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REVIEW ARTICLE

Fibrinogen-like 1: A hepatokine linking liver physiology to hematology

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Abstract

A recent study identified the critical contribution of the hepatokine FGL1 to the regulation of iron metabolism during the recovery from anemia. FGL1 is secreted by hepatocytes in response to hypoxia to sequester BMP ligands and repress the transcription of the iron-regulatory hormone hepcidin. This process ensures the proper supply of iron to the bone marrow for new red blood cell synthesis and the restoration of physiological oxygen levels. FGL1 may therefore contribute to the recovery from common anemias and cause iron overload in chronic anemias with ineffective erythropoiesis, such as ß-thalassemia, dyserythropoietic anemia, and myelodysplastic syndromes. However, FGL1 has also been described as a regulator of hepatocyte proliferation, glucose homeostasis, and insulin signaling, as well as a mediator of liver steatosis and immune evasion. Chronic exposure to elevated levels of FGL1 during anemia may therefore have systemic metabolic effects besides iron regulation and erythropoiesis. Here, we are providing an overview of the proposed functions of FGL1 in physiology and pathophysiology.

INTRODUCTION

The hepatokine FGL1 has recently been described as a regulator of iron metabolism during anemia and expanded erythropoiesis.[1] While the growth factor erythropoietin (EPO) orchestrates multiple stages of erythropoiesis, mounting evidence indicates that chronic EPO exposure is accompanied by systemic alteration of metabolism, such as insulin sensitivity and glucose tolerance in both humans[2,3] and mice.[4–6] Interestingly, FGL1 has also been described as a mediator of liver homeostasis and insulin signaling and chronically elevated levels of FGL1 during anemia may have systemic effects. Whether FGL1 is involved in the metabolic effects attributed to EPO is unknown. In this review, we provide a detailed overview of the proposed roles of FGL1 in pathophysiology.

Fibrinogen-like 1 (FGL1) structure

FGL1, also known as hepassocin (HPS), liver fibrinogen-related gene-1 (LFIRE1), or hepatocyte-derived fibrinogen-related protein (HFREP1),[7] is a 34 kDa secreted protein initially identified in 1993 in a human hepatocellular sample.[7] The active form of FGL1 is a 68 kDa homodimer linked by disulfide bonds.[8,9] FGL1 belongs to the fibrinogen-related protein (FREPs) family and is composed of a short N-terminal coil-coil domain (CCD) and a C-terminal globular fibrinogen-like domain (Figure 1). The globular domain of approximately 200 amino acids shares a high homology with fibrinogen β and γ chains and is found in proteins, such as angiopoietin (ANGPT) 1, 2, 3, and 4; angiopoietin-like (ANGPTL) 1, 2, and 6; and fibrinogen-like 2 (FGL2), ficolin 2, or tenascin. While FGL1 belongs to the fibrinogen superfamily, it lacks the platelet binding site, the cross-linking region, and the thrombin-sensitive site[7] and therefore does not participate in the blood clotting process. However, FGL1 interacts with the fibrin matrix during clot formation and this interaction may potentiate its function.[10] The activity of growth factors, such as basic fibroblast growth factor or vascular endothelial growth factor (VEGF), is regulated through their association with fibrin[10] but the exact consequences of the FGL1/fibrin association are unknown.

The regulation of FGL1 expression

In physiological conditions, FGL1 is expressed primarily by hepatocytes and to a lesser extent in the pancreas.[9] FGL1 expression also becomes detectable in the bone marrow during expanded erythropoiesis,[1] in brown and white adipose tissue after partial hepatectomy,[11] in the gastric mucosa (stomach),[12] and in the lung[13] after irradiation. Baseline *FGL1* expression is controlled by the transcription factor HNF1α in synergy with other factors, such as high mobility group box 1 (HMGB1) and cAMP response element-binding protein (CREB)-binding protein[14] (Figure 2). Additional transcription factors influence the regulation of FGL1 transcription and its

