

GDF15: Immunomodulatory Role in Hepatocellular Carcinoma Pathogenesis and Therapeutic Implications

Yi-Ning Du¹, Jin-Wei Zhao²

¹Department of Medical Sciences, Li Ka-shing School of Medicine, University of Hong Kong, Hong Kong, People's Republic of China; ²Department of Hepatopancreatobiliary Surgery, Second Hospital of Jilin University, Jilin University, Changchun, Jilin Province, People's Republic of China

Correspondence: Jin-Wei Zhao, Department of Hepatopancreatobiliary Surgery, Second Hospital of Jilin University, Jilin University, No. 218, Ziqiang Road, Changchun, 130000, Jilin Province, People's Republic of China, Tel +8615844089385, Email zhaojinweizjwei@126.com

Abstract: Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related deaths globally and the sixth most common cancer worldwide. Evidence shows that growth differentiation factor 15 (GDF15) contributes to hepatocarcinogenesis through various mechanisms. This paper reviews the latest insights into the role of GDF15 in the development of HCC, its role in the immune microenvironment of HCC, and its molecular mechanisms in metabolic dysfunction associated steatohepatitis (MASH) and metabolic associated fatty liver disease (MAFLD)-related HCC. Additionally, as a serum biomarker for HCC, diagnostic and prognostic value of GDF15 for HCC is summarized. The article elaborates on the immunological effects of GDF15, elucidating its effects on hepatic stellate cells (HSCs), liver fibrosis, as well as its role in HCC metastasis and tumor angiogenesis, and its interactions with anticancer drugs. Based on the impact of GDF15 on the immune response in HCC, future research should identify its signaling pathways, affected immune cells, and tumor microenvironment interactions. Clinical studies correlating GDF15 levels with patient outcomes can aid personalized treatment. Additionally, exploring GDF15-targeted therapies with immunotherapies could improve anti-tumor responses and patient outcomes.

Keywords: growth differentiation factor 15, GDF15, hepatocellular carcinoma, HCC, immune suppression, immunotherapy

Introduction

GDF15, also known as macrophage inhibitory factor-1 (MIC-1), prostate-derived factor (PDF), and others, is a member of the transforming growth factor (TGF) superfamily.¹⁻³ It forms a pro-GDF15 homodimer in the endoplasmic reticulum (ER) through disulfide bond formation. After proteolytic secretion in the ER, mature homodimeric GDF15 is released from its propeptide.⁴

Other members of the GDF family, such as GDF1, GDF3, GDF5, GDF7, and GDF10 are predominantly regulators of cell growth and differentiation in embryonic and adult tissues, and even play a role in skeletal morphogenesis. Silencing of GDF1 expression significantly inhibited the GDF1-SMAD2/3 pathway, demonstrating the role of GDF1 in inhibiting gastric cancer progression.⁵ GDF3 regulates cell fate in stem cells and early embryos.⁶ GDF5 would be involved in the pathogenesis of osteoarthritis.^{7,8} GDF7 can promote the differentiation in vitro of two dorsal sensory interneuron classes.⁹

GDF15 was first identified from a cDNA library enriched for genes linked to macrophages.¹ Paralkar et al discovered it using a probe for human transforming growth factor placental (hTGF-PL), naming it GDF15.¹⁰ Its role in cancer has been suggested, showing various functions mainly involved in regulating anti-inflammatory and apoptotic pathways.¹¹

GFRAL is a specific receptor for GDF15, belonging to the GDNF family receptor α (GFR- α) group within the TGF- β superfamily.³ It's mainly found in hindbrain neurons, exclusively serving as the receptor for GDF15 in the brainstem.¹² To instigate intracellular signaling upon GDF15 activation, GFRAL necessitates binding to the co-receptor proto-oncogene tyrosine-protein kinase receptor RET, and signals through the extracellular-signal related kinase (ERK) and

AKT/protein kinase B pathways, without activating canonical TGF- β signaling pathways.^{13,14} The disparity has been attributed to the presence of TGF- β impurities in several commercially available recombinant GDF15 preparations, considering that TGF- β may induce significant biological effects within the millimolar concentration range.¹⁵

HCC is the third most prominent contributor to cancer-associated mortality worldwide.¹⁶ Data based on prevalence and population attributable scores from different regions suggest that 44% of HCC cases worldwide were attributable to chronic hepatitis B infection, with the majority of cases occurring in Asia.¹⁷ Hepatitis C was responsible for 21% of cases.¹⁷ The development of HCC is largely determined by a variety of key risk factors, including alcohol, obesity, smoking, persistent infection with hepatitis B and C viruses, and the combined effects of MASH and MAFLD transformation.^{18,19}

GDF15 is associated with obesity, aging, cardiovascular disease and progression of various cancers such as colorectal and ovarian cancers, and GDF15 can inhibit DC to promote immune escape from ovarian cancer.^{20–23} Recently, GDF15 was found to play an important role in immunosuppression in HCC.²⁴ However, little is known about the interaction between GDF15 and immune cells in the tumor microenvironment.

