REVIEW

The Role of Fibroblast Growth Factor 19 in Hepatocellular Carcinoma

Zhongguang Chen,^{*} Lili Jiang,[†] Lifan Liang,[‡] Kelly Koral,[§] Qian Zhang,[¶] Lei Zhao,[¶] Songjian Lu,[‡] and Junyan Tao[§]

From the Department of Pharmacy,^{*} Central Hospital of Linyi, Yishui, Shandong, P.R. China; the Ultrasound Medical Department,[†] Jinan Central Hospital Affiliated to Shandong University, Jinan, P.R. China; the Departments of Biomedical Informatics[‡] and Pathology,[§] University of Pittsburgh, Pittsburgh, Pennsylvania; and the Department of Infectious Diseases,[¶] Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, P.R. China

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Address correspondence to
Songjian Lu, Ph.D. or Junyan
Tao, Ph.D., University of Pitts-
burgh School of Medicine, 200
Lothrop St, S-432 BST, Pitts-
burgh, PA 15261. E-mail:
songjian@pitt.edu or jut24@pitt.edu.

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 extra-cellular 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. (*Am J Pathol* 2021, 191: 1180–1192; <https://doi.org/10.1016/j.ajpath.2021.04.014>)

Hepatocellular carcinoma (HCC) is the fifth most common type of cancer and is the third leading cause of cancer-related deaths worldwide. In 2018, the World Health Organization predicted that approximately 83% of the 841,000 new patients with liver cancer are from Eastern Asia. According to the American Cancer Society, there will be 42,230 newly diagnosed cases and 30,230 deaths from HCC and intrahepatic bile duct cancer in the United States in 2021 (<https://cancerstatisticscenter.cancer.org>, last accessed February 28, 2021). Currently, the most effective therapy for HCC is liver resection or transplantation. However, recurrence after surgical resection remains a major concern. Treatment options for HCC are limited and generally ineffective.¹ Sorafenib and regorafenib are currently the most effective drugs for patients with unresectable HCC.^{2,3} Drugs recently approved by the US Food and Drug Administration, such as nivolumab, stivarga, ramucirumab, cabozantinib, and lenvatinib, the combination

of target chemotherapy, and immunity check point inhibitors are now available. However, because of limited efficacy and poor 3-month survival rates for patients with HCC taking sorafenib or regorafenib, surgical resection or liver transplantation have been preferred as curative treatments for early-stage HCC. The molecular mechanisms underlying HCC development remain poorly understood, and there is an urgent need to develop novel therapeutics against this deadly malignant neoplasm.

Among various well-known and newly discovered onco-genes related to HCC, fibroblast growth factor (FGF) 19 is overexpressed in HCC.⁴ The current review provides a detailed overview on the FGF19 signaling pathways in HCC development, including its role as a biomarker in various

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animal models as well as the potential clinical application of targeting FGF19 as a novel therapeutic option. Bioinformatics software and a data platform were used to help in the data mining of studies on the clinical relevance of *FGF19* in HCC.

FGF19 as a Biomarker in HCC

The FGF family has >20 different members, including paracrine FGF (*FGF1/2* subfamily, *FGF4/5/6*, *FGF8/17/18*, *FGF3/7/10/22*, *FGF9/16/20* subfamily), intracrine FGF (*FGF11/12/13/14* subfamily), and endocrine FGF (*FGF19/21/23* subfamily).⁵ These growth factors have a variety of functions, which include regulating metabolism, cell proliferation, differentiation, and survival. Although some studies have found that *FGF19* is also involved in hepatoblastoma⁶ and cholangiocarcinoma,⁷ this review focuses on HCC because it covers >85% of primary liver cancer.

The activity of FGF19 is predominantly fibroblast growth factor receptor (FGFR) 4-dependent, although a few studies indicate its role in an FGFR4-independent manner. Structure and function analysis shows that the N-terminal of FGF19 is important for FGFR activity and binding, and the c-terminal of FGF19 is responsible for the binding to β -klotho.

Approximately 7% of patients with HCC from The Cancer Genome Atlas (TCGA) database (25 of 353 patient samples) have a high copy number of FGF19. Patients with a higher expression of FGF19 in HCC had shorter median survival compared with a low-expression cohort (54.1 versus 108.6 months), although no significant difference was found between the two cohorts and no significant difference in the overall survival (OS) between those with high FGF19 expression and those with low FGF19 expression (<http://kmplot.com/analysis>, last accessed April 2, 2021) (Figure 1A). Male patients with alcohol risk factors have lower OS when compared with those with higher or lower FGF19 expression (20 versus 47.4 months, $P = 0.016$) (Figure 1B). However, in the nonalcoholic liver disease–related HCC cohort, patients with HCC with higher expression of FGF19 had better OS when compared with patients with HCC with lower FGF19 expression (71 versus 49.7 months, $P = 0.096$). These results indicate the dual and complicated role of FGF19 in HCC development, and point towards screening of potential risk factors before adjusting FGF19 expression for HCC treatment. These results were also verified in animal experiments in nonalcoholic fatty liver disease.⁸ Meanwhile, patients with stage I HCC with higher FGF19 expression had lower OS compared with those with lower FGF-19 expression (OS data are not available, $P = 0.049$). When compared with patients with stage I and II HCC from TCGA, higher *fgf19* expression groups had lower median OS (81.9 versus 108.6 months, $P = 0.079$). Although no correlation was found in

the advanced stage HCC group, these results point towards the use of FGF19 as an early-stage biomarker and target therapy for HCC.