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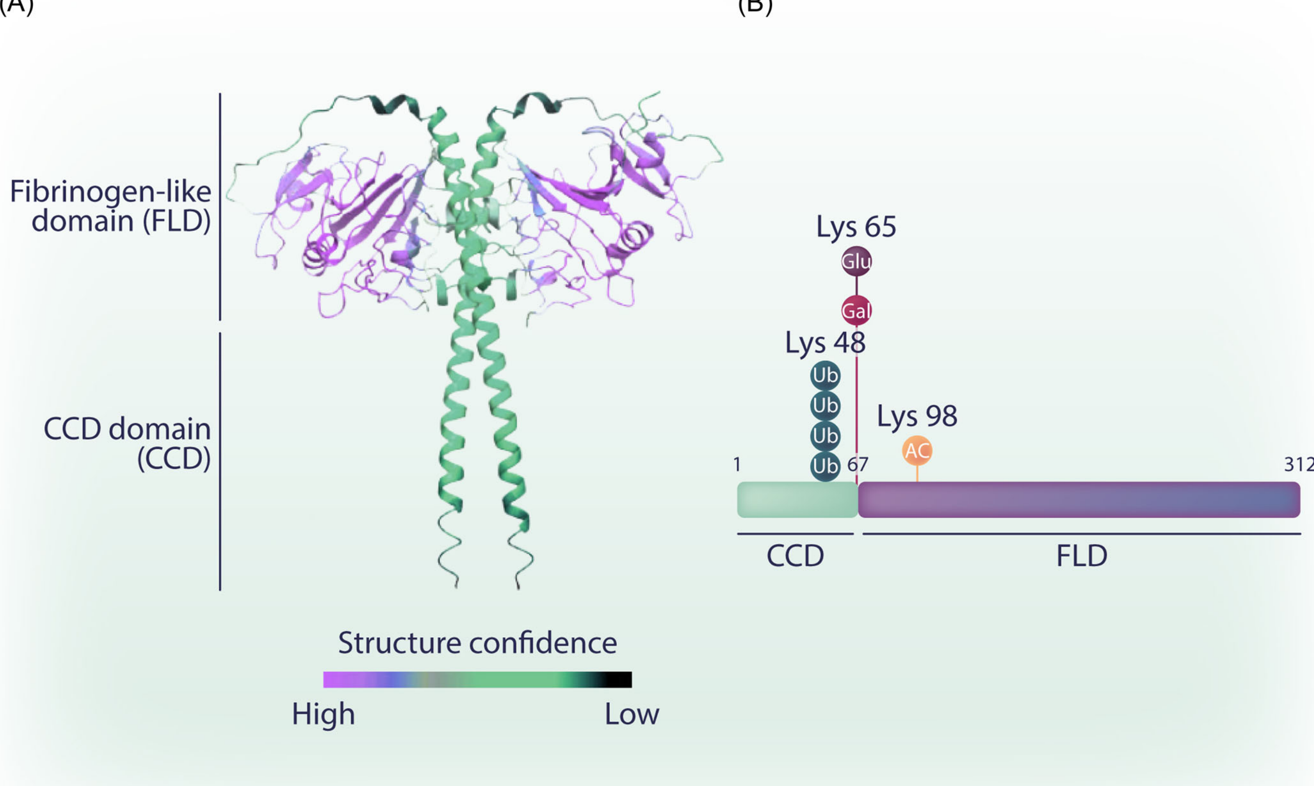

FIGURE 1 Fibrinogen-like 1 (FGL1) structure. (A) FGL1 dimer structure prediction by Alphafold2 shows interaction by the linear coil-coli domain (CCD) leading to functional dimers. (B) Schematic representation of post-translational modifications regulating FGL1 protein stability.

