REVIEW ARTICLE

RECORDS OF PHARMACEUTICAL AND BIOMEDICAL SCIENCES

Targeting Glutamine Metabolism: A Novel Approach to Combat Hepatic Fibrosis

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Abstract

Liver disease causes around 2 million deaths globally each year, with half due to fibrosis and half due to viral hepatitis and hepatocellular carcinoma. Fibrosis ranks as the 11th leading cause of death worldwide. Egypt has the highest prevalence of hepatitis C virus (HCV) infection, impacting 15%-25% of its rural population. Liver fibrosis results from excessive extracellular matrix protein accumulation, primarily collagen, driven by activated hepatic stellate cells (HSCs). These cells transform into fibrogenic myofibroblast-like cells following liver injury. Emerging antifibrotic therapies aim to reduce fibrogenic cell accumulation and extracellular matrix deposition. Glutaminase (GLS) is an enzyme that converts glutamine, the most abundant amino acid in circulation, into glutamate, releasing ammonia. Humans have four GLS isoforms, with GLS-1 and GLS-2 being significant. Glutamine metabolism influences HSC proliferation and activation, with glutamine converting to α-ketoglutarate for the Krebs cycle and pyrroline-5-carboxylate, which stimulates collagen production. In healthy livers, GLS-2 predominates, while in fibrotic livers, GLS-2 levels decrease, and GLS-1 increases in stromal cells. Inhibiting GLS-1 may reduce HSC proliferation and fibrosis. The selective GLS-1 inhibitor CB-839 is in clinical trials, and the small molecule 968 inhibits GLS-2. These inhibitors help elucidate the roles of GLS isoforms and offer new strategies for targeting glutamine metabolism in liver fibrosis.

Keywords: Hepatic fibrosis; glumatinase; CB-839; 968-compound.

1. Background

Hepatic fibrosis is a result of the wound-healing response of the liver to repeated injury. This process is associated with an inflammatory response and a limited deposition of extracellular matrix (ECM). Fibrosis occurs when ECM proteins accumulate in excessive amounts, leading to scarring that distorts the normal layout and stiffness of the tissue **(Tao et al., 2023)**.

Hepatic fibrosis was historically thought to be a passive process due to the collapse of the hepatic parenchyma and its substitution with collagen-rich tissue **(Obembe et al., 2022)**. Currently, it is considered a model of the wound-healing response to chronic liver injury **(Shah et al., 2023)**. Therefore, researchers have been stimulated to

identify antifibrotic therapies.

Hepatic stellate cells (HSCs) are resident perisinusoidal cells distributed throughout the liver, with a marked range of functions. In normal liver, their most distinctive feature is the presence of cytoplasmic droplets that store retinyl esters. They also participate in immune-tolerance, vaso-regulation, drug detoxification, extra-cellular matrix homeostasis and the preservation of hepatocyte mass through secretion of mitogens **(Sufleţel et al., 2020)**.

Glutamine (Gln) is the most prevalent free amino acid in blood. It is neutral, polar, and conditionally essential, participating in various cellular activities **(Meynial-Denis, 2016)**. The enzyme L-glutaminase (GLS) primarily metabolizes Gln, converting it into glutamate (Glu) and releasing ammonia. As an endopeptidase, GLS breaks peptide bonds within proteins and is mainly located in the mitochondria, where it generates Glu for the TCA cycle **(Katt et al., 2017)**. Humans have four glutaminase isoforms. GLS-1 produces two kidney-type, while GLS-2 generates two liver-type glutaminases **(Tan et al., 2021)**.

L-glutaminase enzyme is strongly linked to tissue fibrosis due to considerable reasons as:

i) glutamate produced by GLS can be converted to α-KG, which then enters TCA cycle in order to generate energy desired for differentiation of quiescent HSCs into myofibroblasts **(Khomich et al., 2019)**.

ii) glutamate can be converted to pyrroline-5-carboxylate (P5C) which then reduced to proline. Hydroxyproline (Hyp) and proline are essential components of collagen structure and stability by forming hydrogen bonds that permits the sharp twisting of the collagen helix **(Palka et al., 2021)**.

iii) glutaminolysis also has a role in the maintenance of the phagocytic and secretory capacities of lymphocytes and macrophages **(Muri & Kopf, 2021)**.

Healthy liver almost solely expresses liver-type GlS-2. During fibrosis, a metabolic switch from GLS-2 to GLS-1 isoform occurs. GLS-2 level is then down-regulated and GLS-1 starts to be up-regulated and accumulates in fibrotic septa **(Khomich et al., 2019)**. Suggesting that inhibiting GLS-1 may halt the proliferative, growth and fibrogenic activity in human HSCs **(Du et al., 2020)**.

