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Review Article

Targeting Glypican-3 for Liver Cancer Therapy: Clinical Applications and Detection Methods

Jin Zhang[1,2], Rong Li[1], Xueqin Tan[1] and Chuang Wang[2,3*]

[1]North Sichuan Medical College, Nanchong, Sichuan, China; [2]Chengdu Yunce Medical Biotechnology Co., Ltd., Chengdu, Sichuan, China; [3]Laboratory of Human Disease and Immunotherapies, West China Hospital, Sichuan University, Chengdu, Sichuan, China

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Abstract

Recent advancements in cancer immunotherapy have highlighted glypican-3 (GPC3) as a prominent target for treating hepatocellular carcinoma (HCC). However, approximately 10% to 30% of HCC patients exhibit low or absent GPC3 expression on the surface of tumor cells, which limits the feasibility of GPC3-targeted therapies. Consequently, it is essential for patients to undergo pre-diagnostic assessments of GPC3 expression in tumor cells to evaluate their suitability for GPC3-directed therapy. Although various methods have been developed to specifically detect GPC3 as a biomarker for treatment and prognosis, the diagnostic approaches currently employed in clinical studies remain relatively limited. Here, we provide a comprehensive overview of the clinical development of GPC3-targeted therapeutics, clinical trials in GPC3-positive HCC, and current methods for detecting GPC3 expression, highlighting their advantages and limitations. Furthermore, we explore the potential of integrating targeted therapy with various GPC3 detection modalities tailored to different pathological stages. This integration not only provides insights into the selection of effective methods for detecting GPC3 expression but also has the potential to significantly improve the clinical outcomes of patients with liver cancer. By simultaneously assessing the advantages and disadvantages of these methods, this review aims to establish a theoretical foundation for the clinical selection of appropriate GPC3 detection strategies for targeted therapy.

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Introduction

Hepatocellular carcinoma (HCC) is characterized by high incidence and mortality rates worldwide, with particularly significant patient populations in Asia.[1,2] Currently, surgical intervention remains the first-line treatment for HCC. Additional therapeutic options include radiofrequency ablation and transarterial chemoembolization.[3] However, the 5-year recurrence rates for patients with HCC after surgery are about 50% to 70%.[4] In addition to surgical options, molecular targeted agents (e.g., sorafenib, lenvatinib, regorafenib, ramucirumab, and cabozantinib) are first-line or second-line systemic treatments for advanced HCC.[5–7] However, drug resistance remains a severe problem for these therapeutics.[8] Natural agents such as celastrol and berbamine may enhance the sensitivity of sorafenib.[8,9] Furthermore, artesunate derivatives have shown anti-HCC activity.[10] Artemisinin synergizes with rhein to inhibit HCC.[11] Early detection of HCC is often challenging due to its occult onset, which is frequently associated with varying degrees of liver cirrhosis and chronic hepatitis.[12,13] Additionally, the sensitivity of existing diagnostic methods, combined with patients' adherence to and awareness of the importance of regular health check-ups, complicates early diagnosis and timely treatment of HCC.[14] Nevertheless, early diagnosis and intervention can substantially increase patient survival rates.[12] Therefore, developing sensitive and efficient detection methods is crucial for improving cure rates and overall survival outcomes.

In addition to surgical treatment and chemotherapy, tumor-targeted immunotherapy has provided new options and renewed hope for patients with HCC, particularly those who have not responded to chemotherapy. Immunotherapy involves targeting specific antigens to activate the antitumor immune response, thereby attenuating the ability of tumor cells to evade attacks from the immune system and modulating the tumor microenvironment.[15] Currently, a range of immunotherapeutic modalities targeting HCC have been developed, including immune checkpoint inhibitors,[16] oncolytic viruses,[17] tumor vaccines,[18] antibody–drug conjugates,[19] and adoptive cell transfer therapies.[20] Furthermore, to optimize the application of immunotherapy in treating HCC, exploring specific antigens and developing diagnostic tools will facilitate early screening and increase cure rates.[21]

Glypican-3 (GPC3) is an oncofetal proteoglycan that accumulates on the surface of tumor cells and is specifically upregulated in HCC patients. Elevated expression of GPC3 is strongly associated with poor prognosis in HCC. GPC3 is also detectable in the serum of HCC patients, whereas its concentration in normal tissues and serum is negligible.[21] The majority of studies and several meta-analyses indicate that serum GPC3 concentrations are increased in HCC patients

Keywords: Glypican-3; GPC3; Hepatocellular carcinoma; Immunotherapy; Detection; Clinical application.