Some findings showed that GDF15 expression was upregulated in HCC tissues, promoting HCC cell invasion and inhibiting HCC cell apoptosis through the TGF- β /Smad pathway.^{25–27} In response to the inconsistent findings, subsequent studies have generally concluded that GDF15 promotes HCC, but does not act by acting on the receptor for TGF- β .²⁸ Overall, the inhibitory effect of GDF15 on HCC involves multiple signaling pathways, and the effect of GDF15 on promoting the development of HCC and immune escape proceeds by suppressing immune responses in the tumor microenvironment.

Role of GDF15 in Occurrence and Development of HCC

GDF15 exerts a substantial impact on various activities related to HCC, including proliferation, migration, invasion, angiogenesis, and other processes associated with carcinogenesis. Numerous recent studies have explored the involvement and underlying mechanisms of GDF15 in the carcinogenic process.

Dual Role of GDF15 in HCC Development and the Immune Microenvironment in HSC Fibrosis and MASH/MAFLD-HCC

Like other members of the GDNF receptor family, GFRAL interacts with its co-receptor RET and signals via the ERK and AKT/protein kinase B pathways.²⁸ Xu et al discovered that reducing GDF15 levels in human liver cancer stem-like cells (LCSCs) inhibited the AKT pathway. Additionally, blocking the AKT/GSK-3 β / β -catenin pathway reduced GDF15-driven proliferation, colony formation, and invasion in these cells.²⁹ Benkhei et al found through microarray analysis that over-expression of GDF15 could up-regulate epidermal growth factor receptor (EGFR), a key cytokine in Hepatitis C Virus (HCV) induction of HCC, and Dong et al discovered that GDF15 activates VEGFR during the development of HCC.^{30,31}

Yu et al found that ectopic expression of GDF15 in Hep3B cells (human HCC cell line) suppressed proliferation and induced apoptosis, thus demonstrating that GDF15 is a potential tumor suppressor gene.³² The experiment lacked exploration of inhibitory mechanisms. Then Suriben et al complemented GDF15 has been shown to translocate into mitochondria and induce ROS production and cell death in HCC cells.³³

GDF15, a key protein induced by the tumor suppressor gene p53, can inhibit tumor growth.³⁴ In 2003, Yang et al discovered that GDF15 is linked to p53 pathway activation in HCT116 human colon cancer cells, a finding also confirmed in p53-wild-type HepG2 and p53-deficient Hep3B cells.^{35,36} This was evidenced by the upregulation of p21, a cyclin-dependent kinase inhibitor that regulates cell cycle progression and senescence.³⁷

However, p21 also has a pro-tumorigenic role in HCC. Marhenke et al. found that the absence of p21 impairs hepatocyte proliferation, while p21 promotes HCC proliferation by facilitating the formation of the cell cycle protein D1-CDK4 complex.³⁸ GDF15 is known to inhibit other cancers in its early stages but promote it in later stages.^{39–41} The dual role of GDF15 in HCC development, potentially influenced by p21's dual role, is rarely reported.

GDF15 is found in both HSCs and HCC cells. HCC is commonly seen in people with fibrotic livers. HSCs act as fibroblasts that produce extracellular matrix (ECM) proteins and are key players in liver fibrosis.⁴² Myojin et al clarified

a study using a mixed culture of hepatoma cells and HSCs, where they knocked out GDF15 in LX-2 cells (a human HSC cell line). They found that without GDF15, LX-2 cells no longer promoted the growth of hepatoma cells.⁴³ This research demonstrates HSCs promote the proliferation of hepatoma cells through autophagy-dependent production of GDF15. This study shows that HSCs promote hepatoma cell proliferation through autophagy-dependent GDF15 production. Additionally, GDF15 induces collagen synthesis in HSCs, increasing liver fibrosis.^{44,45}

The study showed a significant increase in GDF15 in human HCC, with more GDF15-expressing HSCs in cancerous regions than in non-cancerous ones, indicating the role of GDF15 in promoting liver tumors.⁴³

When the liver is damaged in MASH or MAFLD, HSCs are activated and become myofibroblast-like cells.^{46,47} These activated HSCs proliferate and secrete extracellular matrix, leading to liver fibrosis and changes in liver structure.^{46,48} Overactivation of HSCs by GDF15 can worsen liver fibrosis and possibly contribute to HCC development.⁴⁹ Activated HSCs also promote tumor cell growth, migration, and invasion by secreting factors like HGF and Osteopontin.^{50,51}

In mice without GDF15, severe liver inflammation and fibrosis occur, showing GDF15 helps prevent MASH progression.⁵² In humans, GDF15 levels correlate with MAFLD severity and indicate fibrosis progression in MASH/MAFLD-HCC.⁵³ Therefore, targeting HSC activation and proliferation may offer new treatment strategies for MASH/MAFLD-HCC. HSCs promote liver fibrosis and possibly tumor development in MASH-HCC, so interventions targeting HSCs could be a new treatment approach.

The Role of GDF15 in HCC Metastasis: Angiogenesis, Metastasis Promotion, and Therapeutic Interactions

Neoangiogenesis supports tumor development, and activating the tyrosine kinase steroid receptor coactivator (Src) is necessary for this process. Treating endothelial cells with recombinant GDF15 increases Src phosphorylation.⁵⁴ Using the Src inhibitor Saracatinib reduces cell migration, tubular formation, and phosphorylation of mitogen-activated protein kinase (MAPK) Erk1/2, P38MAPK, c-Jun N-terminal kinase, AKT, and nuclear factor kappa-B (NF- κ B) in GDF15-treated cells.^{31,55} Additionally, inhibitors of AKT, P38 MAPK, ERK1/2, or JNK block GDF15-triggered Src signaling.³¹ These findings suggest GDF15 promotes angiogenesis by activating Src and its downstream signals.