CB-839 is currently used in clinical trials as a selective GLS-1 inhibitor as it inhibits GLS tetramer formation. Although GLS-2 is insensitive to CB-839 class drugs, it is inhibited by the small molecule 968 that acts as a glutaminase C (GAC) dimer formation inhibitor. It inhibits both GLS-1 and GLS-2 with a high selectivity, ≥ 3 folds, for GLS-2 **(Lukey et al., 2019; Ding et al., 2021; Yang et al., 2021)**. Using these two inhibitors, we can differentiate, localize and report the critical role of each isoform of GLS and advance our understanding of how to target aberrant glutamine metabolism in fibrotic liver.

2. Pathophysiology of liver fibrosis

Fibrosis is produced by a pathological excess in the deposit of ECM, including collagen types 1 and 2 along with chronic inflammation. This sequence is initiated by parenchymal cell destruction resulting from a large variety of hepatotoxic and injurious agents **(Kumar et al., 2021)**.

Activation of HSC is the common pathway leading to hepatic fibrosis. Once they are activated, they release chemokines and other leukocyte chemoattractants while up-regulating the expression of important inflammatory receptors such as intercellular adhesion molecule-1 (ICAM-1), chemokine receptors, and mediators of lipopolysaccharide signaling **(Shetty et al., 2018)**.

Hepatic stellate cell activation consists of two major phases **(Figure 1)**:

i) Initiation, also called "preinflammatory stage", which refers to early changes in gene expression and phenotype that render the cells responsive to other cytokines and stimuli.

ii) Perpetuation that results from the effects of these stimuli on maintaining the activated phenotype and generating fibrosis **(Tsuchida & Friedman, 2017)**.

2.1. Initiation phase

The earliest changes observed during stellate activation result from paracrine stimulation by all neighboring cell types **(Figure 2)**, including sinusoidal endothelium, Kupffer cells and infiltrating inflammatory monocytes, hepatocytes, and platelets **(Higashi et al., 2017)**.

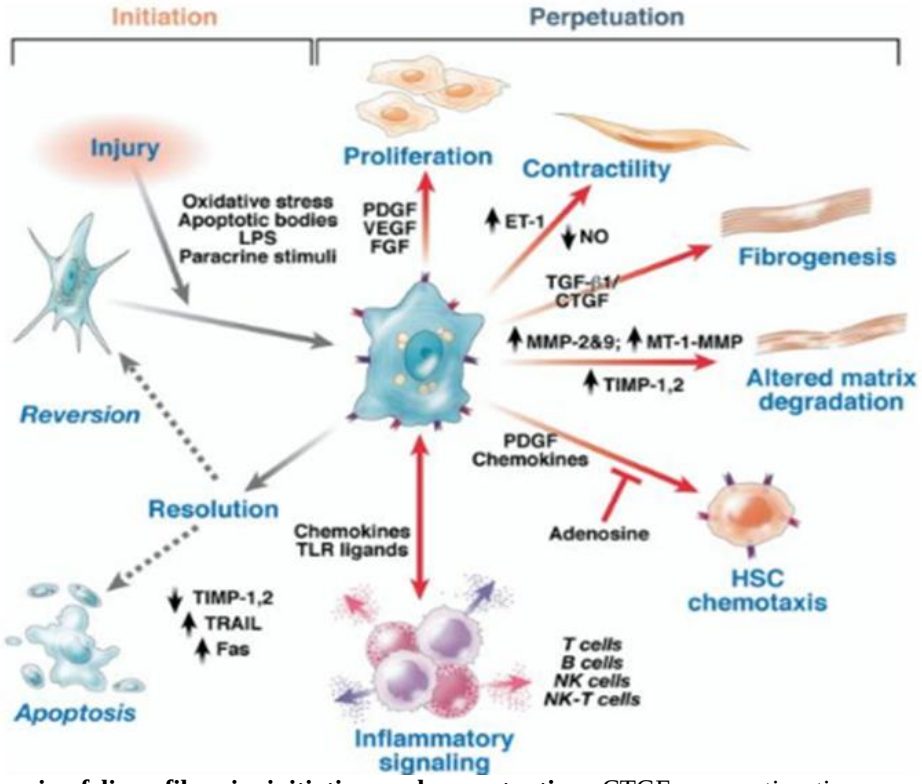

Figure 1. Pathogenesis of liver fibrosis, initiation and perpetuation. CTGF: connective tissue growth factor. ET-1: endothelin-1. FGF: fibroblast growth factor. LPS: lipopolysaccharide. MMP: matrix metalloproteinase. MT-MMP: membrane type matrix metalloproteinase. NK: natural killer. NK-T: natural killer-T cells. NO: nitric oxide. PDGF: platelet-derived growth factor. TGF-β1: transforming growth factor-β1. TIMP-1.2: tissue inhibitor of matrix metalloproteinase-1.2. TLR: toll like receptor. TRAIL: tumor necrosis factor-related apoptosis-inducing ligand. VEGF: vascular endothelial growth factor **(Tsuchida & Friedman, 2017)**.

