lactate, reduces myofibroblast activation, and activated fibroblasts positively regulate the expression of PFKFB3 via feedback, increasing the cellular glycolytic flux (122). Recent studies have revealed the important role of myofibroblast activation in hepatic fibrosis and certain studies have investigated the mechanism of PFKFB3-regulated myofibroblasts in renal fibrosis, pulmonary fibrosis and myocardial fibrosis (122-125); however, to the best of our knowledge, there are no specific studies on the mechanism of PFKFB3-mediated glycolysis in hepatic fibrosis and the related pathways. According to the current study of glycolysis in liver fibrosis, PFKFB3-mediated glycolysis may promote myofibroblast activation in liver fibrosis by regulating the lactate content in cells. TGF-β, an important regulator of liver fibrosis, is also a key factor in myofibroblast activation; thus, it is not clear whether the regulatory pathways of PFKFB3-mediated glycolysis and TGF-β are relevant to the activation of myofibroblasts in liver fibrosis. However, further studies are needed to confirm the role of myofibroblast activation in liver fibrosis (Table I) (121,126).

6. Prospects for PFKFB3-mediated glycolysis

PFKFB3 is an important regulator of key enzymes of glycolysis, and with recent studies on glycolysis in disease, selective inhibition of PFKFB3 in disease has attracted the attention of researchers (19). PFKFB3 is closely related to cancer development, cancer cell proliferation, vascular invasiveness, drug resistance and the tumor microenvironment; thus, more studies on the application of PFKFB3 in cancer have been conducted (21,127). PFKFB3 inhibitors can effectively regulate glycolysis in tumor cells, tumorigenesis, proliferation and therapy, providing new ideas for the development of chemotherapeutic drugs (127). Studies on the role of PFKFB3 in tumor drug resistance and the tumor microenvironment have revealed that PFKFB3 is associated with resistance to rectal cancer treatment with oryzaplatin and that synergistic PFKFB3 inhibition therapy with carboplatin and paclitaxel in drug-resistant cell lines of gynecological cancers can reduce tumor weight (128,129). In addition, PFKFB3 is strongly associated with bone marrow endothelial progenitor cell injury after chemotherapy and radiotherapy and may be a potential therapeutic target for myelosuppressive injury in the treatment of myelosuppression after chemoradiotherapy (130). In addition to its application in tumor therapy, recent studies have revealed that PFKFB3 is a specific indicator of tumor recurrence in patients with colon cancer, which may provide a reference for the prognosis and treatment of patients with colon cancer (131). PFKFB3 is widely expressed in tissues and PFKFB3-mediated glycolysis plays an important role in the genesis, development and treatment of other diseases in addition to tumorigenesis and therapeutic development. Recent studies have shown that the hypoxia-inducible factor 1α-PFKFB3 pathway plays an important role in diabetes mellitus and diabetic retinopathy and that the use of PFKFB3 inhibitors impairs retinal neovascularization. In addition, PFKFB3 has been demonstrated to have a variety of potential protective mechanisms in the treatment of neovascular ophthalmopathies; therefore, the use of targeted PFKFB inhibitors may provide a new direction for exploring therapeutic targets for neovascular ophthalmopathy (132,133). PFKFB3-driven glycolytic reprogramming has been found to be strongly associated with an excessive inflammatory response and high mortality in patients with sepsis in studies of infectious diseases; therefore, the use of PFKFB3 inhibitors alone, or in combination, offers new combinatorial therapeutic targets for the treatment of sepsis and related complications (134,135). In autoimmune diseases, increased PFKFB3 expression has been found to promote the development of inflammatory bowel disease, and the use of the PFKFB3 inhibitor PFK15 effectively reduces the infiltration of immune cells in the colon and the severity of colitis, which provides new ideas for the exploration of therapeutic agents for inflammatory bowel disease (136). In rheumatoid arthritis (RA), the expression level of PFKFB3 plays different roles in different cells. Inhibition of PFKFB3

expression in fibroblast-like synoviocytes in RA may be effective in ameliorating RA symptoms through the inhibition of glycolysis, but forced overexpression of PFKFB3 in T cells from patients with RA restores glycolytic flux and protects against excessive apoptosis. Thus, using PFKFB3 inhibitors to target PFKFB3-mediated glycolysis to treat RA is possible, but the choice of treatment modality and dosage requires further investigation (137).

In the study of fibrotic diseases, PFKFB3-mediated glycolysis also plays an important role in the pathogenesis of renal fibrosis, pulmonary fibrosis, myocardial fibrosis and peritoneal fibrosis. In the pathogenesis of renal fibrosis, PFKFB3 drives renal fibrosis by promoting histone lactylation-mediated activation of the NF-κB family (138). PFKFB3-mediated lactate, a glycolytic product of PFKFB3, promotes the activation of renal fibroblasts and the subsequent development of renal fibrosis; therefore, inhibition of PFKFB3 may be a new strategy for the treatment of chronic kidney disease (124). In studies of idiopathic pulmonary fibrosis, anirutinib was found to exert an effective antifibrotic effect through the downregulation of PCBP3, reduction in PFKFB3 translation and inhibition of glycolysis in myofibroblasts, and anirutinib may constitute a novel and effective candidate for the treatment of pulmonary fibrosis (122). In this study, metformin was also shown to inhibit PFKFB3-mediated aerobic glycolysis by modulating the AMPK/mTOR pathway, thereby reducing collagen synthesis in lung fibroblasts, providing a new reference for exploring therapeutic agents for pulmonary fibrosis (139). In a study of pulmonary fibrosis treatment, silymarin (SIN) was shown to inhibit PFKFB3-mediated glycolysis and thus inhibit the activation of lung fibroblasts; thus, SIN, a PFKFB3 inhibitor, has become a promising antifibrotic agent for pulmonary fibrosis in clinical practice (123). In myocardial fibrosis, PFKFB3-mediated glycolysis promotes EndoMT and the use of PFKFB3 inhibitors such as salvianolic acid C may have therapeutic potential to counteract the EndoMT-associated fibrotic response through metabolic modulation (9). Studies of post-myocardial infarction myocardial fibrosis revealed that OTU deubiquitinase 4 upregulated PFKFB3 to promote post-myocardial infarction cardiac fibrosis and that the inhibition of PFKFB3 helps ameliorate ischemia-induced cardiac fibrosis; therefore, the use of PFKFB3 inhibitors may provide a new therapeutic option for the treatment of post-myocardial infarction cardiac fibrosis (125). Studies on the role of PFKFB3 in peritoneal fibrosis have revealed that high glucose (HG) induces peritoneal epithelial-mesenchymal transition (EMT) and hyper glycolysis in cells and that peritoneal fibrosis is accompanied by increased phosphorylation of STAT3 and increased expression of PFKFB3. HG/STAT3/PFKFB3 may contribute to the progression of peritoneal fibrosis by regulating fibrosis and angiogenesis, and the use of PFKFB3 inhibitors attenuates HG-induced peritoneal EMT. Thus, targeting PFKFB3 inhibitors may offer a new possibility for delaying the treatment of peritoneal fibrosis in patients on peritoneal dialysis (140).