Because of heterozygous and complicated risk factors of HCC development, fewer single genes could be used to predict HCC survival. However, they were sufficient to predict the outcome combined with various risk factors and molecular biology events behind the diseases progress. In addition, the diagnosis of HCC at an early stage is important for therapy outcome, and the development of new efficient biomarkers for HCC diagnosis has been the goal of bench and clinical researchers for many years. In particular, with the development of high-throughput sequencing techniques, new molecular biomarkers have been discovered from the tissue or blood of patients with HCC. One study found that the expression of FGF19 in the serum could be used as a biomarker to detect HCC and predict the treatment outcome.⁹ FGF19 is up-regulated at both the mRNA and protein levels, and can be used as a biomarker for a specific subtype of HCC, depending on the cause of the disease.¹⁰ In addition, FGF19 copy number may be an additional predictor of response to sorafenib in combination with FGF3/FGF4 amplification¹¹ because FGF-3/FGF-4 amplification serves as a predictor of response to sorafenib treatment in patients with HCC.¹²

Role of FGF19 in Tumorigenesis in Various Mouse Models

Aberrant FGF/FGFR signaling plays a role in tumorigenesis as indicated by various mouse genetic models and human genetic studies.^{13–15} FGF19 plays an important role in liver regeneration after acetaminophen-induced liver injury,¹⁶ as well as reducing liver injury in multidrug resistance 2–deficient mice.¹⁷

Eight to ten-month old FGF19 transgenic mice spontaneously develop HCC, and 2 to 4-month old transgenic mice have remarkably higher levels of 5-bromo-2'-deoxyuridine–labeled hepatocytes than those in the age-matched wild-type mice.¹⁸ Furthermore, recombinant FGF19 protein also induced a significantly higher 5-bromo-2'-deoxyuridine–labeling index in healthy mice injected with endogenous FGF19.^{18,19}

The mouse ortholog of human *FGF19* is *Fgf15*; however, these two hormones exert different effects, depending on the system being studied. Zhou et al^{17,20} found that both *Fgf15* and FGF19 hormones repressed bile acid (BA) synthesis. Because mouse FGF15 and human FGF19 share approximately 50% amino acid identity, they exhibit fundamentally different biological activities in mice. However rodent models can be used to evaluate the safety of farnesoid X receptor (FXR) activators or FGF19 inhibitors. Murine *Fgf15* lacked the protective effects characteristic of human FGF19 in db/db mice. In addition, unlike FGF19, *Fgf15* does not induce HCC in db/db, diet-induced obese, and

Figure 1 Fibroblast growth factor 19 (FGF19) amplification in liver cancer and correlation of its expression with survival ratio. **A:** No significant difference was found in the overall survival between high FGF19 expression and low FGF19 expression group [hazard ratio (HR) = 1.29; $P = 0.24$]. **B:** Patients with higher FGF19 expression had a lower survival ratio when analyzed by alcohol risk factors ($P = 0.016$). **C:** For patients with stage 1 hepatocellular carcinoma, the higher FGF19 expression group had a lower survival ratio ($P = 0.049$).

multidrug resistance 2—deficient mice models of metabolic diseases.²⁰

Humans express FGF19 in the liver and the intestine, but mice express Fgf15 only in the intestine. Livers of mice repopulated with human hepatocytes grow larger than normal in healthy mice because the human hepatocytes do not recognize Fgf15 produced in mouse intestine, resulting in promoting BA synthesis and BA pool in mice. However, in FGF19 transgenic mice, the transplanted human hepatocytes grow to normal size.²¹ These results indicate that mouse Fgf15 does not bind to human FGFR4, whereas FGF19 overexpression in mice liver is active, and FGF19 binds to mouse Fgfr4 (Z. Chen et al, unpublished data). Additional models are needed to explore the mechanisms behind these differences. Studies in translational medicine study can be used to interpret the results from mice experiments.

FGF19 Mechanisms for HCC Improvement

Under normal conditions, FGF19/15 regulates liver BA and lipid metabolism, in addition to playing a vital role in liver regeneration after partial hepatectomy or acetaminophen-induced injury in mice.¹⁶ Amplification of FGF19 stimulates hepatocellular protein synthesis and proliferation. Although the downstream signaling pathways that mediate FGF19-dependent tumorigenesis are still unclear, extracellular signal—regulated protein kinase (ERK) and β -catenin have been implicated in hepatocyte proliferation, survival, migration and invasion, as well as angiogenesis.²² Therefore, the current review provides an update of the recent studies on the FGF19 pathway in primary liver cancer.