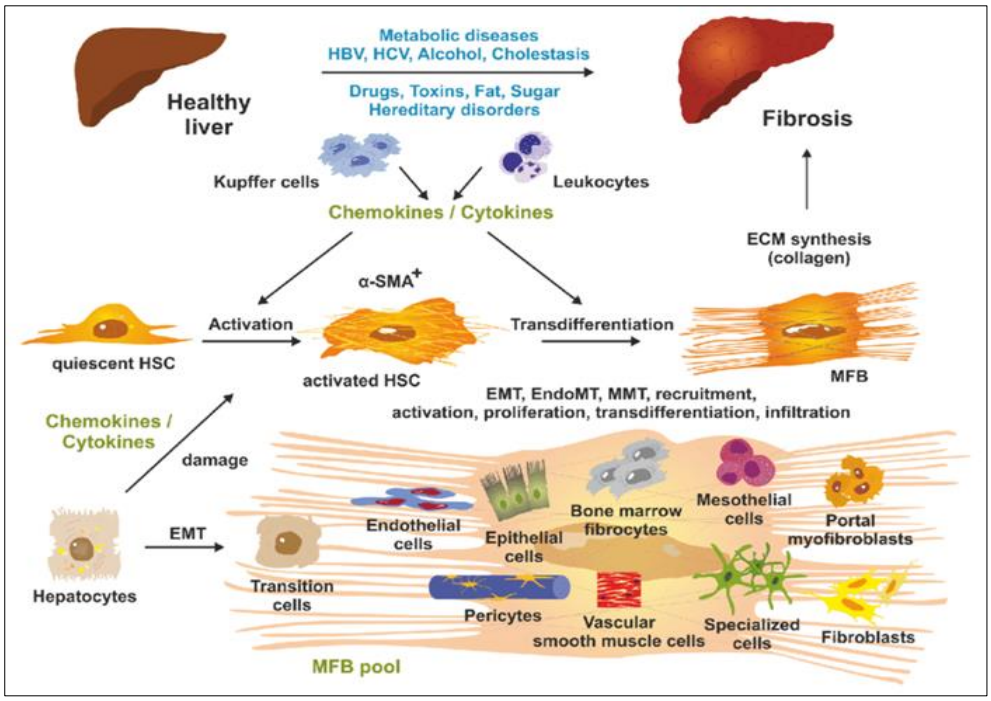

Figure 2. Initiation of liver fibrosis. α-SMA: α-smooth muscle actin. EMT: epithelial mesenchymal transition. HBV: hepatitis B virus. HCV: hepatitis C virus. HSC: hepatic stellate cells. MFB: myofibroblast. MMP: matrix metalloproteinase **(Weiskirchen et al., 2018)**.

Early injury to endothelial cells stimulates production of cellular fibronectin, which has an activating effect on HSC. Endothelial cells are also likely to participate in conversion of profibrogenic TGF-β1 from the latent to the active form **(Dewidar et al., 2019)**.

Platelets are another important source of paracrine stimuli, including platelet-derived growth factor (PDGF), TGF-β1, and epidermal growth factor (EGF). TGF-β1, derived from both paracrine and autocrine sources, is the best characterized and most potent fibrogenic cytokine **(Mukai et al., 2018)**.

Monocyte infiltration and activation also contribute to stellate cell activation. Kupffer cells stimulate matrix synthesis, cell proliferation and release of retinoids by stellate cells through the actions of cytokines, especially TGF-β1, reactive oxygen intermediates and lipid peroxides. On the other hand, activated macrophages can lead to stellate cell apoptosis by different mechanisms **(George et al., 2019)**.

Hepatocyte apoptosis following injury also promotes stellate cell initiation and activation of Kupffer cells through a process mediated by Fas protein **(Figure 3)**. Proapoptotic stimulus induces hepatocyte apoptosis. These apoptotic cells release lipid signals for their uptake by Kupffer cells and HSCs. Engulfment of the apoptotic bodies by HSCs and Kupffer cells enhances their expression of pro-fibrogenic genes and of death ligands (e.g., Fas ligand). Persistent activation of these cells promotes generation of chemokines (Interleukin-8, macrophage inflammatory protein-2 and growth-related oncogene) that promotes neutrophil infiltration into liver and stimulate hepatic inflammation **(Cheng et al., 2019)**.

Cytochrome P450 2E1 (CYP2E1) may have an important role in the generation of reactive oxygen species (ROS) that stimulates HSCs. CYP2E1-derived ROS are responsible for the increased collagen production and it is involved in the pathogenesis of liver injury in alcoholic liver disease since CYP2E1 is alcohol-inducible **(Ceni et al., 2014)**.