Owing to the widespread expression of PFKFB3 in a wide range of diseases, PFKFB3 serves as an important target for disease treatment, and to date, numerous novel compounds have been designed, synthesized and evaluated for their PFKFB3 inhibitory activity (141). 3PO, a traditional PFKFB3 inhibitor, has not been further evaluated in clinical trials because of its poor solubility and selectivity, although its antitumor efficacy has been demonstrated in numerous cell and animal experiments (127). PFK15 is a 3PO derivative and has better selectivity than 3PO (142). PFK15 has been shown to exhibit antitumor activity in a variety of tumor models and synergistic antitumor effects have been observed when PFK15 is combined with phenelzine (141,143). PFK158, a derivative of PFK15, is a specific inhibitor of PFKFB3 that exhibits broad antitumor activity in models of breast, ovarian, lung, myeloma, glioblastoma, melanoma, pancreatic and colon cancers, significantly inhibiting tumor growth (141). PFK158 is the first PFKFB3 inhibitor evaluated in humans and the first of its kind in a phase I clinical trial (no. NCT02044861) (144). In addition to the three PFKFB3 inhibitors, there are phenoxyindole, biaryl sulfonamide, aminoquinoxaline, benzopyrone, pyridazinone, pyrrolopyrimidinone, benzindole and peptide derivatives, which are PFKFB3 inhibitors based on the modification of the structure of PFKFB3 and its ligands (141). Compounds 53 and 55 also act as PFKFB3 inhibitors (141). Despite the wide variety of PFKFB3 inhibitors, only one has entered the clinical stage, but with the continuous development of PFKFB3 inhibitors and the discovery of their roles in different diseases, PFKFB3 has promising applications in the diagnosis, treatment and determination of disease prognosis.

7. Conclusion

Liver fibrosis is a special manifestation of chronic liver disease and numerous studies have suggested that treatment of liver fibrosis can reverse liver fibrosis, which is highly important for the treatment of chronic liver disease with improved prognosis (56). In recent years, the metabolic reprogramming of cells has been a hot research topic. The process of liver fibrosis involves the proliferation and activation of various cells, phenotypic transformation and the secretion of related cytokines, which require glycolysis to provide intermediate metabolites and energy support for the proliferation and activation of cells (18). PFKFB3 synthesizes and hydrolyzes F-2.6-BP to regulate PFK-1, a key enzyme in glycolysis. By studying the role of PFKFB3-mediated glycolysis in cells involved in hepatic fibrosis and the related mechanisms, the present review explored interventions for hepatic fibrosis through the regulation of PFKFB3-mediated glycolysis. At present, there are more relevant studies on the mechanism of liver fibrosis, but to the best of our knowledge, no studies have investigated the overall mechanism of liver fibrosis, and PFKFB3-mediated glycolysis plays an important role in a wide range of cells involved in liver fibrosis, including HSCs, hepatic sinusoidal endothelial cells, lymphoid cells, macrophages and myofibroblasts (7). PFKFB3 promotes glycolysis in different cells through different mechanisms and thus participates in the regulation of the liver fibrosis process, and a wide range of PFKFB3 inhibitors are currently available for the therapeutic application of PFKFB3 inhibitors in the treatment of a variety of diseases (141). In addition, it may be possible to intervene in the expression of the PFKFB3 gene and the target of the PFKFB3 protein to explore the therapeutic mechanisms of liver fibrosis; in summary, PFKFB3 is a promising target for the future treatment of liver fibrosis.

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Authors' contributions

QL: Writing-original draft (lead). JL: Investigation (equal). XL and LZ participated in the literature search and analysis of the data to be included in the review. SY and YW were involved in the design of the study and assisted in the preparation of the figures and table. BT: Funding acquisition (supporting); writing-review and editing (supporting). HJ: Funding acquisition (supporting); project administration (supporting); resources (supporting); supervision (supporting); writing-review and editing (supporting). All authors have read and approved the final manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

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Competing interests

The authors declare that they have no competing interests.

