Section A -Research paper

Role of Fibroblast Growth Factor 19 in Hepatocellular Carcinoma

Nahla Ibrahim EL-Attar, Rania Ahmed Ghonaim, Nahla El-sayed EL-Gammal, Tasneem Abd EL-Hamid EL-Sherief, Eman Abdelaziz

Clinical Pathology Department, Faculty of Medicine, Zagazig University, Egypt
Corresponding author: Tasneem Abd EL-Hamid EL-Sherief
Email: tasneemelsherief@gmail.com

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Abstract:

Hepatocellular carcinoma (HCC) is the fifth most common type of cancer and the third leading cause of cancer-related deaths worldwide. Liver resection or liver transplantation is the most effective therapy for HCC because drugs approved by the US Food and Drug Administration to treat patients with unresectable HCC have an unfavorable overall survival rate. Therefore, the development of biomarkers for early diagnosis and effective therapy strategies are still necessary to improve patient outcomes. Fibroblast growth factor (FGF) 19 was amplified in patients with HCC from various studies, including patients from The Cancer Genome Atlas. *FGF19* plays a syngeneic function with other signaling pathways in primary liver cancer development, such as epidermal growth factor receptor, Wnt/β-catenin, the endoplasmic reticulum–related signaling pathway, STAT3/IL-6, RAS, and extracellular signal–regulated protein kinase, among others. The current review presents a comprehensive description of the FGF19 signaling pathway involved in liver cancer development. The use of big data and bioinformatic analysis can provide useful clues for further studies of the FGF19 pathway in HCC, including its application as a biomarker, targeted therapy, and combination therapy strategies.

Keywords: Fibroblast Growth Factor 19, Hepatocellular Carcinoma.

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Introduction:

Fibroblast growth factor (FGF) are a family of growth factors, involved in variety of biological processes such as embryonic development, cell growth, morphogenesis, tissue repair, tumor growth and invasion. They possess metabolic, mitogenic, cell survival and angiogenic activities. FGFs typically bind to cell surface receptors, Fibroblast Growth Factor Receptors (FGFRs) **(1).**

Additionally, to booster their interactions with FGFRs, FGFs show high affinity for cell surface polysaccharides-glycosaminoglycan heparan sulphate. The interactions of FGF with FGFR and heparan sulphate proteoglycans are essential for

receptor dimerization, phosphorylation and subsequent signal transduction **(2).**

FGF19, an atypical member of the FGF family was initially characterized by its reduced affinity towards heparan sulphate mediated binding and unique and exclusive specificity towards FGFR4 and the trans-membrane receptor, b-klotho. To compensate for the loss of heparan sulfate-mediated high-affinity interactions observed with the receptors of other FGFs, the FGF19 subfamily members instead use single-transmembrane-containing Klotho proteins to facilitate their interactions with and activations of FGFR4 **(3).**

Reduced affinity of FGF19 towards heparin sulphate allows it to be poorly tethered to the pericellular proteoglycan and ultimately makes it free to diffuse from the extracellular matrix (ECM). This diffusion and escape from the ECM help to explain why FGF19 functions as an endocrine hormone in addition to performing paracrine or autocrine functions. As an endocrine hormone, FGF19 play regulatory roles in bile acid synthesis and homeostasis and also in glucose and lipid metabolism **(4).**

As a paracrine effector, FGF19 is involved in embryogenesis, growth, differentiation and angiogenesis while as a mediator of autocrine functions it promotes invasion and proliferation of metastatic tissues. Although FGF19 transcripts are found in brain, cartilage, skin, kidney, gall bladder and intestine, its expression is primarily found in ileum **(5).**

FGF19 is secreted into the circulation and transported back to the liver to repress the bile acid biosynthetic pathway. The endocrine regulation of hepatic bile acid metabolism by intestinal FGF19 is based on the fact that even though FGF19 is expressed in the ileum, its receptor FGFR4 is highly expressed in the liver **(1).**

Interest into FGF19 as a metabolic regulator sparked from the observation that transgenic mice expressing FGF19 showed reduced adiposity, liver triglycerides and glucose levels and increased fatty acid oxidation and improved insulin profile **(6).**

Metabolic Roles of FGF19:

FGF19 in Bile acid metabolism:

The synthesis of bile acids is a major pathway of cholesterol catabolism in mammals. Bile acid synthesis is initiated via hydroxylation of the cholesterol molecule at the 7th position, by the action of cholesterol 7α-hydroxylase (CYP7A1), which is an Endoplasmic Reticulum (ER) localized enzyme **(2).**