Wnt/ β -Catenin as a Downstream Target Gene in FGF19-Amplified HCC

β -Catenin is regulated by FGF19 during epithelial-mesenchymal transition (EMT) in HCC cells,²³ and the expression of FGF19 is significantly increased and negatively associated with E-cadherin expression in HCC tissues and cell lines. Teng et al²⁴ showed that FGF19 regulates glycogen synthase kinase 3 β (GSK3 β) to activate the stress-regulated transcription factor called nuclear factor (erythroid-derived 2)—like 2 (Nrf2 or NFE2L2) in several HCC cells. Therefore, the FGFR4-GSK3 β -Nrf2 signaling cascade could be a potential therapeutic candidate for HCC treatment. However, FGF19 amplification is mutually exclusive to β -catenin and AXIN1 mutations in a subtype of patients with HCC,²⁵ demonstrating that β -catenin can play a dependent or independent role in HCC with FGF19 amplification, depending on other risk factors.

ER Stress Involved in FGF19 Amplification in HCC

Endoplasmic reticulum (ER) stress promotes tumor cell escape from immunosurveillance,²⁶ is associated with liver cancer and other liver diseases,^{27,28} and is involved in diethylnitrosamine-induced HCC in rats.²⁹ FGF19 overexpression regulates ER stress and tumor cell proliferation via activation of the FGFR4-GSK3 β -Nrf2 signaling pathway.²⁴

Stat3/IL-6 Activation in FGF19-Induced HCC

The IL-6/STAT3 axis is involved in FGF19-driven HCC in mice.³⁰ FGF19 increases IL-6 production in the liver microenvironment followed by STAT3 activation in

hepatocytes. Inhibition of STAT3, IL-6, or JAK expression abolishes FGF19-induced tumorigenesis. However, the regulatory functions of FGF19 in BA, glucose, and energy metabolism remain intact.

Activation of Ras and ERK Signaling Pathway in FGF19-Amplified HCC

Ras and ERK play key roles in HCC development³¹ and can be activated by FGF19.^{32,33} FGF19 is the upstream stimulus for the Ras-ERK-Mnk1 signaling cascade, leading to activation of phosphorylation of eukaryotic initiation factor 4B and eukaryotic initiation factors on Ser209,³⁴ which can mediate mRNA binding to the ribosome to promote translation initiation. Exogenous FGF19 also increases the phosphorylation of ERK1/ERK2 and up-regulates phosphorylation of Ser235 and Ser236 of the ribosomal protein S6 (rpS6), which is involved in protein synthesis in the liver of mice fasted overnight³⁴ (Figure 2A).

EGFR Signaling Pathway in HCC Induced by FGF19 Activation

A recent study found that the epidermal growth factor receptor (EGFR) ligand amphiregulin (AR) plays a central role in HCC proliferation, survival, and drug resistance. AR expression is frequently up-regulated in HCC tissues and cells.³⁵ Latasa et al³⁶ found that FGF19 induced AR expression through activation of β -catenin signaling in HCC, resulting in up-regulation of cyclin D1 (CCND1). These results were used to estimate the network of FGF19 and EGFR in patients with HCC in TCGA data sets (liver hepatocellular carcinoma, TCGA, PanCancer Atlas, 372 total samples) through cBioPortal (<https://www.cbioportal.org>, last accessed April 6, 2021); the signal pathway involved in FGF19-EGFR, including EGFR, FGFR4, neurotrophic receptor tyrosine kinase 1 (NTRK1), Ki-ras2 Kirsten rat sarcoma viral oncogene homolog, Ras like without CAAX 1, *Raf-1* proto-oncogene, serine/threonine kinase, and mitogen-activated protein kinase (MAPK1); and the network of FGF19 and EGFR with LENS (<https://hagrid.dbmi.pitt.edu/LENS>, April 6, 2021), a web-based tool for predicting the network of protein-protein interactions through one or two lists of genes of interest.³⁷ The results indicate that FGF19 and EGFR crosstalk through signal pathways including EGFR-RAF1-PDLIM2-FGFR4-FGF19, EGFR-ERRFI1-FGFR4-FGF19, EGFR-STAT1-FGFR4-FGF19, EGFR-(NTRK1)-PLCG1-FGFR4-FGF19, and EGFR-(NTRK1)-(MAPK1)-STAT3-FGFR4-FGF19 (only the connecting nodes are shown) (Figure 2B). FGF19 and EGFR signaling can both contribute to HCC development and progression through direct and indirect shared signaling pathways.

Bioinformatic Analysis to Determine the Secondary Hit Involved in FGF19 Amplification—Induced Primary Liver Cancer

In HCC models generated in mice using sleeping beauty transposon technology, one oncogene or tumor suppressor gene perturbation is usually not sufficient to induce liver cancer development, and additional gene changes are required to induce liver cancer. The understanding of the progression of FGF19-amplified HCC would be improved by determining the secondary hit and molecular modification events necessary for liver carcinogenesis.