References

1. Devarbhavi H, Asrani SK, Arab JP, Nartey YA, Pose E and Kamath PS: Global burden of liver disease: 2023 Update. J Hepatol 79: 516-537, 2023.
2. Huang DQ, Terrault NA, Tacke F, Gluud LL, Arrese M, Bugianesi E and Loomba R: Global epidemiology of cirrhosis-aetiology, trends and predictions. Nat Rev Gastroenterol Hepatol 20: 388-398, 2023.
3. Gilgenkrantz H, Mallat A, Moreau R and Lotersztajn S: Targeting cell-intrinsic metabolism for antifibrotic therapy. J Hepatol 74: 1442-1454, 2021.
4. Parola M and Pinzani M: Liver fibrosis in NAFLD/NASH: From pathophysiology towards diagnostic and therapeutic strategies. Mol Aspects Med 95: 101231, 2024.
5. Pei Q, Yi Q and Tang L: Liver fibrosis resolution: from molecular mechanisms to therapeutic opportunities. Int J Mol Sci 24: 9671, 2023.
6. Campana L, Esser H, Huch M and Forbes S: Liver regeneration and inflammation: From fundamental science to clinical applications. Nat Rev Mol Cell Biol 22: 608-624, 2021.
7. Kisseleva T and Brenner D: Molecular and cellular mechanisms of liver fibrosis and its regression. Nat Rev Gastroenterol Hepatol 18: 151-166, 2021.
8. Wang FD, Zhou J and Chen EQ: Molecular mechanisms and potential new therapeutic drugs for liver fibrosis. Front Pharmacol 13: 787748, 2022.
9. Zeng H, Pan T, Zhan M, Hailiwu R, Liu B, Yang H and Li P: Suppression of PFKFB3-driven glycolysis restrains endothelial-to-mesenchymal transition and fibrotic response. Signal Transduct Target Ther 7: 303, 2022.
10. Yang Q, Zong X, Zhuang L, Pan R, Tudi X, Fan Q and Tao R: PFKFB3 inhibitor 3PO reduces cardiac remodeling after myocardial infarction by regulating the TGF-β1/SMAD2/3 pathway. Biomolecules 13: 1072, 2023.
11. Song C, Wang S, Fu Z, Chi K, Geng X, Liu C, Cai G, Chen X, Wu D and Hong Q: IGFBP5 promotes diabetic kidney disease progression by enhancing PFKFB3-mediated endothelial glycolysis. Cell Death Dis 13: 340, 2022.
12. Jiang A, Liu J, Wang Y and Zhang C: cGAS-STING signaling pathway promotes hypoxia-induced renal fibrosis by regulating PFKFB3-mediated glycolysis. Free Radic Biol Med 208: 516-529, 2023.
13. Hu X, Xu Q, Wan H, Hu Y, Xing S, Yang H, Gao Y and He Z: PI3K-Akt-mTOR/PFKFB3 pathway mediated lung fibroblast aerobic glycolysis and collagen synthesis in lipopolysaccharide-induced pulmonary fibrosis. Lab Invest 100: 801-811, 2020.
14. Zhao X, Kwan JYY, Yip K, Liu PP and Liu FF: Targeting metabolic dysregulation for fibrosis therapy. Nat Rev Drug Discov 19: 57-75, 2020.
15. Horn P and Tacke F: Metabolic reprogramming in liver fibrosis. Cell Metab 36: 1439-1455, 2024.
16. Hammerich L and Tacke F: Hepatic inflammatory responses in liver fibrosis. Nat Rev Gastroenterol Hepatol 20: 633-646, 2023.
17. Cogliati B, Yashaswini CN, Wang S, Sia D and Friedman SL: Friend or foe? The elusive role of hepatic stellate cells in liver cancer. Nat Rev Gastroenterol Hepatol 20: 647-661, 2023.
18. Qu H, Liu J, Zhang D, Xie R, Wang L and Hong J: Glycolysis in chronic liver diseases: Mechanistic insights and therapeutic opportunities. Cells 12: 1930, 2023.
19. Jones BC, Pohlmann PR, Clarke R and Sengupta S: Treatment against glucose-dependent cancers through metabolic PFKFB3 targeting of glycolytic flux. Cancer Metastasis Rev 41: 447-458, 2022.
20. Baker SA and Rutter J: Metabolites as signalling molecules. Nat Rev Mol Cell Biol 24: 355-374, 2023.
21. Shi L, Pan H, Liu Z, Xie J and Han W: Roles of PFKFB3 in cancer. Signal Transduct Target Ther 2: 17044, 2017.
22. Mejias M, Gallego J, Naranjo-Suarez S, Ramirez M, Pell N, Manzano A, Suñer C, Bartrons R, Mendez R and Fernandez M: CPEB4 increases expression of PFKFB3 to induce glycolysis and activate mouse and human hepatic stellate cells, promoting liver fibrosis. Gastroenterology 159: 273-288, 2020.
23. Alvarez R, Mandal D and Chittiboina P: Canonical and non-canonical roles of PFKFB3 in brain tumors. Cells 10: 2913, 2021.
24. Calderone V, Gallego J, Fernandez-Miranda G, Garcia-Pras E, Maillo C, Berzigotti A, Mejias M, Bava FA, Angulo-Urarte A, Graupera M, *et al*: Sequential functions of CPEB1 and CPEB4 regulate pathologic expression of vascular endothelial growth factor and angiogenesis in chronic liver disease. Gastroenterology 150: 982-997.e30, 2016.
25. Hu KF, Shu CW, Lee CH, Tseng CJ, Chou YH and Liu PF: Comparative clinical significance and biological roles of PFKFB family members in oral squamous cell carcinoma. Cancer Cell Int 23: 257, 2023.
26. Zodda E, Tura-Ceide O, Mills NL, Tarragó-Celada J, Carini M, Thomson TM and Cascante M: Autonomous metabolic reprogramming and oxidative stress characterize endothelial dysfunction in acute myocardial infarction. Elife 12: e86260, 2023.
27. Wang Y, Tang S, Wu Y, Wan X, Zhou M, Li H and Zha X: Upregulation of 6-phosphofructo-2-kinase (PFKFB3) by hyperactivated mammalian target of rapamycin complex 1 is critical for tumor growth in tuberous sclerosis complex. IUBMB Life 72: 965-977, 2020.
28. Boscaro C, Carotti M, Albiero M, Trenti A, Fadini GP, Trevisi L, Sandonà D, Cignarella A and Bolego C: Non-genomic mechanisms in the estrogen regulation of glycolytic protein levels in endothelial cells. FASEB J 34: 12768-12784, 2020.