Oxidative stress mediated by NADPH oxidase activates HSCs as well as Kupffer cells or resident liver macrophages **(Sancho et al., 2012)**. In addition, nitrosative stress from hepatocyte mitochondrial injury and induction of nitric oxide synthase-2 (NOS-2) may be an overlooked pathway of liver injury. Hypoxia is also a component of the injury milieu and elicits fibrogenic and angiogenic signaling **(Ramos-Tovar & Muriel, 2020)**.

Direct effects of hepatotrophic viruses may also contribute to stellate cell activation. In particular, HCV proteins can activate stellate cells through several mechanisms. HSCs express several coreceptors that interact with HCV proteins, promoting liver fibrogenesis. In addition, HSCs have the ability to engulf apoptotic bodies of hepatocytes induced by the virus and trigger a profibrogenic response. HSCs enhance differentiation and accumulation of regulatory T cells. HSCs-activated natural killer cells could produce interferon-γ (IFN-γ) that inhibits HCV replication. Importantly, HSCs possess functional Toll-like receptor-3 and retinoic acid-inducible gene-I that can be activated by their ligands. Whereas, accelerated fibrosis in chronic HBV may engage specific immune cell types, especially natural killer cells **(Ray & Ray, 2019)**.

2.2. Perpetuation phase

Perpetuation phase results from paracrine and autocrine stimulation. It involves at least seven discrete changes in cell behavior: proliferation, chemotaxis, fibrogenesis, contractility, matrix degradation, retinoid loss, and white blood cell chemoattractant and cytokine release **(Lee & Friedman, 2011)**.

2.2.1. Proliferation

One of the most potent stellate cell mitogens is PDGF. Induction of PDGF receptors in stellate cell activation increases responsiveness to this potent mitogen. Other compounds with mitogenic activity in stellate cells and with a potential role in fibrogenesis include vascular endothelial cell growth factor (VEGF), thrombin and its receptor, EGF, TGF-β1 and fibroblast growth factor (FGF) **(Wallace et al., 2015)**.

2.2.2. Chemotaxis

Stellate cells can migrate towards cytokine chemoattractants. These chemoattractants include PDGF, methyl-accepting chemotaxis proteins (MCP-1), and chemokine receptor (CXCR3). In contrast to PDGF, adenosine blunts chemotaxis,

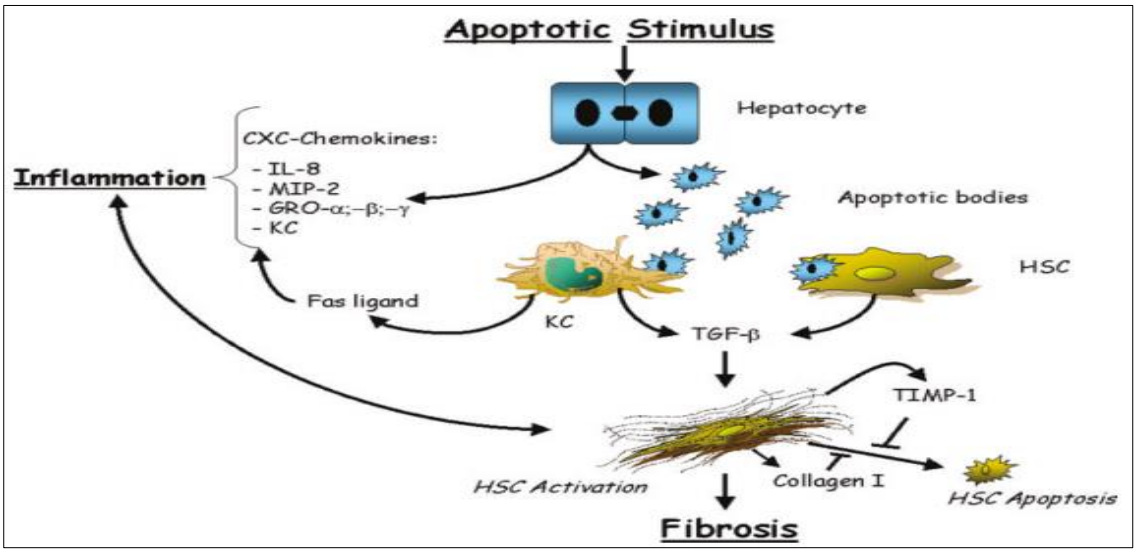

Figure 3. The association between apoptosis and inflammation on liver fibrosis. IL-8: Interleukin-8, MIP-2: Macrophage inflammatory protein-2, GRO: Growth-related oncogene, TGF-β1: Transforming growth factor-β1, HSCs: Hepatic stellate cells, TIMP: Tissue inhibitor of matrix metalloproteinases **(Canbay et al., 2004).**

thereby providing a counter-regulatory pathway that fixes cells at sites of injury **(Zhu et al., 2021)**.