In vivo experiments with nude mice showed that HCC cells damaged by chemotherapy can induce angiogenesis through GDF15.³¹ GDF15 released by these HCC cells promotes proliferation, migration, and tube formation in endothelial cells, aiding residual tumor growth after chemotherapy.

However, in a diethylnitrosamine-induced HCC mouse model, high GDF15 expression was seen in small HCC foci, but not in advanced tumors. There was no significant difference in tumor area or invasiveness between GDF15 knockout mice and wild-type mice, indicating that suppressing GDF15 did not affect tumor formation, growth, or invasiveness in this model.⁵⁵

Effects of GDF15 on multiple immune cell subsets on the promotion of neovascularization. GDF15 promotes the differentiation of neutrophil into the N2 phenotype for the production of pro-angiogenic factors.^{56,57} GDF15 promotes the secretion of multiple pro-angiogenic growth factors (VEGF, FGF and EGF), angiogenic chemokine (C-X-C motif) ligands, and angiogenesis-associated factors (TGF- β and TNF- α) after macrophage polarization towards the M2 phenotype.⁵⁸

Angiogenesis is essential for providing nutrients and oxygen to growing tumors. Drugs targeting the receptor VEGF or receptor tyrosine kinases can effectively inhibit angiogenesis is one of the main therapeutic strategies for HCC.

GDF15 and Cachexia

Cachexia, marked by severe weight loss and muscle wasting, often occurs in advanced HCC and significantly reduces life expectancy.⁵⁹ Key contributors to cachexia include inflammation, metabolic dysregulation, and anorexia.⁶⁰ The combination of anorexia and inflammation disrupts metabolism, leading to cachexia. Elevated levels of circulating GDF15 are linked to reduced food intake in cachexia patients.⁶¹

The GDF15/GFRAL pathway is key in regulating energy balance and anorexia. GDF15 reduces appetite, lean body mass (LBM), and fat mass (FM) by binding to the neuroreceptor GFRAL in the brainstem. This binding inhibits food intake, adjusts energy use, and promotes weight loss.⁶² GDF15 acts on the appetite center in the brainstem and

hypothalamus.⁶⁰ It binds specifically to GFRAL and RET, forming a receptor complex that activates intracellular signaling through phosphorylation.⁶³

The study conducted by Johnen et al provides evidence supporting the role of GDF15 in inducing tumor-related anorexia and weight loss. This effect involves the modulation of various biological factors, including hypothalamic function, ERK1, ERK2, activation of transcription-3, neuropeptide Y, and morphine-like neuropeptides.⁶³

Signaling Pathway of GDF15 in HCC

GDF15 activates the AKT/GSK-3β/β-catenin signaling pathway by inducing AKT phosphorylation, which inhibits GSK-3β. This inhibition prevents β-catenin degradation, causing its accumulation and movement into the nucleus. In the nucleus, β-catenin activates oncogenic targets like cyclin D1, myelocytomatosis (MYC), metalloproteinase-2 (MMP-2), and MMP-7, leading to epithelial-to-mesenchymal transition (EMT), tumor growth, and metastasis.²⁹ The phenomenon of EMT has been identified as a critical determinant in the metastasis and migration of HCC cells.^{64,65}

The mRNA synthesized by the HCV encodes proteins that collaborate with GDF15 to induce the activation of the downstream MAPK pathway.⁶⁶ The EGFR-ERK signaling pathway significantly impacts HCC progression.⁶⁷ High EGFR expression in HCC is linked to metastasis and lower survival rates. EGFR activation and MAPK signaling contribute to the expression of pro-inflammatory and angiogenic proteins involved in liver cancer development.

GDF15 significantly increases in HCC cells when exposed to chemotherapy or hypoxia. This upregulation, triggered by DNA damage, activates p38 MAPK, JNK, and ERK1/2 pathways, as shown in Figure 1. In the liver, JNK phosphorylation disrupts mitochondrial function, raises oxidative stress, and produces reactive oxygen species, leading