Impact of FGF19 Amplification on Chromatin Accessibility

As a member of the FGF family, FGF19 may direct changes in higher-order chromatin organization.³⁸ However, direct evidence of a role of FGF19 in chromatin organization has not been found. The processed assay for transposase-accessible chromatin was downloaded using sequencing (ATAC-seq) data on tumor samples from the Xena browser, along with the copy number variation status of FGF19 in corresponding samples, to determine whether FGF19 plays a role in accessibility. Pearson correlation was captured between FGF19 copy number variation status and ATAC-seq binding signals. Some of the peaks have a strong correlation with FGF19, thereby supporting the theory that FGF19 can direct changes in chromatin organization (Figure 3A). Notably, some of the peaks were located near the same genes, such as *MYEOV*, *FGF4*, and *RP11*. Peaks with *P* values smaller than 10^{-1} were selected and merged on the gene level. Ninety nine genes were input in the chromatin immunoprecipitation sequencing (ChIP-seq) enrichment analysis³⁹ on EnrichR,⁴⁰ using the transcription factor ChIP-seq with *P* < 0.01.⁴¹ FGF19 can regulate hepatic glucose metabolism by inhibiting CBP.⁴² The relationship of FGF19 with other transcription factors, including AHR, RUNX1, and RUNX2, is also supported by the literature.^{43,44} However, this relationship could be reversed in which the transcription factors regulate FGF19 expression. For example, a master transcriptional activator, SREBP2, is found to negatively regulate FGF19 in intestinal cells.⁴⁵ Although ERG and ARNT were well studied as tumor drivers or protectors, there is no evidence of their functional relationship with FGF19. FGF19 can influence chromatin assembly, which can affect the development and progression of HCC; however, additional studies are warranted to determine the specific transcription factors involved in this process.

Finding HCC Driver Partners for FGF19

Because manipulating FGF19 alone is not sufficient to induce HCC development, modifying an additional HCC

Figure 2 Molecular mechanisms of fibroblast growth factor 19 (FGF19) in liver cancer development. **A:** Signaling pathways related to FGF19 activation in liver cancer development. Binding of FGF19 to its co-receptor β -Klotho and FGFR4, FGF19-FGFR4 activated downstream signaling, including phosphatidylinositol 3-kinase (PI3K)/AKT/mechanistic target of rapamycin (mTOR), RAS/extracellular signal-regulated protein kinase (ERK) pathway, leading to cell growth and proliferation, and had an effect on protein and glycogen synthesis, which are often activated in liver cancer. The **blue arrow** indicates activation, and the **red blunt line** indicates inhibition. **B:** The network between FGF19 and EGFR was analyzed with LENS. The nodes in the network were taken from the pathway involved in FGF19 and EGFR in the patient analysis with cBioPortal (<https://www.cbioportal.org>): EGFR-RAF1-PDLIM2-FGFR4-FGF19, EGFR-ERRF1-FGFR4-FGF19, EGFR-STAT1-FGFR4-FGF19, EGFR-(NTRK1)-PLCG1-FGFR4-FGF19, EGFR-(NTRK1)-(MAPK1)-STAT3-FGFR4-FGF19 (only the connecting nodes discussed here are shown). ABL1, ABL proto-oncogene 1; CAV1, caveolin 1; DIRAS3, DIRAS family GTPase 3; eIF4B, eukaryotic initiation factor 4B; eIF4E, eukaryotic initiation factor 4E; ERRF1, ERBB receptor feedback inhibitor 1; FRS, fibroblast growth factor receptor substrate; GAB1, GRB2 associated binding protein 1; GRB2, growth factor receptor bound protein 2; IRS1, Insulin Receptor Substrate 1; KRAS, Ki-ras2 Kirsten rat sarcoma viral oncogene homolog; MAPK1, mitogen-activated protein kinase; Mnk1, mTORC1, mammalian target of rapamycin complex 1; NTRK1, neurotrophic receptor tyrosine kinase 1; OSGIN1, oxidative stress induced growth inhibitor 1; PIK3R1, phosphoinositide-3-kinase regulatory subunit 1; PLCG1, phospholipase C gamma 1; PTPN11, protein tyrosine phosphatase non-receptor type 1; RAC, Rac family small GTPase; RAF, *Raf-1* proto-oncogene, serine/threonine kinase; RALGDS, Ral Guanine Nucleotide Dissociation Stimulator; RAP1A, Ras-related protein Rap-1A; RASA1, RAS P21 protein activator 1; RIT1, Ras like without CAAX 1; rpS6, ribosomal protein S6; RSK, resistant to spore killer; S6K, ribosomal protein S6 kinase B1; SH2B1, SH2B adaptor protein 1; SHC3, SHC adaptor protein 3; SOS, son of sevenless; THRSF, thyroid hormone responsive; TSC2, TSC complex subunit 2; UBB, ubiquitin B.