2.2.3. Fibrogenesis

Stellate cells influence fibrosis by increasing matrix production and scar formation. The expression of collagen type-1 is regulated post-transcriptionally in HSCs and the most potent stimulus for its production is TGF-β1, which is derived from both paracrine and autocrine sources. TGF-β1 also stimulates the production of other matrix components including cellular fibronectin and proteoglycans **(Dewidar et al., 2019)**.

Stellate cells have three classes of peroxisome proliferator-activated receptor gamma (PPARs), (α, β and γ). PPAR-γ is found to be significantly down-regulated in HSCs activation. PPAR-γ plays an important role in fibrogenesis by different mechanisms **(Li et al., 2021)** as PPAR-γ down-regulates type-I collagen mRNA via a direct effect on transcriptional regulation of this gene in stellate cells **(Shafiei et al., 2011)**. PPAR-γ has been shown to down-regulate α-SMA and inhibit proliferation of HSCs **(Alatas et al., 2020)**. It also decreases the accumulation of activated HSCs (**Li et al., 2021)**. PPAR-γ is also considered a potent modulator in lipid metabolism and acts as anti-inflammatory protein **(Ahmadian et al., 2013)**. PPAR-γ appears to antagonize mothers against deca-pentaplegic homolog-3 (SMAD-3)/TGF-β1 signaling, both have a critical role in fibrogenesis **(Wen et al., 2022)**.

Moreover, PPAR-γ has an anti-inflammatory effect via liver-x-receptor activation **(Hassan et al., 2021)**.

2.2.4. Contractility

Contractility of stellate cells may be a major determinant of early and late increase in portal resistance during liver fibrosis. The major stimulus for stellate cell contraction is endothelin-1, whose receptors are expressed on quiescent and activated HSCs. Upon activation of HSCs, the predominant type of endothelin receptor increases its sensitivity to autocrine endothelin-1 **(Ying et al., 2017)**. Moreover, when HSC becomes contractile, it develops increased expression of the cytoskeletal protein α-SMA **(Khomich et al., 2019)**.

2.2.5. Matrix degradation

Changes in matrix protease activity have an important role in ECM remodeling accompanying liver injury. Because stellate cells express virtually all the components required for pathologic matrix degradation, they have a key role not only in matrix production, but also matrix degradation **(Cabral-Pacheco et al., 2020)**.

MMP-2, MMP-9, and MMP-13 are the most commonly reported MMPs related to the regulation of liver fibrogenesis. Matrilysin (MMP-7) is also associated with tissue remodeling during the progression of liver fibrosis in biliary atresia.

MMP-9 promotes HSC apoptosis. On the other hand, α-SMA-positive myofibroblast-like cells increase their expression of fibrillar collagen and MMPs such as MMP-2 and MMP-9, as well as tissue inhibitor of matrix metalloproteinases (TIMPs) in fibrotic tissue. Consequently, fibrillar collagen accumulates especially collagen I and III **(Geervliet & Bansal, 2020)**.

2.2.6. Retinoid loss

Activation of stellate cells is accompanied by the loss of the characteristic perinuclear retinoid (vitamin A) droplets. Retinoid is stored as retinyl esters, whereas the form of retinoid released outside the cell during activation is retinol, suggesting that there is intracellular hydrolysis of esters prior to export **(Trivedi et al., 2021)**.

Retinoid loss is required for acceleration of stellate cells activation. The enzyme patatin-like phospholipase domain-containing protein-3 (PNPLA-3) has been proposed as a “gatekeeper" of stellate cell retinoid content. Polymorphisms in this gene have also been linked to risk of non-alcoholic fatty liver disease (NAFLD) **(Tomita et al., 2014)**.

2.2.7. Inflammatory signaling and leukocyte chemoattraction

Stellate cells can amplify the inflammatory response by inducing infiltration of mono- and polymorphonuclear leukocytes. Stellate cells also express toll-like receptors, indicating a capacity to interact with bacterial lipopolysaccharide, which in turn stimulates stellate cells **(Sufleţel et al., 2020)**.

Stellate cells can also function as antigen-presenting cells that can stimulate lymphocyte proliferation or apoptosis. Stellate cells produce neutrophil chemoattractants, which could contribute to the neutrophil accumulation characteristic of alcoholic liver disease **(Wang et al., 2021)**.

3. L-Glutaminase enzyme

Glutamine (Gln), the most abundant free amino acid presents in human blood, is a neutral, polar and conditionally essential amino acid that has considerable functions in diverse biochemical processes as protein & lipid synthesis, regulation of acid-base balance in the kidney, cellular energy source, nitrogen and carbon donation for different anabolic processes, citric acid cycle refilling and also serves as an important mean for nontoxic transportation of ammonia in the blood circulation **(Cruzat et al., 2018)**.