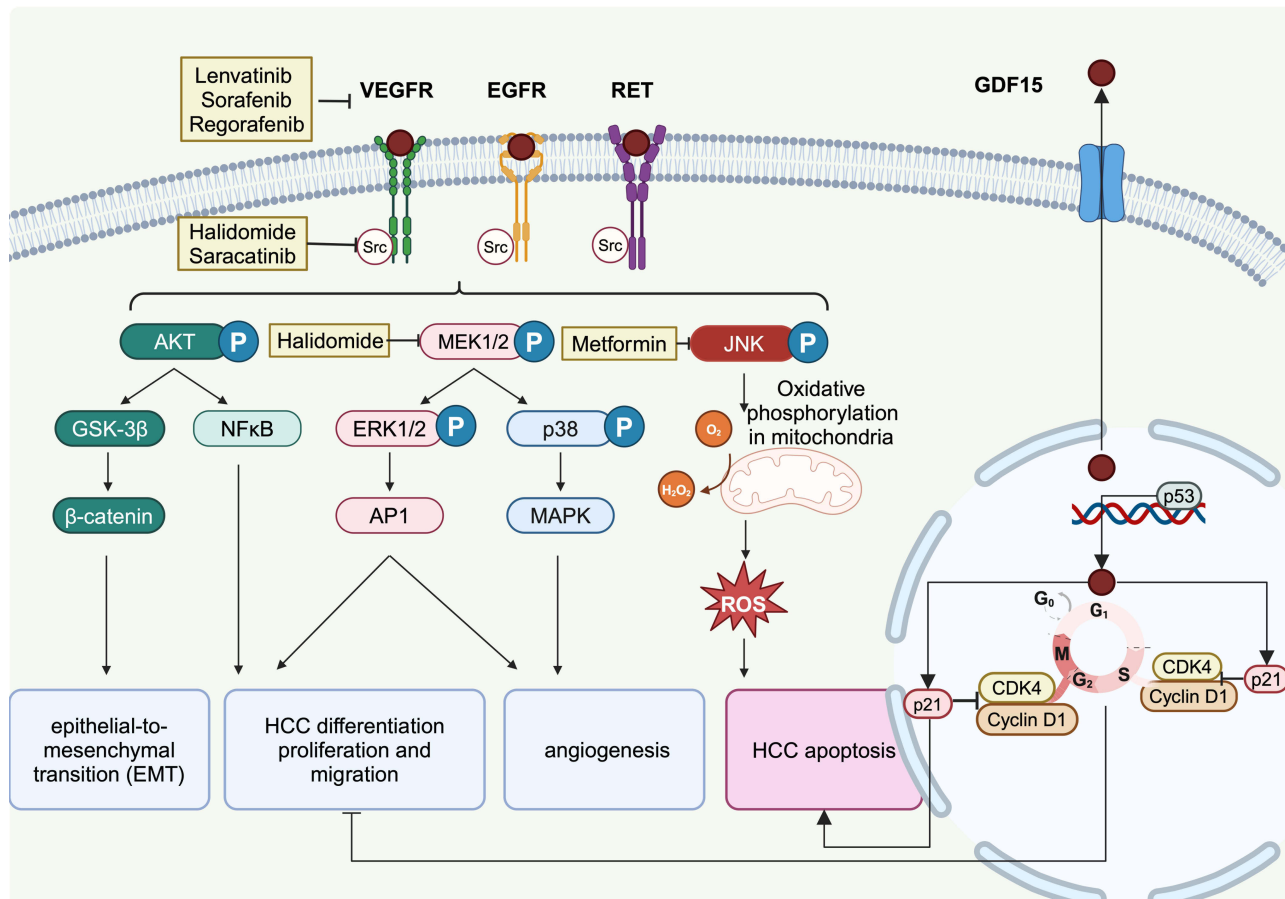


Figure 1 Signaling pathway of the GDF15 expression. GDF15 induces proliferation and pro-angiogenic effects in HCC through the phosphorylation of Src and downstream AKT, MAPK, and NF-κB signaling pathways. GDF15 activates p53 pathway to inhibit HCC. P21 promotes HCC proliferation by facilitating the formation of the cell cycle protein D1-CDK4 complex. Created with BioRender.com.

to cell death.⁶⁸ Consequently, GDF15 has been associated with an unfavorable prognosis in HCC. Urakawa et al found that introducing recombinant human GDF15 increased cell proliferation and activated Akt and ERK1/2 phosphorylation in ESCC cell lines. This suggests GDF15 may be linked to abnormal tumor growth and a poorer prognosis.⁶⁹

Tumor stem cells, known as CSCs, are specific cell groups within tumors that drive invasion, recurrence, metastasis, and resistance to treatments. HSCs play a crucial role in the tumor microenvironment by secreting ECM proteins during liver damage and inflammation, leading to fibrous tissue formation and aiding HCC progression. The interaction between HSCs and CSCs promotes cancer development through various mechanisms.⁴⁴ Furthermore, GDF15 induces the synthesis of collagen in HSCs, thereby augmenting fibrogenic processes in the liver.⁴⁵

Immunoregulatory Effect of GDF15 in HCC

The genetic elimination of GDF15 in HCC induces a transition of the immunosuppressive TME to an inflammatory state. GDF15 plays a role in promoting suppressive functionality of immune cells as illustrated in Figure 2.

Treg Cell

The harmful impact of GDF15 on HCC involves its immunosuppressive role through Treg cells. Wang et al discovered that GDF15 binds to the CD48 receptor on Treg cells, inhibiting the ERK- Activator protein 1 (AP1) pathway downstream of the T cell receptor (TCR).²⁴ This downregulates STIP1 homology and U-Box containing protein 1 (STUB1), preventing FOXP3