secretion in pathological conditions. Induction of endoplasmic reticulum (ER) stress by palmitate in primary mouse hepatocytes stimulated *Fgl1* expression through the p38/C/EBPβ pathway.[15] FGL1 expression is also increased by STAT3 in response to the accumulation of unsaturated fatty acid[16] and by the transfer RNA (tRNA)-derived fragment tRF-Glu49 in cervical cancer.[17] FGL1 promoter contains two HIF-binding sites,[1] suggesting that its expression is directly regulated by hypoxia-inducible factors. Of note, *Fgl1* mRNA expression was induced in hepatocytes incubated in hypoxic conditions (2% O_2) or treated with the prolyl hydroxylase inhibitor DMOG.[1] Similar observations were made in the livers of mice treated chronically with the prolyl hydroxylase inhibitor vadadustat[1] or subjected to hypoxic conditions for 32 days.[18] *FGL1* expression was reduced in human MCF7-cells lacking HIF1α or HIF2α.[19] Whether a certain HIF subunit is specific for the activation of the *FGL1* promoter is unknown. However, the hepatic zonation of *Fgl1* expression correlated with HIF target genes (e.g., *Pck1*, *Eno1*, or *Gapdh*) but not with the gradient in oxygen concentration indicating that *Fgl1* expression is not under the control of HIF in steady-state condition.[20] A relatively short 1 h intracellular half-life has been reported for FGL1 in immortalized cell lines HEK293T and HepG2.[21] Intracellular FGL1 protein stability is sensitive to post-translational modifications, such as the acetylation and glucosyl-galactosyl-hydroxylation (GGH) of lysine (Lys) residues. Acetylation of Lys98 targets FGL1 for proteasomal degradation whereas its removal by the NAD-dependent deacetylase sirtuin 2 stabilizes FGL1 and ensures its secretion in hepatocellular carcinoma (HCC).[22] Likewise, GGH is a unique and specific glycosylation observed in the collagen-like domain present in proteins, such as collagen[23] or adiponectin.[24] Although FGL1 does not contain any collagen-like domain, the absence of Lys65 GHH leads to the reduction of intracellular levels of FGL1 but its secretion is not altered.[25] FGL1 intracellular stability is also regulated by the E3 ubiquitin ligase FBXO38, which mediates FGL1 Lys48 ubiquitinylation and subsequent degradation by the proteasome.[21] Once secreted, the protein half-life in the bloodstream is unknown. Little is known about single nucleotide polymorphism (SNP) in the FGL1 gene except for SNP rs484373, which may be associated with cell cytotoxicity of the hepatitis C virus (HCV).[26]

FGL1 in liver physiology

FGL1 is secreted into the bloodstream by hepatocytes[27] and can function in a paracrine or autocrine manner on hepatocytes. The proposed hepatic functions encompass various aspects of liver functions, such as the regulation of glucose and iron metabolism.

FGL1 and glucose homeostasis

Several lines of evidence have linked FGL1 and glucose homeostasis. Plasma FGL1 concentration was increased in patients with impaired fasting glucose, impaired glucose tolerance, and newly diagnosed diabetes compared to healthy patients.[28] The same authors suggested that this increase was observed independently of liver steatosis.[29] Patients suffering a hyperglycemic crisis (i.e., uncontrolled diabetes) showed increased levels of circulating