***Correspondence to**: Chuang Wang, Laboratory of Human Disease and Immunotherapies, West China Hospital, Sichuan University, Chengdu, Sichuan 611130, China. ORCID: https://orcid.org/0009-0001-5746-7942, Tel: +86-13281256620, E-mail: wangchuang0012@163.com.

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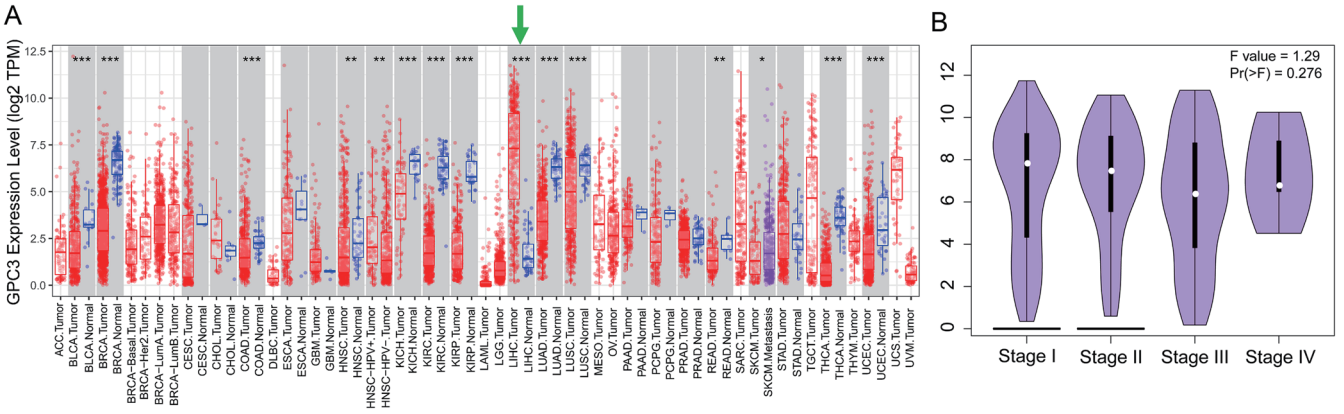

Fig. 1. GPC3 expression across different tumors and pathological stages of HCC. (A) TIMER2.0 was used to analyze the differences in GPC3 expression between various tumors and normal tissues. (B) GPC3 expression in different pathological stages of HCC. ACC, adenoid cystic carcinoma; BLCA, bladder cancer; BRCA, breast cancer; CESC, cervical squamous cell carcinoma; CHOL, cholangiocarcinoma; COAD, colon adenocarcinoma; DLBC, diffuse large B-cell lymphoma; ESCA, esophageal carcinoma; GBM, glioblastoma multiforme; GPC3, glypican-3; HCC, hepatocellular carcinoma; Her2, human epidermal growth factor receptor 2; HNSC, head and neck squamous cell carcinoma; HPV, human papillomavirus; KICH, kidney chromophobe; KIRC, kidney renal clear cell carcinoma; KIRP, kidney renal papillary cell carcinoma; LAML, acute myeloid leukemia; LGG, brain lower grade glioma; LIHC, liver hepatocellular carcinoma; LUAD, lung adenocarcinoma; LumA, luminal A; LumB, luminal B; LUSC, lung squamous cell carcinoma; MESO, mesothelioma; OV, ovarian serous cystadenocarcinoma; PAAD, pancreatic adenocarcinoma; PCPG, pheochromocytoma and paraganglioma; PRAD, prostate adenocarcinoma; READ, rectum adenocarcinoma; SARC, sarcoma; SKCM, skin cutaneous melanoma; STAD, stomach adenocarcinoma; TGCT, testicular germ cell tumors; THCA, thyroid carcinoma; THYM, thymoma; UCEC, uterine corpus endometrial carcinoma; UCS, uterine carcinosarcoma; UVM, uveal melanoma.