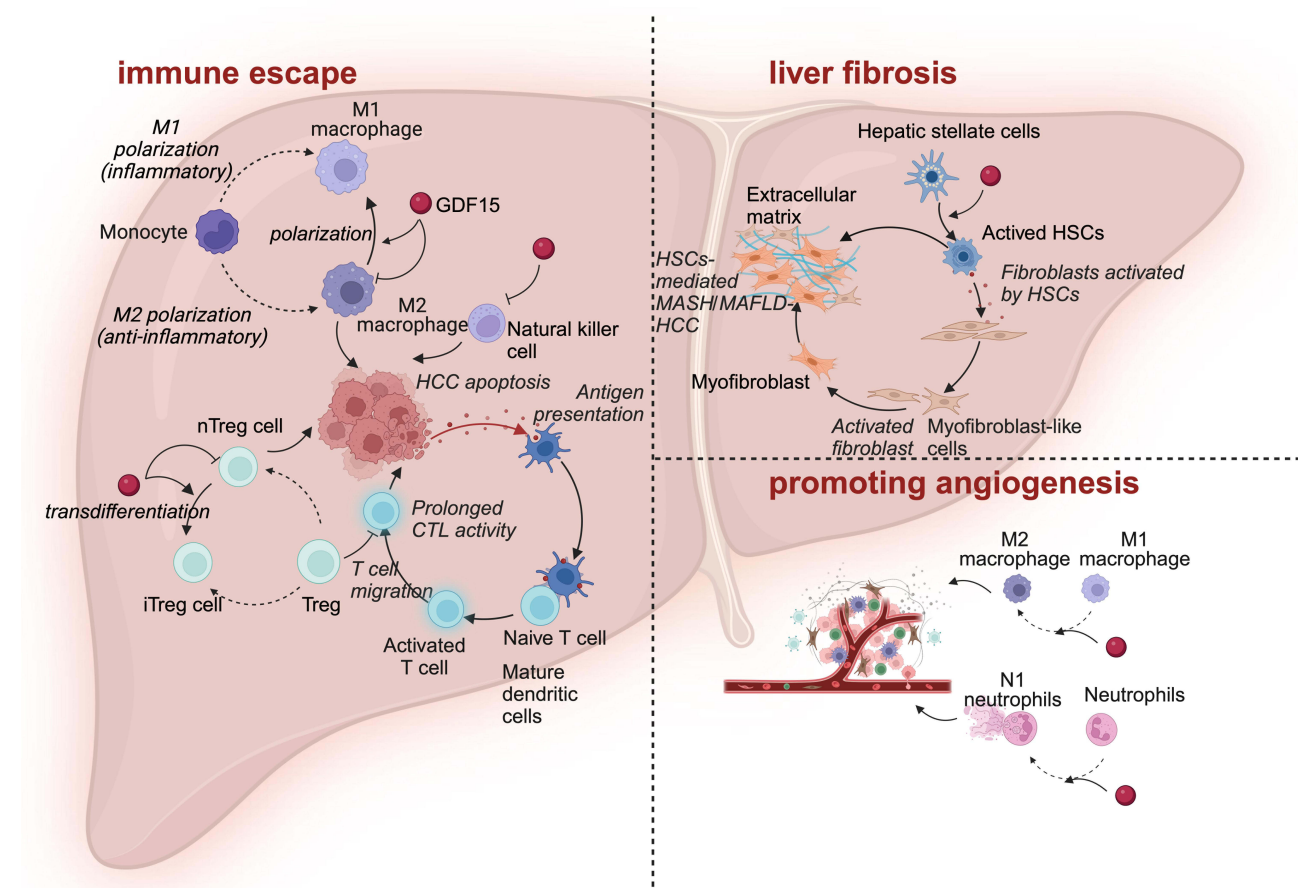


Figure 2 Effect of GDF15 on immune cells in liver cancer immune microenvironment. GDF15 promotes HCC development in the tumor microenvironment through immune escape, hepatocyte fibrosis, and tumor angiogenesis pathways. GDF15 inhibits M2-type cell action and promotes M2 to M1 conversion, inhibits nTreg-type cell action and promotes nTreg to iTreg reprogramming, inhibits DC maturation and T-cell activation, and inhibits NK cells, which diminishes the pro-apoptotic effects of immune cells and promotes immune escape. GDF15 promotes hepatocyte fibrosis through activation of hepatic stellate cells and will promote the transformation of MASH/MAFLD to HCC. GDF15 promotes the transformation of M1 to M2 and the differentiation of neutrophils into the N1 phenotype, which promotes tumour angiogenesis and exacerbates HCC. Created with BioRender.com.

polyubiquitination, leading to FOXP3 accumulation and HCC development. GDF15 also reduces phosphorylation levels of lymphocyte-specific protein tyrosine kinase (Lck) and ERK, lowers STUB1 expression, and decreases nuclear levels of cjun and c-fos proteins.²⁴ Unlike TGF- β , GDF15 enhances FOXP3 protein expression without affecting FOXP3 mRNA, enhancing nTreg function and inducing iTreg production to suppress immunosuppressive responses.