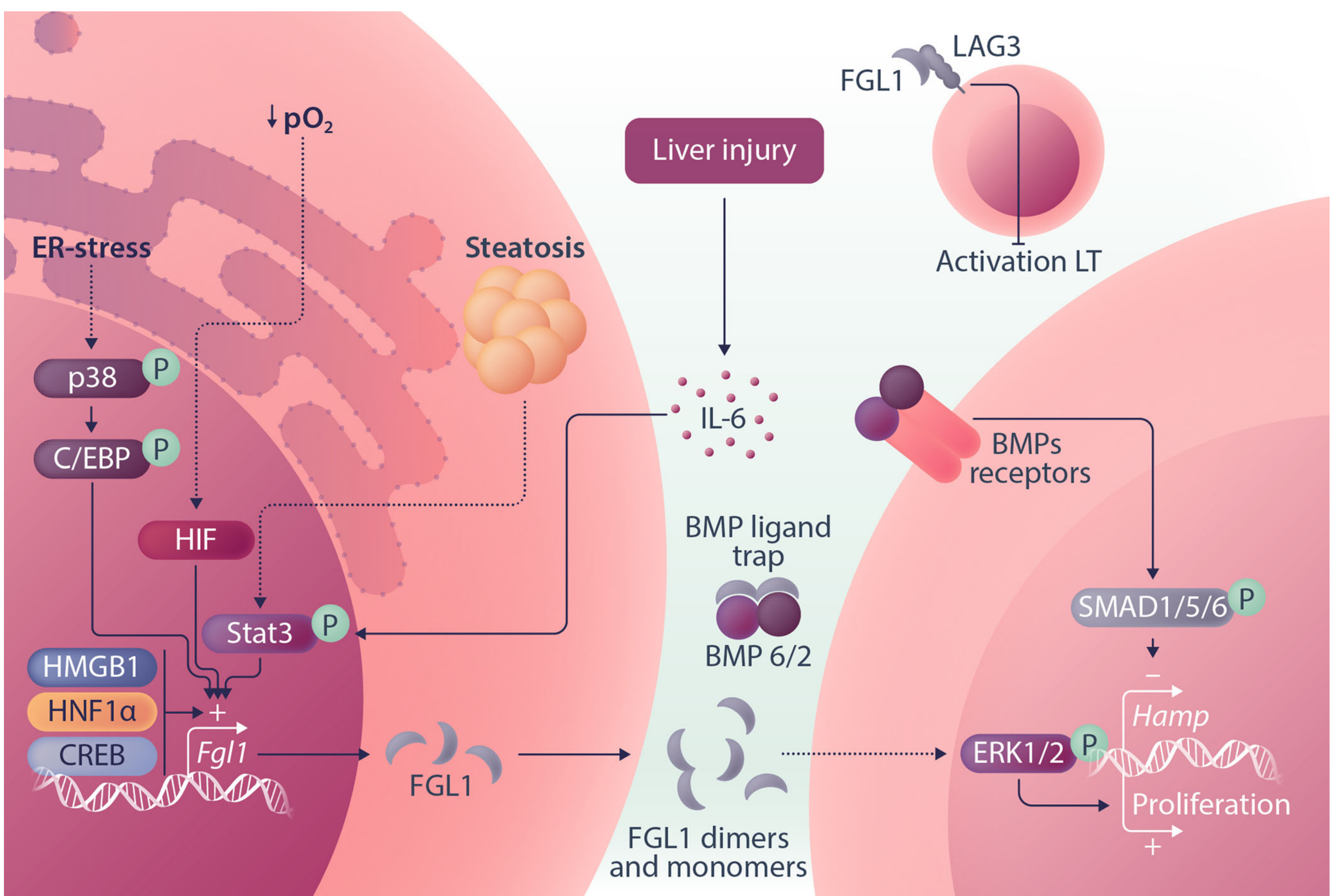

FIGURE 2 Hepatic regulation and functions of fibrinogen-like 1 (FGL1). In hepatocytes, FGL1 expression is governed by multiple stimuli. At the intracellular level, ER stress, hypoxia, and steatosis increase *Fgl1* expression through the p38-C/EBP, HIF, and STAT3 pathways. *Fgl1* expression is also controlled by an HMGB1-HNF1α dimer. During acute inflammation, the pro-inflammatory cytokine interleukin (IL)-6 induces *Fgl1* expression. FGL1 is secreted as monomers or dimers and acts both as a hepatokine or a ligand trap. FGL1 can act on surrounding hepatocytes to promote proliferation through an ERK1/2-dependent pathway. The binding of FGL1 to its cognate receptor LAG3 on T-cells prevents their activation, thus limiting the anticancerous response. During anemia and hypoxia, secreted FGL1 antagonizes the BMP signaling pathway by preventing the interaction between BMP ligands and their receptor complexes, thereby repressing *Hamp* expression and increasing iron availability for erythropoiesis.

FGL1 that returned to normal along with liver function after a standard dose of insulin.[29] FGL1 levels were also increased and normalized after the glycemia was corrected using metformin (an AMP-activated protein kinase activator) or rosiglitazone (an agonist of PPARγ)[28] in a mouse model of diet-induced obesity and diabetes. *Fgl1* expression was also increased in the liver of mice with specific ablation of insulin receptors.[30] Collectively, these studies suggest that *Fgl1* expression correlates with changes in glucose homeostasis. Two studies reported that mice deficient for *Fgl1* exhibited increased body weight[11,31] when fed a standard chow diet but different conclusions were reached regarding glucose metabolism. Indeed, in the first study 3–4 months WT and *Fgl1*$^{-/-}$ male mice were compared after an overnight fasting period, and *Fgl1*$^{-/-}$ mice presented with increased plasma glucose levels and glucose intolerance compared to their WT counterparts.[11] The second study compared animals at 8 weeks of age after a 4-h fasting period and no difference in plasma glucose levels and glucose tolerance was observed between genotypes.[31] However, when mice were fed a high-fat diet for 16 weeks, ablation of *Fgl1* was accompanied by a decrease in glucose tolerance.[31] In contrast with these observations, transient knockdown of *Fgl1* using short hairpin RNA in WT mice fed a high-fat diet or in genetically obese *Ob/Ob* mice resulted in decreased fasting blood glucose and insulin levels and improved glucose tolerance.[28] Similar to the observed discrepancies in mice deficient for *Fgl1*, two studies evaluating the effect of recombinant FGL1 administration on glucose metabolism have also yielded opposite observations. In mice subjected to a hyperglycemic crisis induced by streptozotocin, FGL1 administration (1 µg/kg once a day for 7 days) reduced circulating levels of transaminases, liver inflammation, and oxidative stress but it did not normalize glucose or insulin levels in comparison to hyperglycemic mice.[29] Identical treatment or the overexpression of FGL1 using a lentiviral vector in wild-type unchallenged mice induced fasting hyper-glycemia and hyper-insulinemia combined with an increased hepatic glucose production and decreased glucose infusion rate,[28] suggesting that the contribution of FGL1 to glucose metabolism differs depending on the metabolic status of the animals. In vitro, treatment of primary mouse hepatocytes or hepatoma cell lines with FGL1 (100 ng/mL) impaired insulin-dependent AKT and ERK1/2 signaling.[28,32] Altogether, these studies indicate that FGL1 may play a role in glucose metabolism but its exact mechanism and pathophysiological relevance are still unclear.