29. Kommagani R, Szwarc MM, Kovanci E, Gibbons WE, Putluri N, Maity S, Creighton CJ, Sreekumar A, DeMayo FJ, Lydon JP and O'Malley BW: Acceleration of the glycolytic flux by steroid receptor coactivator-2 is essential for endometrial decidualization. PLoS Genet 9: e1003900, 2013.
30. Novellasdemunt L, Bultot L, Manzano A, Ventura F, Rosa JL, Vertommen D, Rider MH, Navarro-Sabate À and Bartrons R: PFKFB3 activation in cancer cells by the p38/MK2 pathway in response to stress stimuli. Biochem J 452: 531-543, 2013.
31. Watanuki S, Kobayashi H, Sugiura Y, Yamamoto M, Karigane D, Shiroshita K, Sorimachi Y, Fujita S, Morikawa T, Koide S, *et al*: Context-dependent modification of PFKFB3 in hematopoietic stem cells promotes anaerobic glycolysis and ensures stress hematopoiesis. Elife 12: RP87674, 2024.
32. Yi M, Ban Y, Tan Y, Xiong W, Li G and Xiang B: 6-Phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 and 4: A pair of valves for fine-tuning of glucose metabolism in human cancer. Mol Metab 20: 1-13, 2019.
33. Shakhpazyan N, Mikhaleva L, Bedzhanyan A, Sadykhov N, Midiber K and Orekhov A: Commentary: PFKFB3 overexpression in monocytes of patients with colon but not rectal cancer programs pro-tumor macrophages and is indicative for higher risk of tumor relapse. Front Immunol 14: 1290459, 2023.
34. Jia W, Wu Q, Shen M, Yu X, An S, Zhao L, Huang G and Liu J: PFKFB3 regulates breast cancer tumorigenesis and Fulvestrant sensitivity by affecting ERα stability. Cell Signal 119: 111184, 2024.
35. Wang Y, Wang X, Du C, Wang Z, Wang J, Zhou N, Wang B, Tan K, Fan Y and Cao P: Glycolysis and beyond in glucose metabolism: Exploring pulmonary fibrosis at the metabolic crossroads. Front Endocrinol (Lausanne) 15: 1379521, 2024.
36. Yang Q, Huo E, Cai Y, Zhang Z, Dong C, Asara JM, Shi H and Wei Q: Myeloid PFKFB3-mediated glycolysis promotes kidney fibrosis. Front Immunol 14: 1259434, 2023.
37. Chandel NS: Glycolysis. Cold Spring Harb Perspect Biol 13: a040535, 2021.
38. Paul S, Ghosh S and Kumar S: Tumor glycolysis, an essential sweet tooth of tumor cells. Semin Cancer Biol 86: 1216-1230, 2022.
39. Qiao Q, Hu S and Wang X: The regulatory roles and clinical significance of glycolysis in tumor. Cancer Commun (Lond) 44: 761-786, 2024.
40. Fendt SM: 100 Years of the Warburg effect: A cancer metabolism endeavor. Cell 187: 3824-3828, 2024.
41. Jaccard A, Wyss T, Maldonado-Pérez N, Rath JA, Bevilacqua A, Peng JJ, Lepez A, Von Gunten C, Franco F, Kao KC, *et al*: Reductive carboxylation epigenetically instructs T cell differentiation. Nature 621: 849-856, 2023.
42. Feng J, Li J, Wu L, Yu Q, Ji J, Wu J, Dai W and Guo C: Emerging roles and the regulation of aerobic glycolysis in hepatocellular carcinoma. J Exp Clin Cancer Res 39: 126, 2020.
43. Ma H, Zhang J, Zhou L, Wen S, Tang HY, Jiang B, Zhang F, Suleman M, Sun D, Chen A, *et al*: c-Src promotes tumorigenesis and tumor progression by activating PFKFB3. Cell Rep 30: 4235-4249.e6, 2020.
44. Galindo CM, de Oliveira Ganzella FA, Klassen G, Souza Ramos EA and Acco A: Nuances of PFKFB3 signaling in breast cancer. Clin Breast Cancer 22: e604-e614, 2022.
45. Li FL, Liu JP, Bao RX, Yan G, Feng X, Xu YP, Sun YP, Yan W, Ling ZQ, Xiong Y, *et al*: Acetylation accumulates PFKFB3 in cytoplasm to promote glycolysis and protects cells from cisplatin-induced apoptosis. Nat Commun 9: 508, 2018.
46. Suematsu M, Nakamura T, Tokumoto Y, Yamamoto T, Kajimura M and Kabe Y: CO-CBS-H2 S axis: From vascular mediator to cancer regulator. Microcirculation 23: 183-190, 2016.
47. McErlean P, Bell CG, Hewitt RJ, Busharat Z, Ogger PP, Ghai P, Albers GJ, Calamita E, Kingston S, Molyneaux PL, *et al*: DNA methylome alterations are associated with airway macrophage differentiation and phenotype during lung fibrosis. Am J Respir Crit Care Med 204: 954-966, 2021.
48. Desideri E, Vegliante R, Cardaci S, Nepravishta R, Paci M and Ciriolo MR: MAPK14/p38α-dependent modulation of glucose metabolism affects ROS levels and autophagy during starvation. Autophagy 10: 1652-1665, 2014.
49. Yuan Y, Wang W, Zhang Y, Hong Q, Huang W, Li L, Xie Z, Chen Y, Li X and Meng Y: Apelin-13 attenuates lipopolysaccharide-induced inflammatory responses and acute lung injury by regulating PFKFB3-Driven glycolysis induced by NOX4-dependent ROS. J Inflamm Res 15: 2121-2139, 2022.