Glutamine is mainly metabolized by GLS which is an amidase enzyme that breaks down Gln into glutamate with the release of ammonia. It acts as an endopeptidase, hydrolyzing the peptide bonds present in the interior of a protein molecule. The enzyme was named after the discovery of its activity by Hans Krebs in 1935. It mainly catalyzes the hydrolysis of the γ-amido bond of L-glutamine (glutaminolysis) and it plays a major role in the nitrogen metabolism of both prokaryotes and eukaryotes **(Cruzat et al., 2018)**.

Glutaminase is seen in the inner membrane of mitochondria and is the predominant glutamine-using enzyme in the brain. Microorganisms are also producers of this commercially important enzyme. It is widely used as an oncolytic enzyme and as an antiretroviral agent because of its capability to degrade small molecules like glutamine. Glutaminase also finds application in the food industry as a flavor-enhancing agent **(Wang et al., 2020)**.

The mammalian solute carrier (SLC) transporters differentiate into 52 different gene families. Fourteen of them are responsible for transporting Gln throughout the plasma membrane; they are sub-divided into four families: SLC1, SLC6, SLC7 and SLC38. SLC38 family is considered the principal transporter for Gln **(Bhutia & Ganapathy, 2016)**. Sodium-dependent neutral amino acid transporter-2 (SNAT-2), a protein encoded by the SLC38A2 gene, is a Na+-coupled influx transporter for Gln which in turn plays a significant role in TGF-β1-induced myofibroblast activation and differentiation **(Morotti et al., 2021)**.

Humans express 4 isoforms of glutaminase. GLS-1 encodes 2 types of kidney-type glutaminase with a high activity and low Km. GLS-2 encodes 2 forms of liver-type glutaminase with a low activity and high Km **(Figure 4) (Simon et al., 2020)**.

The GLS-1 gene encodes two isoforms, known as kidney glutaminase or KGA, and a shorter spliced form named glutaminase C or GAC. On the other hand, the GLS-2 gene codes for the liver isozymes, named LGA, as well as for a longer isoform named GAB originally described in breast cancer cells **(de Los Santos-Jiménez et al., 2021)**.

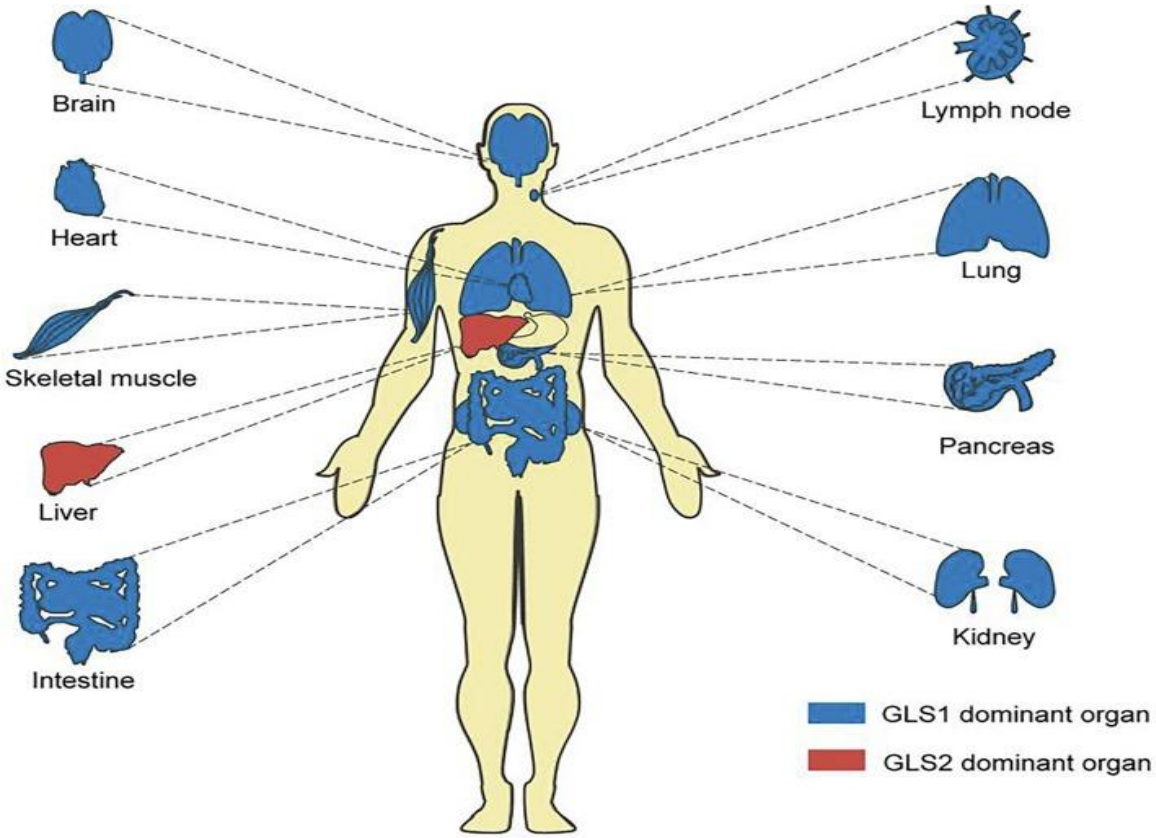

Figure 4. Tissue distribution of GLS1 and GLS2. This figure illustrates the primary organs associated with GLS1 and GLS2 dominance, emphasizing their significance in key systems including the lymphatic, respiratory, and digestive systems **(Ding et al., 2021)**.