FGL1 and iron metabolism

In addition to glucose metabolism, the critical role of FGL1 in the regulation of iron metabolism has recently been uncovered. A human adult body contains between 3 to 5 g of iron (Fe), mostly distributed between red blood cells (hemoglobin; 60%–70%) and the liver (20%). While essential for oxygen transport and storage, excess iron can lead to the production of free radicals and cause irreversible tissue damage, preferentially in the liver and the heart. Most of the plasma iron is provided by the recycling of senescent red blood cells by splenic macrophages. Iron is then directed to the bone marrow for new red blood cell synthesis. Iron losses caused by sweat, blood loss, or the shedding of intestinal and skin epithelial cells are compensated by the absorption of dietary iron by duodenal enterocytes. The release of iron from enterocytes, macrophages, and hepatocytes into the bloodstream is ensured by the sole iron exporter ferroportin.[33,34] The expression of ferroportin at the cell surface is controlled by the liver-produced hormone hepcidin.[35] Hepcidin binds to ferroportin and promotes its occlusion and degradation to prevent iron efflux into the bloodstream.[36] Hepcidin expression is mainly regulated by the BMP-SMAD signaling pathway.[37] During stress erythropoiesis and anemia, hemoglobin synthesis and therefore the iron requirements for the bone marrow can increase up to 10-fold. The erythroid hormone erythroferrone (ERFE)[38] is thus secreted by erythroid precursors to sequester BMP ligands in the liver perisinusoidal space and inhibit the BMP-SMAD signaling directing hepcidin expression.[39] This, in turn, leads to ferroportin stabilization at the cell surface, which stimulates iron uptake and mobilization of iron from stores. Through this process, ERFE facilitates the recovery from anemia induced by bleeding,[38] hemolysis,[40] chronic inflammation,[41] and malaria.[42] Conversely, ERFE contributes to iron overload in iron-loading anemias, such as thalassemia[43–45] or myelodysplastic syndromes.[46] However, ERFE is a stress hormone and evidence suggested that another factor was responsible for hepcidin suppression during the recovery from anemia. It was recently observed that *Fgl1* expression was induced by hypoxia in the liver of mice subjected to a single blood withdrawal and in thalassemic mice. Interestingly, treatment of mouse primary hepatocytes or hepatoma cell lines (HepG2 and Hep3B) with recombinant FGL1 (10–25 µg/mL) or administration of FGL1 into mice (1 mg/kg) led to a robust suppression of liver hepcidin mRNA expression. Mice deficient for *Fgl1* showed significantly elevated hepcidin levels at baseline and 36 h after bleeding compared to WT mice. It was further demonstrated that similar to ERFE, FGL1 is a BMP antagonist that directly binds to BMP6 to repress the BMP-SMAD signaling pathway and hepcidin expression.[1] Interestingly, fibrinogen has been described as an agonist of the BMP/SMAD signaling pathway[47,48] but it does not directly bind BMP ligands. Whether other members of the FREP family interfere with the SMAD signaling and the potential interaction of FGL1 with other BMP ligands are unknown.

FGL1 in pathophysiology

While FGL1 promotes the recovery from anemia in mice, in humans its exact contribution to other forms of anemia and iron-loading anemias, such as thalassemia and myelodysplastic syndromes, has yet to be determined. Interestingly, FGL1 may also be involved in the progression of steatosis, fibrosis, and certain cancers.