50. Lin S, Li Y, Wang D, Huang C, Marino D, Bollt O, Wu C, Taylor MD, Li W, DeNicola GM, *et al*: Fascin promotes lung cancer growth and metastasis by enhancing glycolysis and PFKFB3 expression. Cancer Lett 518: 230-242, 2021.
51. Lin S, Taylor MD, Singh PK and Yang S: How does fascin promote cancer metastasis? FEBS J 288: 1434-1446, 2021.
52. Zhang LF, Deng WQ, Huang QW, Zhang JJ, Wang Y, Zhou TJ, Xing L and Jiang HL: Vicious cycle-breaking lipid nanoparticles remodeling multicellular crosstalk to reverse liver fibrosis. Adv Mater 36: e2311474, 2024.
53. Moreno-Lanceta A, Medrano-Bosch M, Fundora Y, Perramón M, Aspas J, Parra-Robert M, Baena S, Fondevila C, Edelman ER, Jiménez W and Melgar-Lesmes P: RNF41 orchestrates macrophage-driven fibrosis resolution and hepatic regeneration. Sci Transl Med 15: eabq6225, 2023.
54. Cai X, Wang J, Wang J, Zhou Q, Yang B, He Q and Weng Q: Intercellular crosstalk of hepatic stellate cells in liver fibrosis: New insights into therapy. Pharmacol Res 155: 104720, 2020.
55. Sinha S, Hassan N and Schwartz RE: Organelle stress and alterations in interorganelle crosstalk during liver fibrosis. Hepatology 79: 482-501, 2024.
56. Roehlen N, Crouchet E and Baumert TF: Liver fibrosis: Mechanistic concepts and therapeutic perspectives. Cells 9: 875, 2020.
57. Odagiri N, Matsubara T, Sato-Matsubara M, Fujii H, Enomoto M and Kawada N: Anti-fibrotic treatments for chronic liver diseases: The present and the future. Clin Mol Hepatol 27: 413-424, 2021.
58. An P, Wei LL, Zhao S, Sverdlov DY, Vaid KA, Miyamoto M, Kuramitsu K, Lai M and Popov YV: Hepatocyte mitochondria-derived danger signals directly activate hepatic stellate cells and drive progression of liver fibrosis. Nat Commun 11: 2362, 2020.
59. Geervliet E, Moreno S, Baiamonte L, Booijink R, Boye S, Wang P, Voit B, Lederer A, Appelhans D and Bansal R: Matrix metalloproteinase-1 decorated polymersomes, a surface-active extracellular matrix therapeutic, potentiates collagen degradation and attenuates early liver fibrosis. J Control Release 332: 594-607, 2021.
60. Casari M, Siegl D, Deppermann C and Schuppan D: Macrophages and platelets in liver fibrosis and hepatocellular carcinoma. Front Immunol 14: 1277808, 2023.
61. Gao J, Wei B, de Assuncao TM, Liu Z, Hu X, Ibrahim S, Cooper SA, Cao S, Shah VH and Kostallari E: Hepatic stellate cell autophagy inhibits extracellular vesicle release to attenuate liver fibrosis. J Hepatol 73: 1144-1154, 2020.
62. Song Y, Wei J, Li R, Fu R, Han P, Wang H, Zhang G, Li S, Chen S, Liu Z, *et al*: Tyrosine kinase receptor B attenuates liver fibrosis by inhibiting TGF-β/SMAD signaling. Hepatology 78: 1433-1447, 2023.
63. de Carvalho Ribeiro M and Szabo G: Role of the inflammasome in liver disease. Annu Rev Pathol 17: 345-365, 2022.
64. Wang F, Chen L, Kong D, Zhang X, Xia S, Liang B, Li Y, Zhou Y, Zhang Z, Shao J, *et al*: Canonical Wnt signaling promotes HSC glycolysis and liver fibrosis through an LDH-A/HIF-1α transcriptional complex. Hepatology 79: 606-623, 2024.
65. Xu F, Liu C, Zhou D and Zhang L: TGF-β/SMAD pathway and its regulation in hepatic fibrosis. J Histochem Cytochem 64: 157-167, 2016.
66. Feng W, Guan Z, Ying WZ, Xing D, Ying KE and Sanders PW: Matrix metalloproteinase-9 regulates afferent arteriolar remodeling and function in hypertension-induced kidney disease. Kidney Int 104: 740-753, 2023.
67. Yao QY, Feng YD, Han P, Yang F and Song GQ: Hepatic microenvironment underlies fibrosis in chronic hepatitis B patients. World J Gastroenterol 26: 3917-3928, 2020.
68. Giarratana AO, Prendergast CM, Salvatore MM and Capaccione KM: TGF-β signaling: Critical nexus of fibrogenesis and cancer. J Transl Med 22: 594, 2024.
69. Liu Y, Meyer C, Müller A, Herweck F, Li Q, Müllenbach R, Mertens PR, Dooley S and Weng HL: IL-13 induces connective tissue growth factor in rat hepatic stellate cells via TGF-β-independent Smad signaling. J Immunol 187: 2814-2823, 2011.
70. Akkız H, Gieseler RK and Canbay A: Liver fibrosis: From basic science towards clinical progress, focusing on the central role of hepatic stellate cells. Int J Mol Sci 25: 7873, 2024.
71. Yan M, Xie Y, Yao J and Li X: The dual-mode transition of myofibroblasts derived from hepatic stellate cells in liver fibrosis. Int J Mol Sci 24: 15460, 2023.