4. Role of L-glutaminase enzyme in pathogenesis of liver fibrosis

Glutamine metabolism plays an important role in regulating the proliferation and activation of HSCs **(Figure 5)**. Gln can be converted to glutamate, by glutaminase enzyme, which then converted to α-ketoglutarate (α-KG), which provides carbon to Krebs cycle (TCA) cycle **(Yoo et al., 2020)**. Glutamate can also be converted to pyrroline-5-carboxylate (P5C), which can stimulate collagen biosynthesis in cultured cells. Reduction of P5C to proline is a critical step for proline biosynthesis, which has enormous effect on collagen synthesis as proline and hydroxyproline together comprise approximately 23% of the collagen molecules **(Chalecka et al., 2021)**.

Differentiation of quiescent HSC into myofibroblasts requires increased glycolysis which leads to accumulation of lactate. Glucose and glutamine metabolisms are interrelated, as both are precursors in the TCA cycle to generate energy required for differentiation, as well as precursors in the production of lipids, nucleotides, and amino acids. Strategies that are targeted at glutamine metabolism may represent a novel therapeutic approach to the treatment of liver fibrosis **(Li et al., 2017)**. Glutaminolysis has also a role in the maintenance of the proliferative, phagocytic and secretory capacities of these cells **(Cruzat et al., 2018)**.

5. L-glutaminase inhibitors

5.1. DON

DON (6-Diazo-5-oxo-L-norleucine) is a glutamine antagonist that binds covalently to the enzyme active site and broadly inhibits glutamine-using enzymes, including L-glutaminase and glutamine amidotransferases involved in de-novo nucleotide synthesis, amino acid synthesis and hexosamine production. However, it is considered a non-selective inhibitior that induces high degree of toxicity, so it is not widely used **(Stine et al., 2022)**.

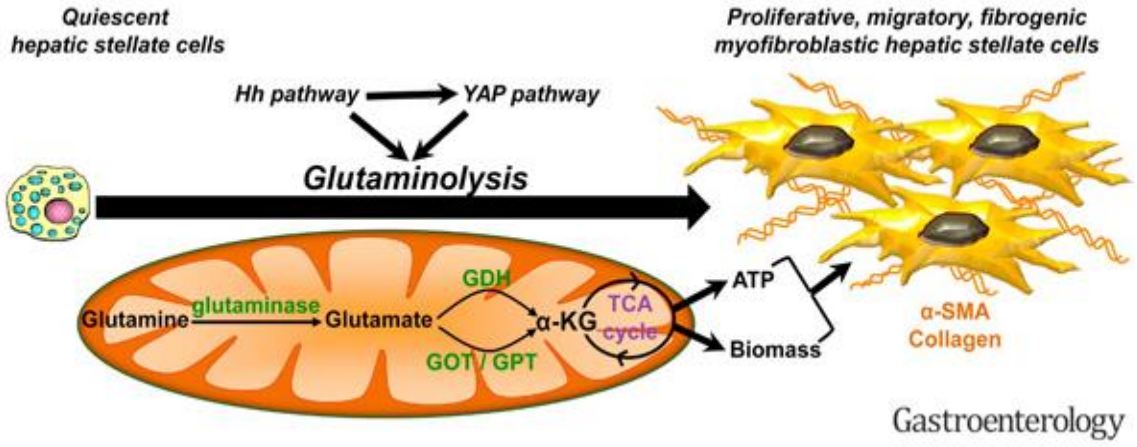

Figure 5. Role of glutaminolysis in hepatic stellate cells activation. α-KG: α-ketoglutarate. α-SMA: α-smooth muscle actin. ATP: Adenosine triphosphate. GDH: Glutamate dehydrogenase. GOT/GPT: Aspartate aminotransferase/alanine aminotransferase. TCA: Tricarboxylic acid cycle **(Du et al., 2018)**.

5.2. JHU-083

JHU-083 is a newly synthesized pro-drug of DON, which can be administered in an inert state and then be activated in-vivo through enzymatic cleavage, thus alleviating the previously reported toxicity of DON. It was synthesized to overcome peripheral toxicity of DON. JHU-083 exerts similar therapeutic effects. However, due to the lack of selectivity and relatively weak potency, DON and its prodrug may not be considered as first-class as therapeutic drugs for specific inhibition of GLS **(Rad et al., 2021)**.