FGL1 and liver regeneration

Acute liver injury refers to a pathological condition caused by sudden and extensive damage to a significant portion of the liver in the absence of any pre-existing liver disease. The initial phase of hepatocyte apoptosis or necrosis is followed by the proliferation of the remnant hepatocytes to restore the original liver mass and function.[49] The proliferation of hepatocytes is controlled by mitogenic factors, such as the hepatocyte growth factor, the epidermal growth factor receptor in coordination with auxiliary mitogens, such as interleukin-6 (IL-6) or the VEGF (reviewed in Michalopoulos and Bhushan[49]). In mice presenting with acute liver failure induced by low doses of lipopolysaccharide combined with D-galactosamine (D-gal[32]) or carbon tetrachloride (CCl_4[50]), the administration of a single dose of recombinant FGL1 reduced hepatocytes apoptosis and promoted their proliferation and survival in a dose-dependent manner.[32,50] Intriguingly, the protective effect was observed with doses of FGL1 ranging from 0.2 to 1 mg/kg in the D-Gal model[32] whereas 0.1–1 µg/kg was sufficient in rats treated with CCl_4.[50] A low dose of 50 ng/mL induced the ERK1/2 signaling pathway in 5 min in human hepatocytes. Similarly, treatment of mouse primary hepatocytes with 20[8] or 50 ng/mL[50] of recombinant FGL1 enhanced ^{3}H-thymidine incorporation and DNA synthesis. FGL1 expression is also induced during liver regeneration, which supports its role in hepatocyte proliferation,[51] presumably through an ERK1/2 mediated pathway.[29,50] Conditions that cause proliferative stress, such as liver-restricted irradiation[52] or partial hepatectomy,[8,11] are also associated with increased circulating levels of FGL1. In the early phase of an injury, the acute phase response protein IL-6 is produced by liver-resident Kupffer cells to promote liver regeneration.[53] Treatment of the human hepatoma cell line HepG2 with IL-6 increased the expression and secretion of FGL1 in conditioned media concomitantly with other canonical acute phase response proteins, such as serum amyloid A1 or fibrinogen alpha chain.[27] In addition, the induction of extrahepatic inflammation by turpentine oil in mice is sufficient to increase FGL1 serum levels in the early stage of the inflammatory response.[27] Interestingly, ER stress and inflammatory stimuli, such as IL-6 and turpentine oil, are potent inducers of not only *Fgl1* but also of hepcidin, whereas FGL1 represses hepcidin during anemia.[1] An acute induction of *Fgl1* (6 h) may not be sufficient to overcome the inflammatory signal stimulating hepcidin and a prolonged upregulation of *Fgl1* (24 h) may be required to exert a negative pressure on hepcidin, as is the case during the recovery from anemia. In terms of hepcidin regulation, it is also possible that the inflammatory pathway is dominant over the *Fgl1* effect. Further work will be necessary to answer this question. However, recombinant FGL1 stimulated the proliferation of primary hepatocytes (human, rat, and mouse) in a dose-dependent manner, suggesting that FGL1 is a mitogenic factor for hepatocytes. In contrast, FGL1 treatment did not promote the proliferation of the immortalized HCC cell lines HepG2 and SMMC 7721.[50] The authors therefore suggested that the mitogenic property of FGL1 is specific for the proliferation of hepatocytes.

FGL1 and metabolic dysfunction-associated steatotic liver disease (MASLD)

MASLD is a major cause of chronic liver disease and HCC.[54,55] The initiation of the pathology manifests by steatosis consecutive to obesity and metabolic syndrome[56] that can progress toward metabolic dysfunction-associated steatohepatitis (MASH), cirrhosis, and HCC. This pathological cascade is accompanied by the secretion of liver-derived factors, such as fetuin-A, fibroblast growth factor 21 (FGF21), or angiopoietin-like protein 3 (ANGPTL3).[57] FGL1 has been proposed as a contributing factor but its role in the pathogenesis of MASLD/MASH is still unclear. Indeed, patients with MASLD presented with significantly higher FGL1 mRNA[58] and protein[59,60]