As in a typical feedback mediated regulation, bile acids repress their own synthesis and CYP7A1 is the enzyme in the bile acid metabolic pathway that is repressed to prevent a potentially harmful expansion of the bile acid pool. Bile acids activate the nuclear receptor, Farnesoid X Receptor (FXR). Upon activation, FXR initiates transcription of a cohort of genes that function to decrease the concentration of bile acids within the liver, where bile acids are produced **(7).**

In liver or ileum FXR also induces expression of Small Heterodimer Partner-1 (SHP-1), an atypical member of the nuclear receptor family that lacks a DNA-binding domain. SHP-1 in turn inhibits expression of Cyp7a1 by reducing the activity of Liver Receptor Homolog 1 (LRH-1), an orphan nuclear receptor that is known to regulate CYP7A1 and the consequent bile acid biosynthetic pathway positively **(3).**

Targeted disruption of the FXR gene impaired bile acid and lipid homeostasis indicating the critical role of FXR in bile acid metabolism. However, deletion of the SHP gene in mice reduced but did not completely eliminate the feedback repression of bile acid synthesis. This observation suggested existence of a SHP independent mechanism of bile acid regulation by FXR **(5).**

For years, there had been discrepancy as to why Cyp7a1 expression in the liver was inhibited in response to enteral but not intravenously administered bile acids. The puzzle was solved by the discovery of the FGF19, when it was realized that in response to enteral bile acids, the ileum secretes FGF19 that binds to FGFR4 in the liver and mediates repression of bile acid synthesis in liver via inhibition of Cyp7a1 gene transcription **(1).**

The importance of the role of FGF19 in maintaining proper bile acid homeostasis has been highlighted further by clinical studies. In humans, serum FGF19 levels and bile acid synthesis is diurnally regulated. After a meal, bile acids released into the intestine bind to and activate FXR, which then induces the expression of FGF19. The postprandial rise in serum bile acids is followed by a synchronous serum FGF19 peak that occurs at a delay of 90-180 minutes after a meal **(4).**

The study supports the view that FGF19 is secreted in the ileum in response to postprandial increase in bile acid flux. In addition, patients with primary bile acid malabsorption syndrome have reduced FGF19 production by the ileum, which is associated with increased bile acid synthesis that spills into the colon to stimulate electrolyte and water secretion **(7).**

The typical symptom of bile acid malabsorption is therefore chronic watery diarrhea, also known as Bile Acid Diarrhea (BAD). In response to bile acids FXR induces the expression of the apical sodium dependent bile acid transporter (ASBT) in the ileum, which is involved in reclamation of bile salts during the enterohepatic circulation of bile acids. In Crohn's disease and in other intestinal bowel disease (IBD), bile acid malabsorption occurs via FGF19 mediated repression of ASBT **(8).**

The upstream element, uAP1 binds a C-Jun homodimer and mediates transcriptional activation, while the downstream dAP-1 site binds a C-Jun/C-Fos heterodimer and mediates transcriptional repression. Binding of FGF19 to its receptor complex, FGFR4/b-klotho is associated with up-regulation and phosphorylation of C-Fos, which then represses ASBT promoter via binding of the dAP-1 element by a C-Jun/C-Fos heterodimer **(3).**

Patients with bile acid malabsorption are usually treated with bile acid sequestrants

such as colestyramine, colestipol or colesevalam. As an alternative treatment measure, stimulation of FGF19 by FXR agonists can be used to reverse FGF19 deficiency, which is considered one of the factors of bile acid malabsorption and the resulting BAD. However, treatment methods involving supraphysiological levels of FGF19 has always been held with skepticism because of FGF19's reputation of promoting tumors **(2).**

- **FGF19 in glucose and lipid metabolism:**

In addition to its role in hepatic and ileal bile acid homeostasis, FGF19 has been suggested to be useful in lowering serum glucose and triglyceride levels. Type 2 Diabetes mellitus occurs when the pancreas produces insufficient amount of the hormone insulin or the body's tissues and organs (such as liver) become resistant to high or normal levels of insulin **(5).**

One of the key problems in insulin signalling pathway is insufficient drug target owing to either lack of suitable ligand binding domains in the target or shared partners in other signalling pathways that regulate cell growth and differentiation. Studies undertaken to circumvent this problem led researchers to propose alternative pathways to insulin signalling for glucose metabolism **(4).**