GDF15 increases FOXP3 protein expression in both mouse and human CD4⁺ T cells.⁷⁰ However, unlike TGF- β , GDF15 does not affect FOXP3 mRNA expression. Instead, GDF15 blocks FOXP3 ubiquitination, enhancing the inhibitory function of natural Treg (nTreg) cells and inducing the production of induced Treg (iTreg) cells to suppress immune responses.^{24,71}

Dendritic Cell (DC)

DCs are crucial for initiating antigen-specific immune responses. GDF15 inhibits DC maturation and function, impairing tumor-specific immune responses. Zhou et al showed that GDF15 prevents surface protrusion formation during DC maturation, reduces the expression of maturation and co-stimulatory factors, and inhibits T cell stimulation and CTL activation by DCs.⁷² This suppression leads to weaker immune responses against tumors. In ovarian cancer, GDF15 targets CD44 in DCs, decreasing their function and aiding immune escape.²³ In HBV-associated HCC, elevated GDF15 near DCs suppresses antigen presentation, contributing to recurrence and poorer prognosis, indicating the role of in immune escape in HCC.⁷³

Macrophage

Hepatic Macrophages play a crucial role in the anti-tumor response during hepatocarcinogenesis. In MAFLD, the loss of CD4⁺ T lymphocytes in the liver promotes hepatocarcinogenesis, but hepatic macrophages can prevent their interaction.⁷⁴ Tumor-derived GDF15 facilitates HCC development by inhibiting the TAK1 to NF- κ B signaling, reducing TNF and NO synthesis, and thus inhibiting macrophage activity.^{40,75} This NF- κ B/GDF15 axis is crucial in early tumorigenesis for evading immunosurveillance.

However, during HCC progression, macrophages shift from an M2 to an M1 phenotype, with M2 macrophages promoting cancer cell proliferation and invasion by suppressing the adaptive immune system. GDF15 drives macrophages towards an M2 phenotype, promoting HCC and alleviating metabolic syndrome.^{76,77} Thus, despite the double-edged role of hepatic macrophages in HCC development and progression, GDF15 always acts in the direction of promoting HCC development.

Natural Killer (NK) Cell

NK cells are crucial in viral hepatitis and HCC by killing cells and secreting cytokines like interferon.⁷⁸ GDF15 helps colon cancer cells evade NK cell surveillance by regulating their activity, in which GDF15 expression is regulated by acetylation.⁷⁹ In HBV-induced HCC, NK cells have a dual role: they are hepatoprotective and antifibrotic, but their activation can also cause liver damage.⁸⁰ NK cell-derived IFN- γ promotes HCC development through the EpCAM-EMT axis in HBV.⁸¹ However, the role of GDF15 in modulating NK cells in HCC remains unclear.

GDF15 as a Target for HCC Diagnosis, Prognosis, and Treatment

To investigate the changes in GDF15 levels at different stages of HCC development in conjunction with serum and tissue studies of clinical cases, and to investigate whether GDF15 can be used as a biomarker for HCC diagnosis as illustrated in [Table 1](#).

GDF15 is a Serum Indicator of HCC

Alpha-fetoprotein (AFP) is a common biomarker for HCC, but only 10–20% of early-stage tumors show abnormal AFP levels.⁸⁶ GDF15 has been shown to be upregulated in HCC, indicating its potential as a diagnostic or prognostic biomarker.⁸² In healthy individuals, serum GDF15 levels typically range from 0.2 to 1.2 ng/mL, with a median of 0.30 ng/mL.^{75,87} In HCC patients, GDF15 levels are significantly higher, ranging from 2551 to 3521 ng/L, compared to those without HCC or with negative biopsy results.⁸²

Table 1 Expression Level of GDF15 in Peripheral Blood of Patients with HCC

Patient Cohort	Patient Numbers	Assay Matrix	Plasma/Serum Threshold	Correlation with Clinical Outcome and GDF15	Reference
Patients with HCC	223	Serum	(6.66±0.67 ng/mL, p<0.0001)	HCC biomarker and HCC diagnosis value by combination with AFP tests.	[17]
HCC samples	40	NA	>2500 pg/mL	Prognostic value in patients with HCC.	[29]
HCC samples	NA	Plasma	Groups of HBV (range 0.07–6.31 ng/mL, mean = 1.39 ng/mL) and HCV (range 0.07–37.88 ng/mL, mean = 4.62 ng/mL)	Diagnostic value for viral hepatitis.	[75]
Patients with HCC	35	Serum	2551 to 3521 ng/l, with a mean of 3047.43 ± 323.86 ng/l	Diagnostic value for viral hepatitis.	[82]
Patients with HBV-associated HCC	110	Serum	1218.22 (641.32–2453.64) pg/mL	Diagnosis value by combination with PIVKA-II tests.	[83]
Male patients with HBV-related HCC	92	Serum	(1625.50 ± 171.83 pg/mL, P<0.001)	GDF15 levels were higher in the BCLC-C and BCLC-B patients than in the BCLC-A patients and controls (all P<0.05).	[84]
Patients with chronic HCV infection	642	Serum	(>1350 pg/mL)	Prognostic value in patients with HCC.	[85]

Serum GDF15 has been validated as a biomarker for HBV-HCC. Monitoring GDF15 along with AFP and PIVKA-II enhances HCC diagnosis sensitivity and specificity.^{83,88} Immunohistochemistry (IHC) testing shows increased GDF15 expression in moderate to severe HCC, correlating with pathological grading, but not with age, gender, or tumor node metastasis (TNM) stage.^{29,89} The Barcelona Clinic Liver Cancer (BCLC) system, the most reliable staging method for HCC, includes five stages: stage 0 (extremely early), stage A (early), stage B (intermediate), stage C (advanced), and stage D (end-stage).^{84,90} A study by Chen et al found elevated GDF15 levels in patients with BCLC-B and BCLC-C compared to healthy individuals and those with BCLC-A.⁹¹

GDF15 inhibits early prostate cancer development but promotes metastasis in later stages. It regulates GDF15 expression throughout tumor development.⁹² GDF15 is linked to the progression and poorer survival in HCC.^{82,88} Elevated GDF15 levels are found in HCV (0.07–37.88 ng/mL) and HBV (0.07–6.31 ng/mL) patients, with much higher levels in some HCC patients (13.83, 35.18, 37.21 ng/mL).⁷⁵ Therefore, GDF15 can serve as a diagnostic marker for HCC due to its significant up-regulation in infected patients.⁸⁵

The expression level of GDF15 in blood or tissues affected by HCC are detailed in [Table 1](#).