driver gene, called the driver partner of FGF19, is needed to induce oncogenesis in primary cells. For instance, overexpressing both FGF19 and Met induced HCC development in mice, which was not seen if only one of these genes was overexpressed (Z. Chen et al, unpublished data). Therefore, finding driver partners for FGF19 is crucial and needs to be addressed. If somatic genomic alterations (SGAs) of two genes/drivers are necessary or sufficient to cause HCC development, then the SGAs of these two genes should co-occur often in HCC tumors. TCGA data cohort has SGAs, including somatic mutation and copy number alteration data for approximately 360 HCC tumors,⁴⁶ which provides useful information to find driver partner candidates with high-alteration co-occurrences with FGF19. Once the genes of interest are found, then the cBioPortal is a convenient tool to check their SGA co-occurrence with FGF19.⁴⁷ However, the high SGA co-occurrences of two genes are not always related to tumor development. Because copy number alterations usually occur from DNA segment copy and paste (gene amplification) or deletion (gene deletion), SGAs of neighboring genes may co-occur often, but are usually not related to HCC development. For example, SGAs of FGF19

and CCND1 are often co-amplified in TCGA HCC tumors because they are neighboring genes on chromosome 11 (Figure 3B). Sawey et al⁴⁸ found that FGF19 and its neighbor CCND1 had high copy numbers in patients with HCC based on a clinical screening study; therefore, FGF19 and CCND1 could be an effective biomarker for patient sensitivity to anti-FGF19 treatment. However, simultaneously overexpressing FGF19 and CCND1 in mouse liver with a sleeping beauty transposon system via hydrodynamic tail vein injection⁴⁹ showed no tumor nodules at 30 weeks after tail vein injection (Z. Chen et al, unpublished data). On the basis of the current experimental results, the network between FGF19 and CCND1 was analyzed for a potential genetic link between them. First, using the network map from the cBioPortal data set, STAT3 was found as a gene linking FGF19 and CCND1. At the same time, the crosstalk between these pathways was explored by using the interactome software known as LENS. More than 30 genes were involved in CCND1 activity, but the shortest path between FGF19 and the CCND1 network was FGF19-FGFR4-STAT3-CCND1 (Figure 3C). These results indicate that STAT3 might be the second tumorigenesis hit to FGF19 or

Figure 3 Bioinformatic analysis of fibroblast growth factor 19 (FGF19) and its partner in liver cancer development. **A:** Pearson correlation between FGF19 copy number variation status and assay for transposase-accessible chromatin using sequencing binding signals analysis. The blue shaded areas around the line indicate standard deviation. **B:** FGF19 and its neighbor genes were often coamplified in The Cancer Genome Atlas hepatocellular carcinoma (HCC) tumors (the figure is generated from cBioPortal, <https://www.cbioportal.org>). **C:** Network analysis between FGF19 and cyclin D1 (CCND1) by LENS. Approximately >30 genes were involved in CCND1 activity, but the shortest path between the FGF19 and CCND1 network was FGF19-FGFR4-STAT3-CCND1. STAT3 might be the secondary hit to FGF19 tumorigenesis role in HCC development. Red circles indicate candidate; gray circles, interactor.

CCND1, and neighboring genes of FGF19 should be excluded when searching for its driver partners.

SGAs of an HCC driver affect cellular behavior by perturbing a signaling pathway, which includes the driver gene as a member. Hence, SGAs of FGF19's driver partner may not overlap significantly with those of FGF19 but overlap significantly with SGAs of another gene on the same signaling pathway, which includes FGF19 as a member. In this case, the information of SGA co-occurrences is not helpful to find the driver partners. One way to address this challenge is to search for signaling pathways in which the SGAs of the pathways and FGF19 alterations co-occur frequently. Different computational models and algorithms have been developed to search for novel signaling pathways using big omics data and TCGA data cohort.⁵⁰ The mutual exclusivity of SGAs in tumors is one of the most important properties that has been heavily relied on to search for pathways.^{51–53} Many network-based models consider functional relations or protein-protein interactions among members in the signaling pathways.⁴¹ The models that consider the causal relations between SGAs that perturb pathways and expression changes of genes regulated by the perturbed pathways are also very important in accurately identifying signaling networks.^{52,54,55} The various

techniques of finding pathways altered in HCC can greatly expand the number of possible candidates for new driver partners of FGF19.

Comprehensive Therapy Strategies in FGF19-Amplified HCC

FGF19 may be a new target for the treatment of HCC. However, large clinical trials studying FGF19 in HCC are still needed. Deep mining of clinically relevant data to determine the role of FGF19 in HCC and fully understanding the complex molecular mechanisms of FGF19 can provide new opportunities for therapeutic strategies to treat advanced HCC.

Targeting FGF19 or Its Receptor FGFR4 and Other Downstream Target Genes

The FGF19/FGFR4/ β KL signaling pathway might be used for precision medicine strategies for treatment of a subset of patients with HCC with the FGF19 amplification signature (Figure 4). As the predominant FGFR expressed in hepatocytes, FGFR4, a well-known regulon of FGF19,^{4,23,56,57} is