FGF19 (in an alternative but overlapping signalling pathway from Insulin) could govern postprandial glucose metabolism in liver, raising interest and hopes about possible therapies involving this molecule. FGF19 can also improve glucose tolerance. Similar to Insulin, FGF19 can also promote protein and glycogen synthesis in liver **(1).**

FGF19 increases phosphorylation of eukaryotic Initiation Factor 4B (eIF4B) and eIF4E proteins, which are components of the eIF4F complex. The eIF4F complex mediates binding of mRNA to the ribosome and phosphorylation of these proteins (eIF4B and eIF4E) promotes initiation of translation **(6).**

FGF19 also increased phosphorylation of ribosomal subunit protein S6 (rpS6). Phosphorylation of rpS6 enhances global protein synthesis. Thus, by inducing phosphorylation of eIF4B, eIF4E and rpS6, FGF19 stimulated hepatic protein synthesis. While insulin increased phosphorylation of further downstream protein kinases AKT and p70 S6 kinase, which are known to stimulate the mTOR pathway, FGF19 increased phosphorylation of p90 ribosomal S6 kinase via activation of ERK1 and ERK2. FGF19 signalling pathway acts in parallel but independent of the insulin pathway to govern glucose metabolism in liver **(2).**

One of the important effects of insulin on intracellular metabolism is its ability to stimulate the synthesis of glycogen in muscle and liver. It does this by negatively regulating glycogen synthase kinase 3α (GSK3α) and GSK3β, which phosphorylate and inhibit the enzyme glycogen synthase (GS), the rate-limiting enzyme in the pathway of glycogen synthesis. In a fashion similar to that of insulin, FGF19 induced phosphorylation of GSK3α and GSK3β and increased glycogen synthase activity **(3).**

In the diabetic liver, there is an overproduction of glucose and atherogenic lipoproteins such as very-low-density lipoproteins (VLDLs) and small dense LDLs probably from impaired secretion of insulin, which promotes fatty acid synthesis (lipogenesis) **(7).**

However, unlike insulin, recombinant FGF19 suppressed the ability of insulin to stimulate fatty acid synthesis. Not only that, but it also suppressed the insulin induced expression of Sterol Regulatory Element Binding Protein-1c (SREBP-1c), a key transcriptional activator of lipogenic genes-the suppression being brought about without any alterations in the insulin signalling pathway **(4).**

The effect of FGF19 on lipid metabolism is somewhat controversial. FGF19 leads to significant improvements in hyperglycemia and hyperlipidemia with increased fatty acid oxidation in models of genetic and acquired insulin resistance. FGFR4 is required for bile acid regulation but not for improvement of glucose tolerance by FGF19 at a pharmacological dose **(8).**

Pharmacologically, FGF19 might be an attractive candidate in the management of diabetes; its future as a drug is currently ridden with holes. First of all, peptide-based diabetic medication was never popular owing to the complexity of measuring the correct dose as well as patient aversion to needles based approach to deliver the protein therapeutic **(1).**

It was with the discovery of the incretin peptide hormone-an intestinal factor that stimulates insulin secretion in response to glucose that a renewed interest has born for injectable peptide based drugs. Secondly, FGF19 production is normal in diabetics, raising doubts about the benefits of boosting its actions **(5).**

Finally, FGF19 production falls in response to administration of bile acid absorption inhibitors like colesevelam, which increases circulating incretins and improves tissue glucose metabolism in both the fasting and postprandial states in a manner different from other approved agents. Nevertheless, it might still be useful to find a therapeutic dose of FGF19, which is effective for treating metabolic disorders but is not in the tumorigenic range **(7).**

FGF19 in tumor and cancer induction:

FGF19 share some of its ability to regulate glucose, lipid, and energy homeostasis, with another related fibroblast growth factor, FGF 21; however, it is only FGF19 that has potential mitogenic and proliferative activity. The FGFR4-FGF19 signalling axis is important in the development and progression of hepatocellular carcinoma (HCC) in humans **(3).**

It is believed that the highly specific interaction of FGFR4 with FGF19 and no other FGFs (such as FGF21) is responsible for its proliferative effects. Investigating the structural differences between FGF19 and FGF21 showed that abolishing a N-terminal five-amino acid region (residues 38-42) in FGF19 that is important for FGFR4 activation and heparan binding completely

prevented FGF19 mediated hepatocyte proliferation **(2).**

Desnoyers group developed an anti-FGF19 monoclonal antibody (IA6) that selectively blocked the interaction of FGF19 with FGFR4. The blocked interaction effectively prevented HCC in FGF19 transgenic mice. This antibody has also been proven to be effective in all models of cancer, where FGF19 was overexpressed along with FGFR4 **(6).**