expression, suggesting that FGL1 is actively secreted in response to metabolic alterations. FGL1 expression was also elevated in the liver of patients with MASH when compared to healthy donors, of patients with cirrhosis secondary to HCV infection, and of patients with the autoimmune disease associated with primary biliary cirrhosis.[61] Conversely, data mining of published datasets (GSE83452, GSE61260, and GSE48452) showed a reduction in *FGL1* expression during the disease progression.[31] Altogether, these studies support a potential contribution of FGL1 in liver steatosis but the exact nature of this contribution is unclear. Interestingly, in line with its iron-regulatory function, BMP inhibition in the liver is presumably an aggravating factor in MASLD to fibrosis progression.[62] Moreover, BMP2 and BMP4 have also been described as regulators of adipogenesis and the BMP-antagonist erythroferrone can inhibit the BMP-SMAD signaling triggered by BMP2 in preadipocytes.[63] Further work is needed to determine whether FGL1 can contribute to steatosis progression by impairing the BMP signaling and adipogenesis.

FGL1 and murine models of MASLD

Studies in mice have yielded mixed messages. *Fgl1* mRNA expression was induced in the liver of mice fed with a high-fat diet containing 35% fat for 12 weeks[59] compared to control mice. Conversely, whole liver microarray analysis of more severe steatosis models revealed that *Fgl1* expression was reduced in the liver of mice fed with a high-fat diet (60% fat), a high-fat diet deficient in methionine and choline (45% fat) or a western diet (42% fat) for 15 weeks.[64] We speculate that these discrepancies suggest that *Fgl1* expression may be stimulated in the early phase of steatosis but that it may progressively decline proportionally to the accumulation of lipids in hepatocytes. It has also been suggested that mice deficient for *Fgl1* are more susceptible to steatosis and fibrosis when fed for 15 weeks with a high fat or methionine- and choline-deficient diet.[31] This phenotype was associated with an alteration of glucose and insulin sensitivity, dyslipidemia, increased hepatocyte apoptosis, and liver fibrosis.[31] Surprisingly, administration of a daily dose of FGL1 (1 mg/kg) for 7 days after the appearance of hepatic lesions (3 weeks MCD diet or 15 weeks HFD diet) was sufficient to mitigate the metabolic alteration, liver inflammation, and fibrosis.[31] Another study reached opposite conclusions. Indeed, a lentiviral-based strategy to overexpress FGL1 in adult C57Bl/6 mice for 14 days induced an MASLD-like phenotype characterized by increased expression of de novo lipogenesis markers (*Fasn*, *Acaca)*, accumulation of triglycerides in the liver, increased plasma concentration of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), and increased mRNA expression of the pro-inflammatory cytokines I *Il1b*, *Il6*, and *Tnfa*. Treatment of eight-week-old *ob/ob* mice with recombinant FGL1 (1 mg/kg) every day for 1 week had no effect on hepatic triglyceride content, steatosis, or ballooning.[32] Moreover, transient overexpression of FGL1 in HepG2 cells increased lipid storage through an ERK1/2-dependent pathway while the knockdown of *Fgl1* knockdown reduced FASN and ACC expression and oleic acid-induced lipid accumulation.[59] These findings are in line with observations made 48 h after partial hepatectomy by Demchev and colleagues. *Fgl1*-deficient mice exhibited significantly higher lipid accumulation in the regenerative liver, which coincides with increased de novo lipogenesis or a defect in lipid utilization. Interestingly, overexpression or knockdown of *Fgl1* in the adipose tissue respectively increased and reduced the mass of adipose tissue.[65] Ablation of *Fgl1* in specific cell types (hepatocytes or adipocytes) may help unravel its contribution to lipid metabolism during steatosis. Overall, these studies indicate that, regardless of the duration or the composition of the diet, a dietary challenge and the accumulation of fat or triglycerides in hepatocytes influence *Fgl1* mRNA expression. However, the exact mechanism, time course, and tipping points in triglycerides or lipid accumulation driving *Fgl1* transcription are unknown. Whether or not the administration of FGL1 can improve MASLD-like phenotype remains to be determined as the use of viral vectors or recombinant FGL1 has yielded contradictory results. The manipulation of FGL1 may be relevant only at a certain stage of progression from steatosis to fibrosis, which is also currently unknown.