GDF15 as a Therapeutic Target for Liver Cancer Development

Given the critical role of GDF15 in HCC, inhibiting the function or expression of GDF15 may become an effective therapeutic strategy. By developing drugs targeting GDF15 or its receptor, its signaling pathway in HCC could be blocked, thereby inhibiting the growth and spread of HCC.

The main agents signaling pathways of HCC are detailed in [Table 2](#).

Lenvatinib

Lenvatinib is a tyrosine kinase inhibitor that blocks receptor dimerization and autophosphorylation, inhibiting pathways like AKT/NF-κB, MEK/ERK, and MEK/MAPK.^{93,94} GDF15 promotes angiogenesis by binding to vascular endothelial growth factor receptors (VEGFR).⁹⁵ Lenvatinib inhibits tumor angiogenesis by blocking these receptors. Phase I clinical trials in Japan, Europe, and the US have shown its effectiveness in various solid tumors.¹⁰⁵

Table 2 The Main Agents and Its Mechanisms in HCC

Anti-HCC Agents	Signaling Pathways	Effects	Reference
Thalidomide	AKT, MAPK, and NF- κ B pathway	Block Src to improve HCC prognosis	[31]
Lenvatinib	AKT/NF- κ B, MEK/ERK, and MEK/MAPK pathway	Block RTKs to inhibit HCC angiogenesis	[93–95]
Regorafenib	STAT3 pathway	Block RTKs to inhibit HCC proliferation	[96,97]
Metformin	JNK pathway	Target JNK to prevent fibrosis-associated HCC development	[45,98]
Huaier polysaccharide	AEG-I/EMT, VRGF and MAPK pathway	Reduce the protein expression of VEGF to inhibit HCC angiogenesis	[99–101]
Quisinosat	MEK pathway	Target CXCR4 to inhibit fibrosis-associated HCC development	[102]
MBL	ERK/ COX-2/ PGE2 pathway.	Target ERK to inhibit HCC-induced HSC activation	[103,104]

A Phase I trial of Lenvatinib (12 mg/day) for HCC in 46 patients showed a median time to progression of 7.4 months, median overall survival of 18.7 months, a response rate of 37%, and a disease control rate of 78%.¹⁰⁶ These significant results in metastatic HCC led to a Phase III trial comparing Lenvatinib with Sorafenib, the first tyrosine kinase inhibitor (TKI) for metastatic HCC.^{107,108} Lenvatinib was found to be superior in overall survival, resulting in its approval as a frontline therapy for advanced HCC due to its effectiveness in blocking abnormal signal pathways.⁹⁴

TKI resistance is a major challenge in advanced HCC management. Most patients on Sorafenib develop resistance within 6 months.¹⁰⁹ Although Lenvatinib is as effective as Sorafenib for overall survival, acquired resistance still limits its efficacy. Targeting lipid metabolism pathways is a promising strategy to overcome treatment resistance in HCC.¹¹⁰

Regorafenib

Regorafenib is an oral multikinase inhibitor similar to sorafenib, targeting kinases involved in angiogenesis and tumor growth. It enhances anti-tumor immunity by regulating macrophages and increasing CD8+ T cell activity, switching macrophages from a tumor-promoting M2 phenotype to a tumor-inhibiting M1 phenotype, thereby blocking the role of GDF15 in HCC proliferation.¹¹¹ Regorafenib also inhibits the STAT3 pathway downstream of GDF15, enhances NK cell activity by upregulating NKG2D ligands, and helps NK cells recognize and kill HCC cells, countering the function of GDF15 in promoting immune escape.^{96,97}

Metformin in Combination with Transarterial Chemoembolization (TACE)

TACE is a key treatment for inoperable HCC. Metformin significantly reduces GDF15 production in HCC cells after treatment by targeting JNK, thereby decreasing fibrosis and GDF15 expression.^{45,98} This counteracts treatment-induced fibrosis and cirrhosis. Additionally, TACE-induced damage can increase GDF15, promoting tumor angiogenesis, which thalidomide can mitigate by inhibiting AKT, MAPK, and NF- κ B signaling via Src inhibition.³¹ Combining thalidomide with TACE improves HCC prognosis, offering better therapeutic outcomes.