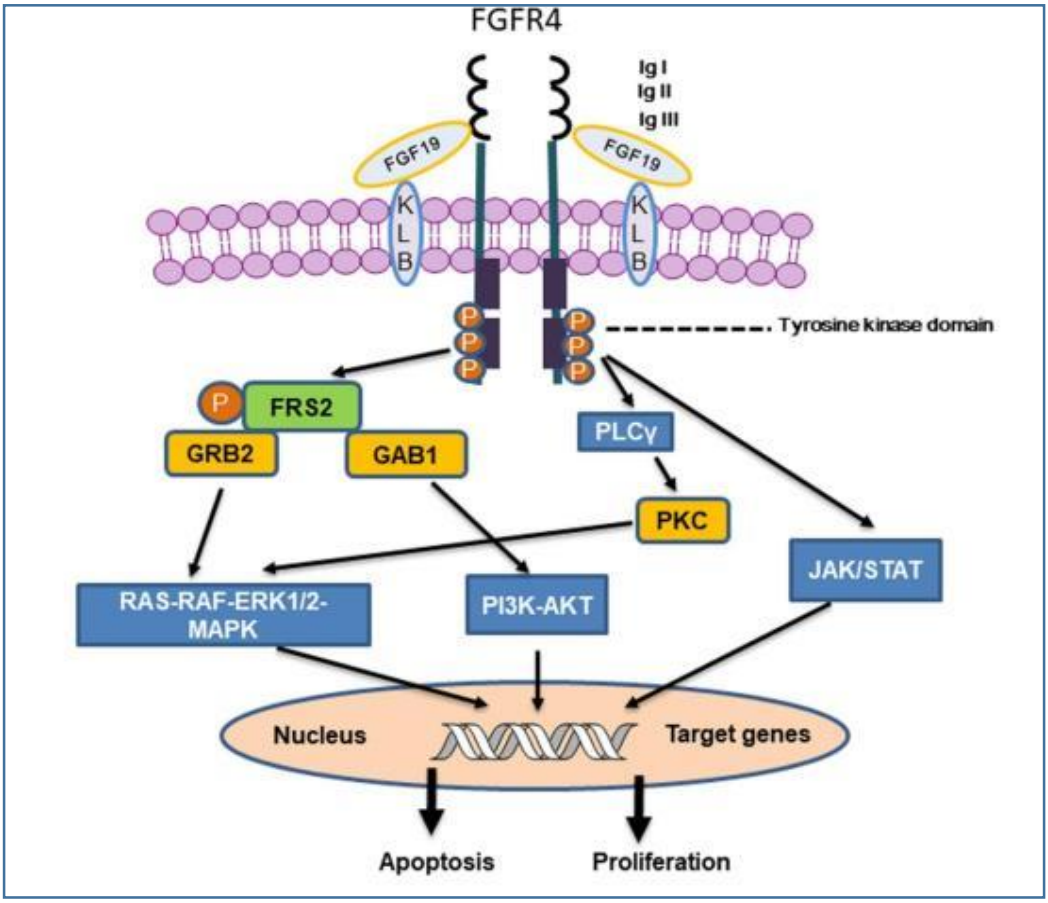

Figure (1): FGF19 in tumor and cancer induction **(9).**

For example, the antibody was effective in preventing tumor formation in colon cancer xenografts models in vivo. FGF19 is over expressed in prostate cancer or breast cancer and treatment with the antibody suppressed tumorigenesis and cancer progression in these cancer types **(4).**

The efficacy of the antibody was linked to inhibition of FGF19-dependent activation of FGFR4 and downstream targets Fibroblast Growth factor Receptor Substrate 2 (FRS2), Extracellular Signal Regulated Kinase (ERK), and β-catenin. Several studies have shown that it is not FGF19 but the overexpression of FGFR4 that is associated

with poor clinical prognosis in HCC and in prostate cancer **(1).**

Angiogenic Roles of FGF19:

FGF19 is a major growth factor expressed during retinal development and lens differentiation. FGF19 expressed in lens cells is necessary for lens fiber differentiation and survival. Knockdown of FGF19 in zebrafish embryos affects lens growth and differentiation of primary fiber cells thus reducing cell viability and increasing degeneration of lens **(8).**

In addition, FGF19 is implicated in the nasal-temporal patterning of the retina and guidance of retinal ganglion cell axons but not neuronal differentiation and lamination in the retina. FGF15 is expressed in the early stage of the neural epithelium development and in later stages in specific groups of neural cells **(7).**