72. Bouguéon M, Legagneux V, Hazard O, Bomo J, Siegel A, Feret J and Théret N: A rule-based multiscale model of hepatic stellate cell plasticity: Critical role of the inactivation loop in fibrosis progression. PLoS Comput Biol 20: e1011858, 2024.
73. Lu JL, Yu CX and Song LJ: Programmed cell death in hepatic fibrosis: Current and perspectives. Cell Death Discov 9: 449, 2023.
74. Li R, Li Z, Feng Y, Yang H, Shi Q, Tao Z, Cheng J and Lu X: PDGFRβ-targeted TRAIL specifically induces apoptosis of activated hepatic stellate cells and ameliorates liver fibrosis. Apoptosis 25: 105-119, 2020.
75. Noom A, Sawitzki B, Knaus P and Duda GN: A two-way street-cellular metabolism and myofibroblast contraction. NPJ Regen Med 9: 15, 2024.
76. Cao Y, Wang S, Zhang M, Lai B and Liang Y: PFKFB3-mediated glycolysis in hepatic stellate cells promotes liver regeneration. Biochem Biophys Res Commun 712-713: 149958, 2024.
77. Kovacs L, Cao Y, Han W, Meadows L, Kovacs-Kasa A, Kondrikov D, Verin AD, Barman SA, Dong Z, Huo Y and Su Y: PFKFB3 in smooth muscle promotes vascular remodeling in pulmonary arterial hypertension. Am J Respir Crit Care Med 200: 617-627, 2019.
78. Cheng D, Chai J, Wang H, Fu L, Peng S and Ni X: Hepatic macrophages: Key players in the development and progression of liver fibrosis. Liver Int 41: 2279-2294, 2021.
79. Wang Z, Du K, Jin N, Tang B and Zhang W: Macrophage in liver fibrosis: Identities and mechanisms. Int Immunopharmacol 120: 110357, 2023.
80. Pei L, Li R, Wang X, Xu D, Gong F, Chen W, Zheng X, Liu W, Zhao S, Wang Q, *et al*: MSCs-derived extracellular vesicles alleviate sepsis-associated liver dysfunction by inhibiting macrophage glycolysis-mediated inflammatory response. Int Immunopharmacol 128: 111575, 2024.
81. Liang W, Huang X and Shi J: Macrophages serve as bidirectional regulators and potential therapeutic targets for liver fibrosis. Cell Biochem Biophys 81: 659-671, 2023.
82. Strickland JD and Copple BL: Modulation of macrophage phenotype to treat liver fibrosis-current approaches and future possibilities. Adv Pharmacol 91: 213-228, 2021.
83. Liu Y, Xu R, Gu H, Zhang E, Qu J, Cao W, Huang X, Yan H, He J and Cai Z: Metabolic reprogramming in macrophage responses. Biomark Res 9: 1, 2021.
84. Soto-Heredero G, Gómez de Las Heras MM, Gabandé-Rodríguez E, Oller J and Mittelbrunn M: Glycolysis-a key player in the inflammatory response. FEBS J 287: 3350-3369, 2020.
85. Duan S, Lou X, Chen S, Jiang H, Chen D, Yin R, Huang X, Yan H, He J and Cai Z: Macrophage LMO7 deficiency facilitates inflammatory injury via metabolic-epigenetic reprogramming. Acta Pharm Sin B 13: 4785-4800, 2023.
86. Leslie J, Macia MG, Luli S, Worrell JC, Reilly WJ, Paish HL, Knox A, Barksby BS, Gee LM, Zaki MYW, *et al*: c-Rel orchestrates energy-dependent epithelial and macrophage reprogramming in fibrosis. Nat Metab 2: 1350-1367, 2020.
87. Fuhrmann DC and Brüne B: miR-193a-3p increases glycolysis under hypoxia by facilitating Akt phosphorylation and PFKFB3 activation in human macrophages. Cell Mol Life Sci 79: 89, 2022.
88. Zhang J, Muri J, Fitzgerald G, Gorski T, Gianni-Barrera R, Masschelein E, D'Hulst G, Gilardoni P, Turiel G, Fan Z, *et al*: Endothelial lactate controls muscle regeneration from ischemia by inducing M2-like macrophage polarization. Cell Metab 31: 1136-1153.e7, 2020.
89. Zhang M and Zhang S: T cells in fibrosis and fibrotic diseases. Front Immunol 11: 1142, 2020.
90. Koda Y, Teratani T, Chu PS, Hagihara Y, Mikami Y, Harada Y, Tsujikawa H, Miyamoto K, Suzuki T, Taniki N, *et al*: $CD8^+$ tissue-resident memory T cells promote liver fibrosis resolution by inducing apoptosis of hepatic stellate cells. Nat Commun 12: 4474, 2021.
91. Barron L and Wynn TA: Fibrosis is regulated by Th2 and Th17 responses and by dynamic interactions between fibroblasts and macrophages. Am J Physiol Gastrointest Liver Physiol 300: G723-G728, 2011.
92. Li N, Yamamoto G, Fuji H and Kisseleva T: Interleukin-17 in liver disease pathogenesis. Semin Liver Dis 41: 507-515, 2021.
93. Savage TM, Fortson KT, de Los Santos-Alexis K, Oliveras-Alsina A, Rouanne M, Rae SS, Gamarra JR, Shayya H, Kornberg A, Cavero R, *et al*: Amphiregulin from regulatory T cells promotes liver fibrosis and insulin resistance in non-alcoholic steatohepatitis. Immunity 57: 303-318.e6, 2024.