Figure 4 Combination therapy strategies based on the role of fibroblast growth factor 19 (FGF19) in hepatocellular carcinoma (HCC). Various risk factors, including virus (hepatitis B virus and hepatitis C virus), heavy alcohol use, nonalcoholic fatty liver disease (NASH), and aflatoxin B, can lead to HCC development. Screening of patients with HCC based on FGF19 expression level allows for patients to be separated into 2 subtypes: one group with low expression of glycogen synthase (GS)—positive (GS+) and programmed cell death 1 (PD-1) and the other group with high expression of GS-negative (GS-) and PD-1. Combination therapy strategies can then be used based on the molecular subtype by combining the FGF19 co-receptor β -Klotho and fibroblast growth factor receptor 4 (FGFR4). FGF19-FGFR4 downstream signaling inhibitor or upstream regulator inhibitors with the PD-1 inhibitor are activated in the GS- population. However, there is a need to combine with the PD-1 inhibitor in the GS+ [β -catenin (CTNNB1) activated] group. AR, amphiregulin; ATF4, activating transcription factor 4; β KL, β -klotho; ERK, extracellular signal-regulated protein kinase; FOXC1, forkhead box C1; FXR, farnesoid X receptor; PI3K, phosphatidylinositol 3-kinase; mTOR, mechanistic target of rapamycin; PD-L1, programmed cell death 1 ligand 1.

frequently overexpressed and involved in HCC development,⁵⁸ promotes EMT, and contributes to sorafenib resistance. In addition, the activation of FGFR4 alone is sufficient to induce hepatocyte proliferation^{19,59} and is required for FGF19-induced hepatocyte proliferation.⁶⁰ FGF19-induced EMT can be markedly attenuated when FGFR4 is knocked out; however, FGF19-induced EMT can not be abrogated when FGF19 is depleted in the presence of GSK3 β inhibitors.²³ This finding suggests that the FGFR4/GSK3 β / β -catenin axis may play an important role in FGF19-induced EMT in HCC cells.

In a preclinical study, anti-FGF19 completely inhibited the development of liver tumors in FGF19 transgenic mice, and the inhibition of FGF19-dependent activation of FGFR4, fibroblast growth factor receptor substrate 2, ERK, and β -catenin was related to the efficacy of the antibody in this model.⁶¹ Meanwhile, more studies have shown FGFR4 to be a potentially useful therapeutic target for HCC.⁵⁷ Knockdown of FGFR4 using siRNA was able to suppress α -fetoprotein production in the liver cancer line HuH7.⁵⁸ Another study found that anti-FGF19 or FGFR4 antibodies are useful for the treatment of HCC.⁶² In an FGF19 transgenic mouse model, blocking FGFR4 inhibited the binding between FGF19 and FGFR4 and, subsequently,

tumor development in mice.⁶³ An FGFR4-specific inhibitor, BLU9931, was developed and demonstrated remarkable antitumor activity in a xenograft HCC mice model with FGF19 overexpression. To reduce the adverse effects of an FGF19/FGFR4 inhibitor on BA synthesis, an FGFR4 targeting antibody, U3-1784, was designed to inhibit FGF19-induced carcinogenesis without affecting the synthesis of BA. This inhibitor had good anti-tumor effects on 10 different liver cancer models without invertible injury.⁶⁴ Conversely, genetic deletion of FGFR4 in mice resulted in faster progression of diethylnitrosamine-accelerated HCC, indicating that FGFR4 may suppress hepatoma proliferation.⁶⁵ Taken together, these findings indicate that the role of FGFR4 in HCC progression needs to be further investigated.

In a clinical trial study, BLU9931 had good therapeutic efficiency on one-third of patients with HCC and FGF19/FGFR4 activation.⁶⁶ A clinical relevance study found that lenvatinib could inhibit HCC development by targeting FGFR4 *in vivo* and *in vitro*, including in the FGF19 overexpressed HCC cell line Hep3B2.1-7.⁶⁷ Another phase 1 trial with a selective oral FGFR4 inhibitor, fisogatinib (BLU-554), of 25 patients found an overall response rate of 17% in FGF19-positive patients and 0% in FGF19-negative

patients. These results indicate that FGFR4 can be used as a targetable driver in FGF19-positive advanced HCC.⁶⁸

Besides FGFR4, some downstream target genes of FGF19/FGFR4 can also be used for the treatment of FGF19-amplified HCC. FGF19 regulates the activation of the small heterodimer partner (SHP)^{69,70} and may also impact mitochondrial efficiency through regulating GC-1 α .⁷¹ An additional function of FGF19 is regulating gene expression through metabolism, for example, by regulating BA through FXR and repressing PON1 expression.⁷² Increase in BA synthesis in response to high cholesterol leads to a negative feedback of FGF19 on BA secretion by inhibiting cholesterol 7 α -hydroxylase (CYP7A1).⁷³ FGF19 can correct BA signaling defects to normalize BA synthesis, the BA pool, and liver size in mice.²¹ In addition, a nontumorigenic FGF19 analogue suppresses BA synthesis in mice to protect against hepatocarcinogenesis.⁷⁴ Therefore, one of these additional signaling pathways can be targeted to treat HCC with an FGF19 amplification.