5.3. BPTES

BPTES, Bis-2-[5-(phenylacetamido)-1,3,4-thiadiazol-2-yl]ethyl sulfide, is specifically inhibits kidney type glutaminase activity through the formation of an inactive complex. It binds to the gating loop of GLS1, which affects substrate binding and the formation of active tetrameric GLS1, causing a conformational change that deactivates GLS1. Though BPTES shows high specificity and efficiency in inhibiting cancer cell proliferation in-vitro, the drawbacks of poor aqueous solubility and low bioavailability in-vivo restrict its further applications in clinical trials. In order to improve drug solubility, several derivatives of BPTES were synthesized through structural modifications **(Wang et al., 2020)**.

5.4. Phycoerythrin

Phycoerythrin, a natural alcoholate purified from physalis, is a KGA inhibitor with comparable inhibitory effect with BPTES but less cytotoxicity. The cyclic structure overlaps with the binding site of Gln, and the molecule is large enough to completely occupy the cavity of the substrate and product of GLS, thus prevents the substrate from entering. Kinetic studies indicate high affinity of phycoerythrin for KGA and show dual inhibitory (competitive and non-competitive) effects of phycoerythrin on KGA activity **(Ding et al., 2021)**.

5.5. Caudatan A

Caudatan A is a natural product isolated from the roots of natural Ohwia caudate, has the same inhibitory activity against KGA to BPTES and better solubility. This compound exerts enzyme inhibition through competitive binding to the KGA active site in aid of the cyclization of isopentyl and bridge ring in caudatan A **(Ding et al., 2021)**.

5.6. THDP17

THDP17 is a thiourea derivative that shows partial non-competitive inhibition of GLS1 and non-significant cytotoxicity in vitro, and significant inhibition on the intestinal GLS activity in-vivo **(Johnson et al., 2018)**.

5.7. Compound-968

Compound 968 is an allosteric inhibitor of GLS1 that may bind between the N and C termini of two GAC monomers at the monomer-monomer interface and inhibit the formation of active GAC dimer. It inhibits both GLS-1 and GLS-2 with a

high selectivity, ≥ 3 folds, for GLS-2. Currently, the anti-tumor effects of compound 968 are widely investigated, but its anti-inflammatory function in the central nervous system remains unknown **(Lin et al., 2022)**.

5.8. CB-839

CB-839, also known as Telaglenastat, is a potent, selective, reversible and orally bioavailable inhibitor of splice variants of GLS1. CB-839 acts as a tetramer formation inhibitior. It possesses high selectivity toward GLS-1 than GLS-2. Currently, numerous clinical trials are either ongoing or completed that apply CB-839 as an anti-tumor and anti-inflammatory drug **(Wicker et al., 2021)**.

5.9. Carbenoxolone

Carbenoxolone (CBX) is a glycyrrhetinic acid derivative with a steroid-like structure, similar to substances found in the root of the licorice plant. Carbenoxolone is used for the treatment of peptic, esophageal and oral ulceration and inflammation. It is a gap junction inhibitor, also effectively suppresses excess Glu production in activated microglia and subsequent neurotoxicity. In addition, CBX directly targets GLS and exhibits GLS isoform selectivity but the mechanism is still unknown **(Buckley et al., 2021)**.

5.10. Others

Ebselen, Chelerythrine chloride and Apomorphine hydrochloride are considered GLS inhibitors. Ebselen and chelerythrine chloride exhibit high inhibitory effects on GLS1 and 5 to 10-fold less activity against GLS-2. In contrast, apomorphine hydrochloride inhibits both GLS1 and GLS2 with similar activity. Although non-selective, these chemicals display 10 to 1500-fold greater affinities and over 100-fold increased inhibition efficiency than DON and BPTES **(Thomas et al., 2013)**.

6. Conclusion

Hepatic fibrosis remains a critical health concern, particularly in regions severely impacted by hepatitis infections, such as Egypt. The pathophysiology of liver fibrosis is complex, involving the activation of HSCs and the excessive accumulation of extracellular matrix proteins, primarily collagen. Research has highlighted the pivotal role of GlS enzyme, particularly its isoforms GLS-1 and GLS-2, in the metabolic reprogramming of HSCs and the progression of fibrosis. The switch from GLS-2 to GLS-1 during fibrotic changes underscores the potential therapeutic benefits of targeting glutamine metabolism.

Emerging antifibrotic agents like CB-839 and compound 968 offer promising avenues for inhibiting GLS activity, thereby potentially reducing HSC proliferation and extracellular matrix deposition. Continued exploration of these inhibitors could not only enhance our understanding of glutamine's role in fibrosis but also pave the way for innovative treatment strategies. Addressing the burden of hepatic fibrosis through targeted therapies may significantly improve patient outcomes and reduce the mortality associated with liver diseases worldwide.

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