94. Hann A, Oo YH and Perera MTPR: Regulatory T-cell therapy in liver transplantation and chronic liver disease. Front Immune 12: 719954, 2021.
95. Patel AM, Liu YS, Davies SP, Brown RM, Kelly DA, Scheel-Toellner D, Reynolds GM and Stamataki Z: The role of B cells in adult and paediatric liver injury. Front Immunol 12: 729143, 2021.
96. Cao J, Liao S, Zeng F, Liao Q, Luo G and Zhou Y: Effects of altered glycolysis levels on $CD8^+$ T cell activation and function. Cell Death Dis 14: 407, 2023.
97. Madden MZ and Rathmell JC: The complex integration of T-cell metabolism and immunotherapy. Cancer Discov 11: 1636-1643, 2021.
98. Icard P, Alifano M, Donnadieu E and Simula L: Fructose-1,6-bisphosphate promotes PI3K and glycolysis in T cells? Trends Endocrinol Metab 32: 540-543, 2021.
99. Simon-Molas H, Arnedo-Pac C, Fontova P, Vidal-Alabró A, Castaño E, Rodríguez-García A, Navarro-Sabaté À, Lloberas N, Manzano A and Bartrons R: PI3K-Akt signaling controls PFKFB3 expression during human T-lymphocyte activation. Mol Cell Biochem 448: 187-197, 2018.
100. Harshan S, Dey P and Raghunathan S: Altered transcriptional regulation of glycolysis in circulating $CD8^+$ T cells of rheumatoid arthritis patients. Genes (Basel) 13: 1216, 2022.
101. Dou Q, Grant AK, Callahan C, Coutinho de Souza P, Mwin D, Booth AL, Nasser I, Moussa M, Ahmed M and Tsai LL: PFKFB3-mediated pro-glycolytic shift in hepatocellular carcinoma proliferation. Cell Mol Gastroenterol Hepatol 15: 61-75, 2023.
102. Li Y, Zhou Y, Xia S, Chen L, Yang T, Zhao D, Zhang Z, Shao J, Xu X, Zhang F and Zheng S: Blockade of KLF5/LDH-A feedback loop contributes to Curcumol inhibition of sinusoidal endothelial cell glycolysis and mitigation of liver fibrosis. Phytomedicine 114: 154759, 2023.
103. Gracia-Sancho J, Caparros E, Fernández-Iglesias A and Francés R: Role of liver sinusoidal endothelial cells in liver diseases. Nat Rev Gastroenterol Hepatol 18: 411-431, 2021.
104. Gracia-Sancho J, Marrone G and Fernández-Iglesias A: Hepatic microcirculation and mechanisms of portal hypertension. Nat Rev Gastroenterol Hepatol 16: 221-234, 2019.
105. Kumar S, Duan Q, Wu R, Harris EN and Su Q: Pathophysiological communication between hepatocytes and non-parenchymal cells in liver injury from NAFLD to liver fibrosis. Adv Drug Deliv Rev 176: 113869, 2021.
106. Pandey E, Nour AS and Harris EN: Prominent receptors of liver sinusoidal endothelial cells in liver homeostasis and disease. Front Physiol 11: 873, 2020.
107. Khan MA, Fischer J, Harrer L, Schwiering F, Groneberg D and Friebe A: Hepatic stellate cells in zone 1 engage in capillarization rather than myofibroblast formation in murine liver fibrosis. Sci Rep 14: 18840, 2024.
108. Baiocchini A, Del Nonno F, Taibi C, Visco-Comandini U, D'Offizi G, Piacentini M and Falasca L: Liver sinusoidal endothelial cells (LSECs) modifications in patients with chronic hepatitis C. Sci Rep 9: 8760, 2019.
109. Lee KC, Wu PS and Lin HC: Pathogenesis and treatment of non-alcoholic steatohepatitis and its fibrosis. Clin Mol Hepatol 29: 77-98, 2023.
110. Hammoutene A, Biquard L, Lasselin J, Kheloufi M, Tanguy M, Vion AC, Mérian J, Colnot N, Loyer X, Tedgui A, *et al*: A defect in endothelial autophagy occurs in patients with non-alcoholic steatohepatitis and promotes inflammation and fibrosis. J Hepatol 72: 528-538, 2020.
111. Maeso-Díaz R, Boyer-Diaz Z, Lozano JJ, Ortega-Ribera M, Peralta C, Bosch J and Gracia-Sancho J: New rat model of advanced NASH mimicking pathophysiological features and transcriptomic signature of the human disease. Cells 8: 1062, 2019.
112. McConnell MJ, Kostallari E, Ibrahim SH and Iwakiri Y: The evolving role of liver sinusoidal endothelial cells in liver health and disease. Hepatology 78: 649-669, 2023.
113. Wei M, Zhang Y, Zhang H, Huang Z, Miao H, Zhang T, Lu B and Ji L: HMGB1 induced endothelial to mesenchymal transition in liver fibrosis: The key regulation of early growth response factor 1. Biochim Biophys Acta Gen Subj 1866: 130202, 2022.
114. Wang L, Guo S, Cao K, Li Z, Li Z, Song M, Wang C, Chen P, Cui Y, Dai X, *et al*: Glycolysis promotes angiotensin II-induced aortic remodeling through regulating endothelial-to-mesenchymal transition via the corepressor C-terminal binding protein 1. Hypertension 80: 2627-2640, 2023.