Targeting Upstream Regulators of FGF19

Another strategy to develop potential therapeutic targets for HCC would be to explore the upstream regulators of FGF19. It is well known that FGF19 is regulated by FXR in the gut-liver axis,^{75,76} which includes FXR-responsive elements in the FGF19 promoter region.⁷⁷ Besides FXR, studies have also found that activating transcription factor 4 regulates FGF19 expression in the small intestine.⁷⁸ Furthermore, additional evidence indicates that FOXC1 regulates the expression of FGF19.⁷⁹ Therefore, many upstream regulators of FGF19 can be studied to determine if any are potential targets for novel HCC treatments.

miRNAs are potential candidates for liver cancer treatment because they play an important role in liver cancer development.⁸⁰ miR-34a down-regulates the FGF19 co-receptor β KL by binding to the 3'-UTR of β KL mRNA *in vitro*. Hepatic β KL, ERK, and glycogen synthase decrease in response to adenoviral-mediated overexpression of miR-34a in mice. Therefore, the miR-34a/ β KL/FGF19 axis may present unique therapeutic targets for FGF19-related human diseases, including metabolic disorders and cancer^{81,82} (Figure 4).

Integration Therapy Strategies with Combination Therapy of Anti-PD-1 and FGF19/FGFR4 Signal Pathway Inhibitors

The efficacy of targeted therapy for patients with HCC is unsatisfactory because of the complexity of HCC.⁸³ The tyrosine kinase inhibitor sorafenib and transarterial chemoembolization are the standard treatments for unresectable HCC,⁸⁴ and limited outcomes have been found with new chemotherapy agents, such as nivolumab, stivarga, ramucirumab, cabozantinib, and lenvatinib. However, all treatment options mentioned above have only a modest effect on

HCC. In addition, many studies have found that HCC development involves modifications in various genes and signaling pathways with heterogenetic characteristics.⁸⁵ Therefore, combination therapy may be the most effective potential stratagem for the treatment of patients with HCC.⁸⁶ In addition to targeting a single gene for HCC treatment, targeting FGF19 and AR/EGFR in combination may enhance therapeutic efficacy.³⁶ With deep mining on various cell lines through miRNA and mRNA sequencing, FGF19 amplification in HCC is characteristic of a proliferation type with good response to an FGFR4 inhibitor.⁸⁷ This study⁸⁷ also found 11 of 34 cell lines (32%) with FGF19 amplification, including HCC.1.2, SNU387, SNU475, Huh7, Li7, JHH7, SNU354, SNU739, SNU761, SNU878, and SNU886, and provided molecular characteristics and potential drug response predictions. This study, along with an additional clinical review,⁸⁸ suggests choosing different treatment strategies based on the molecular subtype of HCC.

Different targeted inhibitors can be used together, including FGF19 or FGFR4 inhibitors and chemotherapy, to change tumor cell proliferation and differentiation. Gao et al⁵⁶ found that co-treatment of ponatinib and sorafenib might be an effective therapeutic approach to eradicate sorafenib-resistant HCC. In addition, blocking the FGF19-FGFR4 axis might improve the efficacy of sorafenib for the treatment of HCC.⁸⁹ Another study found that the cyclin-dependent kinase 4/6 inhibitor palbociclib, in combination with the selective FGFR4 inhibitor H3B-6527, could significantly reduce tumor development in a xenograft model of HCC.⁹⁰

Immune therapy in combination with targeting therapy to FGF19, FGFR4, and other chemotherapy might be another effective therapeutic option. Immunity plays a vital role in carcinogenesis, including HCC development. Anti-programmed cell death 1 (PD-1), and anti-programmed cell death ligand 1 (PD-L1), are some of the most studied immune therapy targets for various tumors. Ectopic FGF19 expression can promote EMT and invasion in epithelial-like HCC cells through repression of E-cadherin expression. Therefore, FGF19 can act as an EMT inducer to exert its tumor-progressing function in addition to promoting proliferation and suppressing anti-HCC immune function. Therefore, inhibiting FGF19 signaling may increase the efficacy of immunotherapy in HCC by suppressing tumor cell immune evasion.

Before selecting specific treatment modalities, molecular diagnosis of the HCC phenotype should be determined to increase treatment efficiency, especially for cold tumors with immunotherapy escape characteristics; for example, β -catenin activation leads to anti-PD-1-resistant HCC.⁹¹ In addition, a recent clinical trial found that the combined application of the PD-L1 antibody atezolizumab and the vascular endothelial growth factor antibody bevacizumab had clinical efficacy in patients with unresectable HCC.⁹² Because FGF19 can regulate β -catenin and Stat3/IL-6, it is difficult to predict the effect of combining an FGF19

inhibitor and anti-PD-1 chemotherapy on patients with HCC and FGF19 amplification (Figure 4). Meanwhile, on the basis of the FGF19 expression and OS correlation analysis from TCGA database, the group with higher FGF19 expression had lower OS in stage I HCC, no difference in stage 2 and 3 HCC, and had higher OS in stage 4 for the higher FGF19 expression HCC. Therefore, further studies are warranted to determine whether this combination strategy would be effective in the treatment of HCC.