FGL1 and cancer

In humans, the *FGL1* locus is located on chromosome 8 in the cytogenic band 8p22-21.3, which is frequently deleted in HCC as it contains an array of tumor-suppressor genes.[66] Increasing evidence indicates that *FGL1* mRNA expression or plasma concentration is increased in human cancers, such as non-small cell lung cancer (NSLC), metastatic melanoma,[67,68] gastric cancer,[69] clear cell renal cell carcinoma,[70] and laryngeal cancer.[71] This upregulation is associated with metastasis development and poor prognosis. It has therefore been proposed that the delivery of an RNA vaccine targeting FGL1 could be used for anti-tumoral vaccination.[72] On the contrary, FGL1 expression is reduced in head and neck, breast, pancreas, or liver cancer and treatment with FGL1-neutralizing antibody reduced tumor size and promoted survival.[67] FGL1 expression is also often downregulated in HCC tissues compared to healthy tissues[14,73] and a low expression of FGL1 in liver cancer patients is associated with a better survival rate.[74] FGL1 may mediate immune evasion by binding to the type I transmembrane lymphocyte activation gene 3 (LAG3, CD223) at the cell surface of T lymphocytes[67] with a dissociation constant (K_D) in the nanomolar order but the downstream signaling is still unclear. Two other studies have reported that the affinity of FGL1 for LAG3 was weak ($K_D = 6.75\ \mu M$[75] and 3.2 μM[76]) and that the effects on T cell suppression relied on the MHCII rather than on FGL1. While the dissociation constant values may vary under experimental settings, it appears that FGL1 can interact with LAG3 with a moderate to low affinity. However, the ablation of FGL1 in mice favored T cell immunity against tumors,[67] thus confirming that FGL1 plays a role in T cell-mediated tumor anti-immunity. Interestingly, knockdown of *FGL1* using antisense oligonucleotides in the hepatoma cell line HepG2 resulted in a mild increase (1.4-fold) in cell number and soft-agar colony formation[73] compared to control cells, independently of a putative effect on T cells. These findings also question the proposed mitogenic property of FGL1.[50] Decreased FGL1 acetylation in HCC may also account for elevated FGL1 protein levels and an inhibitory tumor microenvironment.[22] FGL1 may be a promising biomarker of cancer progression and a therapeutic target but its potential contribution to hematological malignancies is unknown.

CONCLUSION

Overall, FGL1 is a hepatokine with a broad range of regulatory functions but, besides iron metabolism, the exact mechanisms by which FGL1 exerts these functions or the consequences of chronic exposures to elevated levels of FGL1 are largely unknown. A major caveat is that a widely different range of recombinant FGL1 protein concentrations has been used in the literature yielding a variety of presumed effects. A possible explanation for these discrepancies is the system used to produce the recombinant protein (i.e., bacteria or eukaryotic cells) as FGL1 is subjected to posttranslational modifications and multimeric conformations, both of which differ between in vitro systems.

The conclusions of these studies are hardly comparable and the use of recombinant FGL1 therefore did not necessarily highlight the physiological functions of FGL1. However, mice deficient for *Fgl1* do not display any gross abnormalities with the exception of a mild increase in body weight and a defect in glucose tolerance and hepatic glucose production.[1,11,31] This suggests that FGL1 has little to no critical role in liver functions in physiological conditions but that it could be a mediator of liver-to-organ communication. The increase in adiposity observed in *Fgl1*-deficient mice occurs without modification of food intake or energy expenditure,[11,31] suggesting that FGL1 could act directly on the adipose tissue. Furthermore, the manipulation of FGL1 expression specifically in adipose tissue showed that the differentiation of adipocytes and the subsequent expansion of adipose tissue was regulated through an FGL1-C/EBPβ loop.[65] These findings strengthen the idea of an intracellular function for FGL1 and the putative existence of a yet-unknown receptor because LAG3 is not expressed in parenchymal cells. Another critical roadblock to studying FGL1 in humans is the lack of a validated diagnostic tool, such as ELISA. Several studies have used commercially available assays but the plasma levels of FGL1 detected in control samples were highly different, ranging from low ng/mL concentration to very high levels (mg/mL). Current data involving healthy controls or patients with pathological conditions should be interpreted with caution. The development of a specific assay to measure FGL1 plasma concentration is necessary to study FGL1 in human pathologies. As an acute phase response protein, FGL1 may be a biomarker of interest in acute inflammatory settings. More work is required to delineate the exact functions of FGL1 and its potential in clinical settings.

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AUTHOR CONTRIBUTIONS

All authors wrote the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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