115. Greuter T, Yaqoob U, Gan C, Jalan-Sakrikar N, Kostallari E, Lu J, Gao J, Sun L, Liu M, Sehrawat TS, *et al*: Mechanotransduction-induced glycolysis epigenetically regulates a CXCL1-dominant angiocrine signaling program in liver sinusoidal endothelial cells in vitro and in vivo. J Hepatol 77: 723-734, 2022.
116. Atherton P, Stutchbury B, Jethwa D and Ballestrem C: Mechanosensitive components of integrin adhesions: Role of vinculin. Exp Cell Res 343: 21-27, 2016.
117. Lovisa S, Fletcher-Sananikone E, Sugimoto H, Hensel J, Lahiri S, Hertig A, Taduri G, Lawson E, Dewar R, Revuelta I, *et al*: Endothelial-to-mesenchymal transition compromises vascular integrity to induce Myc-mediated metabolic reprogramming in kidney fibrosis. Sci Signal 13: eaaz2597, 2020.
118. DeLeve LD: Liver sinusoidal endothelial cells in hepatic fibrosis. Hepatology 61: 1740-1746, 2015.
119. Kim HY, Sakane S, Eguileor A, Carvalho Gontijo Weber R, Lee W, Liu X, Lam K, Ishizuka K, Rosenthal SB, Diggle K, *et al*: The origin and fate of liver myofibroblasts. Cell Mol Gastroenterol Hepatol 17: 93-106, 2024.
120. Yang W, He H, Wang T, Su N, Zhang F, Jiang K, Zhu J, Zhang C, Niu K, Wang L, *et al*: Single-cell transcriptomic analysis reveals a hepatic stellate cell-activation roadmap and myofibroblast origin during liver fibrosis in mice. Hepatology 74: 2774-2790, 2021.
121. Wang W, Zhang Y, Huang W, Yuan Y, Hong Q, Xie Z, Li L, Chen Y, Li X and Meng Y: Alamandine/MrgD axis prevents TGF-β1-mediated fibroblast activation via regulation of aerobic glycolysis and mitophagy. J Transl Med 21: 24, 2023.
122. Chen W, Zhang J, Zhong W, Liu Y, Lu Y, Zeng Z, Huang H, Wan X, Meng X, Zou F, *et al*: Anlotinib inhibits PFKFB3-driven glycolysis in myofibroblasts to reverse pulmonary fibrosis. Front Pharmacol 12: 744826, 2021.
123. Nie Z, Wu J, Xie J and Yin W: Sinomenine ameliorates bleomycin-induced pulmonary fibrosis by inhibiting the differentiation of fibroblast into myofibroblast. Heliyon 10: e33314, 2024.
124. Yang Q, Huo E, Cai Y, Zhang Z, Dong C, Asara JM and Wei Q: PFKFB3-mediated glycolysis boosts fibroblast activation and subsequent kidney fibrosis. Cells 12: 2081, 2023.
125. Wang F, Yin X, Fan YM, Zhang X, Ma C, Jia K, Zhou W, Tang Z, Qi LW and Li J: Upregulation of glycolytic enzyme PFKFB3 by deubiquitinase OTUD4 promotes cardiac fibrosis post myocardial infarction. J Mol Med (Berl) 101: 743-756, 2023.
126. Dewidar B, Meyer C, Dooley S and Meindl-Beinker AN: TGF-β in hepatic stellate cell activation and liver fibrogenesis-updated 2019. Cells 8: 1418, 2019.
127. Kotowski K, Rosik J, Machaj F, Supplitt S, Wiczew D, Jabłońska K, Wiechec E, Ghavami S and Dzięgiel P: Role of PFKFB3 and PFKFB4 in cancer: Genetic basis, impact on disease development/progression, and potential as therapeutic targets. Cancers (Basel) 13: 909, 2021.
128. Kashyap A, Umar SM, Dev JRA, Mathur SR, Gogia A, Batra A, Deo SVS and Prasad CP: Combination of 3PO analog PFK15 and siPFKL efficiently suppresses the migration, colony formation ability, and PFK-1 activity of triple-negative breast cancers by reducing the glycolysis. J Cell Biochem 124: 1259-1272, 2023.
129. Edelmann M, Fan S, De Oliveira T, Goldhardt T, Sartorius D, Midelashvili T, Conrads K, Paul NB, Beißbarth T, Fleischer JR, *et al*: Tumor vessel normalization via PFKFB3 inhibition alleviates hypoxia and increases tumor necrosis in rectal cancer upon radiotherapy. Cancer Res Commun 4: 2008-2024, 2024.
130. Lyu ZS, Tang SQ, Xing T, Zhou Y, Lv M, Fu HX, Wang Y, Xu LP, Zhang XH, Lee HY, *et al*: The glycolytic enzyme PFKFB3 determines bone marrow endothelial progenitor cell damage after chemotherapy and irradiation. Haematologica 107: 2365-2380, 2022.
131. Larionova I, Patysheva M, Iamshchikov P, Kazakova E, Kazakova A, Rakina M, Grigoryeva E, Tarasova A, Afanasiev S, Bezgodova N, *et al*: PFKFB3 overexpression in monocytes of patients with colon but not rectal cancer programs pro-tumor macrophages and is indicative for higher risk of tumor relapse. Front Immunol 13: 1080501, 2023.
132. Vezza T and Víctor VM: The HIF1α-PFKFB3 pathway: A key player in diabetic retinopathy. J Clin Endocrinol Metab 106: e4778-e4780, 2021.
133. Min J, Zeng T, Roux M, Lazar D, Chen L and Tudzarova S: The role of HIF1α-PFKFB3 pathway in diabetic retinopathy. J Clin Endocrinol Metab 106: 2505-2519, 2021.
134. Xiao M, Liu D, Xu Y, Mao W and Li W: Role of PFKFB3-driven glycolysis in sepsis. Ann Med 55: 1278-1289, 2023.
135. Liu D, Xiao M, Zhou J, Wang P, Peng J, Mao W, Hu Y, Liu Y, Yin J, Ke L and Li W: PFKFB3 promotes sepsis-induced acute lung injury by enhancing NET formation by $CXCR4^{hi}$ neutrophils. Int Immunopharmacol 123: 110737, 2023.
136. Zhou Z, Plug LG, Patente TA, de Jonge-Muller ESM, Elmagd AA, van der Meulen-de Jong AE, Everts B, Barnhoorn MC and Hawinkels LJAC: Increased stromal PFKFB3-mediated glycolysis in inflammatory bowel disease contributes to intestinal inflammation. Front Immunol 13: 966067, 2022.
137. Zuo J, Tang J, Lu M, Zhou Z, Li Y, Tian H, Liu E, Gao B, Liu T and Shao P: Glycolysis rate-limiting enzymes: Novel potential regulators of rheumatoid arthritis pathogenesis. Front Immunol 12: 779787, 2021.
138. Wang Y, Li H, Jiang S, Fu D, Lu X, Lu M, Li Y, Luo D, Wu K, Xu Y, *et al*: The glycolytic enzyme PFKFB3 drives kidney fibrosis through promoting histone lactylation-mediated NF-κB family activation. Kidney Int 106: 226-240, 2024.
139. Tang CJ, Xu J, Ye HY and Wang XB: Metformin prevents PFKFB3-related aerobic glycolysis from enhancing collagen synthesis in lung fibroblasts by regulating AMPK/mTOR pathway. Exp Ther Med 21: 581, 2021.
140. Fu J, Li N, He M, Huang D and Zhang P: STAT3 signaling mediates peritoneal fibrosis by activating hyperglycolysis. Am J Transl Res 14: 7552-7565, 2022.
141. Wang Y, Qu C, Liu T and Wang C: PFKFB3 inhibitors as potential anticancer agents: Mechanisms of action, current developments, and structure-activity relationships. Eur J Med Chem 203: 112612, 2020.
142. Zlacká J, Murár M, Addová G, Moravčík R, Boháč A and Zeman M: Synthesis of glycolysis inhibitor PFK15 and its synergistic action with an approved multikinase antiangiogenic drug on human endothelial cell migration and proliferation. Int J Mol Sci 23: 14295, 2022.
143. Shi WK, Zhu XD, Wang CH, Zhang YY, Cai H, Li XL, Cao MQ, Zhang SZ, Li KS and Sun HC: PFKFB3 blockade inhibits hepatocellular carcinoma growth by impairing DNA repair through AKT. Cell Death Dis 9: 428, 2018.
144. Thirusangu P, Ray U, Sarkar Bhattacharya S, Oien DB, Jin L, Staub J, Kannan N, Molina JR and Shridhar V: PFKFB3 regulates cancer stemness through the hippo pathway in small cell lung carcinoma. Oncogene 41: 4003-4017, 2022.