Conclusions and Future Prospects

Under normal conditions, FGF19 functions as a postprandial hormone to regulate hepatic protein synthesis, glycogen synthesis, and gluconeogenesis, and adjusting BA homeostasis.⁹³ BAs are regulators of lipid and glucose metabolism and modulate inflammation in the liver. BA can increase the expression of FGF19 through activating FXR. As a consequence, the activated FGF19 can produce negative feedback on BA synthesis in homeostasis to prevent accumulation of toxic BA in human livers via repressing transcription of CYP7A1 in a SHP-dependent manner⁹⁴ or SHP-independent manner.⁹⁵ Meanwhile, FGF19 also stops the gallbladder from filling with bile.⁹⁴ Besides regulating the global BA production level, FGF19-FGFR4- β -klotho-negative feedback also influences BA composition. FGF19 overexpressed in mice leads to the shift of BA synthesis from the classic pathway (CYP7A1 as the rate-limiting and major regulatory enzyme) to the alternative BA synthesis pathway (cholesterol is converted to chenodeoxycholic acid). Its ortholog *Fgf15*, in the intestinal response to BA, had a similar function in mice.⁹⁶

BA and FGF19 expression has a negative feedback regulation. FGF19 has lower expression in the liver but higher expression during cholestasis, and high-level BA is associated liver cirrhosis, which will develop into HCC progression. Therefore, the precise feedback negative regulation loop might be broken under some specific conditions during HCC development, leading to later-stage HCC. Patients with the higher FGF19 expression have better OS because FGF19 can regulate BA synthesis, and results in patients with stage I HCC are not different. In addition, the feedback negative regulation loop also provides a clue on how to screen the expression of BA and FGF19 in patients with HCC before using FGF19 targeting as a therapeutic method. Several studies show that selecting a specific FGF19 variant with nontumor function might be useful when targeting FGF19-involved HCC without adverse effects on BA homeostasis when completing binding to FGFR4.^{74,97} While a specific inhibitor can decrease FGF19 expression in the liver, can nontumor functional FGF19 analog can also be used to reduce FGF19-FGFR4 activated in HCC.

Under abnormal physical conditions, careful evaluation of targetin FGF19 is important to estimate its potential

therapeutic values. Although human *FGF19* and mice *Fgf15* are identical genes, *FGF19* and *Fgf15* has big differences based on structure and molecular biology. *Fgf15* increases the hydrophilic BA pool in rodents, whereas FGF19 increases hydrophobic BA in humans. In mice, there is no FGFR4 for *Fgf15*. Such differences can provide us a chance to explore the role of FGF19 in mice, especially to evaluate the therapeutic effective and safety of FGF19 analogous agents or inhibitors in the HCC population with FGF19 amplification. Under normal conditions, FGF19 is expressed in the small intestine and at very low concentration in the liver. FGF21 is expressed in the liver. Such phenomena might be attributable to a negative regulation of FGF19 in the adult liver and up-regulated FGF19 in HCC development. Determination of whether there is any compensatory effect of tumor cell proliferation and metastasis can also provide a direction for further study.

Studies on the molecular mechanism of FGF19 amplification signature in HCC provided additional potential targets for HCC treatment, including GSK3 β and Nrf2 inhibitor,⁵⁶ autophagy, and mammalian target of rapamycin complex 1 inhibitor. In one of the HCC mice models, up-regulated FGF19 appeared to reduce tumor cell autophagy by increasing mechanistic target of rapamycin expression (Z. Chen et al, unpublished data). In addition, several studies have found that epigenetic changes are also involved in HCC development because of FGF19 amplification. In HCC, overexpression of FGF19/FGFR4 significantly correlates with epithelial cell adhesion molecular expression, which is a marker of hepatic cancer stem cells within the fatty liver—steatosis—cirrhosis—HCC sequence.⁹⁸ *SNHG16* is up-regulated and associated with poor prognosis in HCC and increases liver cancer cell proliferation via the miR-302a-3p/FGF19 axis.⁹⁹ As mentioned above, different bioinformatic analysis platforms and tools can be used to explore FGF19 tumor-driver patterns and look for potential transcription-binding factors as novel therapeutic targets. In addition, further clinical studies on the efficacy of combination therapy for the treatment of HCC are needed.

In conclusion, FGF19 can be a potential biomarker for primary HCC diagnosis and therapy response.¹¹ FGF19 and its receptor FGFR4 and the signaling pathways involved in its activation, including EGFR, wnt/ β -catenin, ERK, and Stat3/IL-6, can also be novel therapeutic targets for the treatment of HCC. Combination therapy strategies with immune check point inhibitors may be designed from the comprehensive analysis of FGF19 expression level and patterns in different HCC populations.

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Authors Contributions

Z.G.C. performed literature search and prepared the manuscript; L.L.J. prepared the section on signal pathway; L.F.L. helped perform the ChIP-seq and ATAC-seq analysis and wrote the section on the role of FGF19 amplification on chromatin accessibility; K.K. and Q.Z. revised the manuscript; L.Z. addressed comments and contributed to writing; S.J.L. wrote the section on FGF19 as HCC driver partner and critically reviewed of the manuscript; J.Y.T. collected literature data, and drafted and critically revised the of manuscript. All authors read and approved the final manuscript.

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