The role of FGF19 in retinal development stems from the fact that it plays a role in forebrain development. Human Fgf19 is expressed preferentially in fetal brain. FGF19 also plays important roles in regulating cell division and patterning of the embryonic brain, spinal cord and the sensory organs **(5).**

FGF19 is also critical for the development of the ventral region of the telencephalon and diencephalon and is involved in the specification of Gamma-Amino Butyric Acid-GABAergic interneurons and oligodendrocytes generated in the ventral telencephalon and diencephalon. It is believed that FGF19 doesnot function alone in this process. A signalling cross talk exists between FGF19 pathway and the Hedgehog (Hh) signalling pathway, which is critical for the specification of the ventral neurons in the forebrain **(3).**

Inhibition of FGF19 functions affected cell proliferation and cell survival in the brain during mid-segmentation stages and led to a reduction in the size of the forebrain, midbrain and cerebellum at 24 hours post fertilization. FGF19 has also been implicated in inner ear development, acting in part by patterning the neuroectoderm. The inner ear develops from the otic placode, which is a patch of thickened cranial ectoderm next to the hindbrain **(4).**

Lateral expression of FGF19 in the mesoderm and neural tube with the presumptive otic placode indicates the direct involvement of FGF19 in otic development. An initiating neural signal activates the otic signalling cascade that finally leads to complex synergistic interactions between FGF19 and wnt-8c **(2).**

Fibroblast Growth Factor 19 (FGF19) and HCC:

Fibroblast growth factors (FGFs) signal through FGF receptor (FGFR) tyrosine kinases to regulate a wide range of biological processes, including cell growth, differentiation, angiogenesis, and metabolism. It is noteworthy that dysregulated FGF/FGFR signalling contributes to cancer development in many types of cancers. Fibroblast growth factor 19

(FGF19), secreted from ileum, negatively regulates bile duct acid synthesis in the liver through FGFR4 activation **(8).**

However, FGF19 production in an autocrine fashion reportedly activates FGF19/FGFR4 signalling and contributes to HCC development. It has been also demonstrated that the overexpression of FGF19 and FGFR4 is associated with unfavourable prognosis in HCC patients. These findings are mainly based on pathological studies, and whether serum FGF19 functions as a biomarker in HCC remains unclear **(1).**

Although bile acid is produced from cholesterol in the liver, hepatic bile acid synthetic levels and its secretion are tightly regulated by enterohepatic circulation. However, cholestasis and liver dysfunction increase the concentrated bile acid in both the blood and bile and induce hepatocyte injury **(3).**

Similar to HCC cells, normal hepatocytes produce FGF19 in an autocrine fashion to protect hepatocytes from the cytotoxicity of bile acid. Considering that FGF19 inhibits bile acid synthesis via the downregulation of cholesterol 7 alpha-hydroxylase (Cyp7a), a mild increase in the serum FGF19 levels of CLD patients may be responsible for the negative feedback of elevated serum bile acid levels **(7).**

Recent advances in research that has used next-generation sequencers has enabled the detection of genomic aberrations. Thus, substantial chromosomal and genetic abnormalities of the driver genes have been reported in a variety of cancers, including HCC. Among them, focal amplification of the FGF19 gene, located on chromosome locus 11q13, has been detected in 20% of all clinical HCC samples **(2).**

In contrast, immunohistochemical analyses have demonstrated that FGF19 overexpression is observed in approximately 50% of all HCC cases. These findings indicates that FGF19 overexpression in HCC tissues may not be accompanied by its copy number gain. Considering that FGF19 is a serum secretory protein produced by HCC cells in an autocrine loop fashion, we investigated the efficacy of serum FGF19 as a tumor marker **(4).**

Unlike the existing markers, FGF19 is a functional protein that is responsible for essential intracellular signal of HCC. FGF19 plays an important role in the proliferation of both, tumor cells and endothelial cells; therefore, it is believed to be promising therapeutic target molecules. In fact, anti-FGF19 antibody treatment is reported to reduce the growth of colon tumor xenografts and prevent HCC development **(1).**

Furthermore, molecular-targeted drugs for advanced HCC, including sorafenib, regorafenib, and lenvatinib, are categorized as multikinase inhibitors; FGFR4, a receptor for FGF19, is one of the most important therapeutic targets. The activation in FGF19/FGFR4 signalling contributes to sorafenib resistance; therefore, abnormal FGF19 production may be associated with the treatment effect of these drugs **(3).**

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