Huaier Polysaccharide (HP)

The poor prognosis and high mortality in HCC result mainly from its tendency to invade and metastasize. Inhibiting these processes effectively can reduce fatality rates. Factors like CSC and EMT contribute to tumor recurrence and metastasis.¹¹² Recent research suggests that LCSCs play a crucial role in HCC relapse and metastasis due to their increased tumorigenicity, metastatic potential, and chemotherapy resistance.¹¹³ Notably, HP has been identified as a substance with anti-invasive and metastatic properties, demonstrated in experiments on human HCC cells. HP's

efficacy in impeding HCC progression and metastasis is attributed to its regulatory impact on the astrocyte elevated gene-1 (AEG-1)/EMT and MAPK pathways.^{99–101}

Potential Therapeutic Targets for HCC

Quisinostat, a novel chemotherapy for HCC, shows promising antitumor activity both *in vitro* and *in vivo*, especially when combined with sorafenib.¹¹⁴ Sorafenib/MEK inhibitor-loaded nanoparticles targeting C-X-C-Motif receptor 4 (CXCR4) effectively suppress hepatic fibrosis and prevent fibrosis-associated HCC development and liver metastasis.¹⁰²

Mannan binding lectin (MBL) interacts with HSCs, inhibiting HCC-induced HSC activation by downregulating the ERK/cyclooxygenase-2(COX-2)/ prostaglandin E2 (PGE2) pathway.^{103,104} Resveratrol inhibits orthotopic HCC tumor growth and reduces the frequency of CD8+CD122+Tregs and M2-like macrophages.¹¹⁵ CD48 is identified as the primary receptor for GDF15 in the immune system, offering a pathway for therapeutic inhibition of GDF15 to achieve successful HCC clearance with minimal side effects.²⁴

Conclusion

This review focuses primarily on the role of GDF15 in the tumor immune microenvironment. GDF15 interacts with its receptor CD48, playing a crucial role in the immune response in cancer and other diseases. Simultaneously, GDF15 directly interacts with immune cells in the tumor microenvironment, primarily through the following mechanisms: disrupting DC activation and maturation; reducing T cell induction and activation by DCs; blocking immune cell extravasation and tumor infiltration; inhibiting T and NK cell cytotoxicity against tumor cells; inducing immune suppression by generating and enhancing regulatory T cell function in tumor tissues. Additionally, in the process of transition from MASH and MAFLD to HCC, GDF15 promotes the occurrence and development of HCC, suggesting that GDF15 may become a new target for anti-tumor immunity. Increasing clinical evidence suggests that serum GDF15 levels can be used for the diagnosis and prognosis of HCC, but substantial clinical data are still needed for confirmation.

Currently, drug research mainly focuses on Lenvatinib and Sorafenib, which act by inhibiting receptor tyrosine kinases (RTKs) to block downstream tumor angiogenesis. However, there is a need for further development of targeted drugs for HCC progression, as GDF15 is involved in the entire process of HCC development, with high GDF15 expression promoting HCC progression and poor prognosis. Interestingly, previous research has highlighted the potential of targeting GDF15 for the treatment of HCC. Therefore, GDF15 provides a new avenue for HCC treatment.

Abbreviations

AEG-1, astrocyte elevated gene-1; AFP, alpha-fetoprotein; AP1, Activator protein 1; BCLC, Barcelona Clinic Liver Cancer; CDK, cyclin-dependent kinases; COX-2, cyclooxygenase-2; CSCs, cancer stem cells; CTL, cytotoxic T-lymphocyte; CXCR4, chemokine C-X-C-motif receptor 4; DC, dendritic cells; EGFR, epidermal growth factor receptor; ECM, extracellular matrix; EMT, epithelial-to-mesenchymal transition; ER, endoplasmic reticulum; ERK, extracellular-signal related kinase; GDF15, growth differentiation factor 15; GFR α -1, GDNF family receptor α -1; GSK, glycogen synthase kinase; HBV-HCC, hepatitis B virus associated hepatocellular carcinoma; HCC, hepatocellular carcinoma; HCV, Hepatitis C Virus; HP, Huaier polysaccharide; HSCs, Hepatic stellate cells; IFN- γ , Interferon- γ ; IHC, Immunohistochemistry; iTreg, induced Treg; JNK, c-Jun N-terminal kinase; LBM, lean body mass; Lck, lymphocyte-specific protein tyrosine kinase; LCSCs, liver cancer stem-like cells; MAPK, mitogen-activated protein kinases; MASH, metabolic dysfunction associated steatohepatitis; MASLD, metabolic dysfunction associated fatty liver disease; MBL, mannan binding lectin; MIC-1, macrophage inhibitory factor-1; MMP-2, matrix metalloproteinase-2; MYC, myelocytomatosis; NAG-1, nonsteroidal anti-inflammatory drugs activity gene-1; NF- κ B, nuclear factor kappa-B; NK, Natural killer; NKG2D, natural killer cell group 2D; NKG2DL, natural killer cell group 2D ligands; NO, Nitric oxide; NSAID, nonsteroidal anti-inflammatory drug; nTreg, natural Treg; PDF, prostate-derived factor; PGE2, prostaglandin E2; PIVKA-II, protein induced by vitamin K absence or antagonist-II; PTGF- β , placental transforming growth factor- β ; RTKs, receptor tyrosine kinases; Src, steroid receptor coactivator; STUB1, STIP1 homology and U-Box containing protein 1; TACE, transarterial chemoembolization; TAK1, TGF- β -activated kinase; TGF, transforming growth factor;

TKI, tyrosine kinase inhibitor; TME, tumor microenvironment; TNF, tumor necrosis factor; TNM, tumor node metastasis; VEGFR, vascular endothelial growth factor receptors.

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Disclosure

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