and significantly greater than those reported in healthy controls and patients with liver cirrhosis or chronic hepatitis.[22–25] GPC3 promotes HCC cell growth, proliferation, and differentiation by activating key signaling pathways such as Wnt/β-catenin, Hedgehog, fibroblast growth factor, and transforming growth factor-beta. Given its critical role in tumor progression and its specific expression in HCC, GPC3 has emerged as an attractive therapeutic target. Targeting GPC3 has shown potential in inhibiting tumor growth and improving patient survival, making it a promising focus for HCC therapy. Compared with other markers for HCC, GPC3 can be detected in more than 70% of HCC patients and is markedly upregulated.[26] The positive rate of GPC3 protein levels in the serum of HCC patients ranges from 36.1% to 95.0%, whereas the positive rate for *GPC3* mRNA is approximately 28% to 100%.[27] Notably, variations and fluctuations in GPC3 levels in the serum of different patients can arise from factors such as detection methods, antibody affinity, and false positivity. Hence, this review begins by providing a comprehensive overview of current clinical studies related to GPC3-targeted therapies, including the diagnostic methods and inclusion criteria employed. Furthermore, it consolidates existing detection techniques for evaluating GPC3 expression in HCC patients, incorporating recent findings from preclinical research. By simultaneously assessing the advantages and disadvantages of these methods, this review aims to establish a theoretical foundation for the clinical selection of appropriate GPC3 detection strategies for targeted therapy.

The role of GPC3 in HCC

The glypican family, comprising six members (glypican-1, glypican-2, GPC3, glypican-4, glypican-5, and glypican-6), is a key part of the heparan sulfate (HS) proteoglycan family. These proteins are anchored to the cell membrane via glycosylphosphatidylinositol and play a role in the extracellular matrix. Among the glypican family members, GPC3 is particularly noteworthy due to its high specificity for HCC. In HCC patients, GPC3 is expressed on the tumor cell surface and can be secreted into serum.[28] Using the TIMER2.0 database,[29] we analyzed GPC3 expression patterns across tumor and normal tissues, revealing statistically significant upregulation in liver HCC (Fig. 1A). Notably, GPC3 expression remains relatively stable across all pathological stages of HCC (Fig. 1B). In-depth research has found that multiple transcription factors are involved in regulating the expression of GPC3 in liver cancer cells. The transcription factor c-Myc enhances GPC3 transcription by binding to its promoter region, thereby increasing the expression of GPC3 at both the mRNA and protein levels.[30] In contrast, ZHX2 binds to the core promoter of *GPC3* and suppresses its transcription in a dose-dependent manner.[31] Interestingly, the hypoxia-inducible factor 1α can promote the extracellular secretion of GPC3.[32] In addition, some miRNAs can directly or indirectly regulate GPC3 expression in HCC. miR-96 and miR-1271 can directly inhibit GPC3 expression, whereas miR-129-1-3p, miR-1291, and miR-1303 promote GPC3 expression.[33] miR-1291 indirectly promotes GPC3 expression by downregulating inositol-requiring enzyme 1α (IRE1α).[34] IRE1α is an endoplasmic reticulum stress sensor that has both kinase and riboendonuclease activities.[35] *GPC3* mRNA can be directly targeted and cleaved by IRE1α.[34]

The molecular weight of GPC3 protein is approximately 70 kDa.[36] Membrane-bound GPC3 plays a critical role in regulating various cellular biological processes, including proliferation, differentiation, and metastasis.[37–39] Studies on zebrafish have demonstrated that GPC3 is cleaved by a furin-like convertase between Arg358 and Ser359, resulting in a 40 kDa N-terminal subunit and a 30 kDa C-terminal subunit, which are linked by disulfide bonds (Fig. 2).[24,36] Furthermore, the C-terminal substructure comprises two HS side chains that are capable of interacting with other regulatory proteins.[40] Interestingly, evidence suggests that furin-dependent GPC3 cleaved domains could serve as both a powerful tool for detecting early-stage HCC and a predictor of HCC prognosis.[41] Additionally, various soluble forms of GPC3 protein have been detected in both cell culture supernatants and serum from HCC patients, indicating that membrane-bound GPC3 may undergo proteolytic cleavage under physiological conditions.[23,25,42,43] However, the mechanism of GPC3 protein

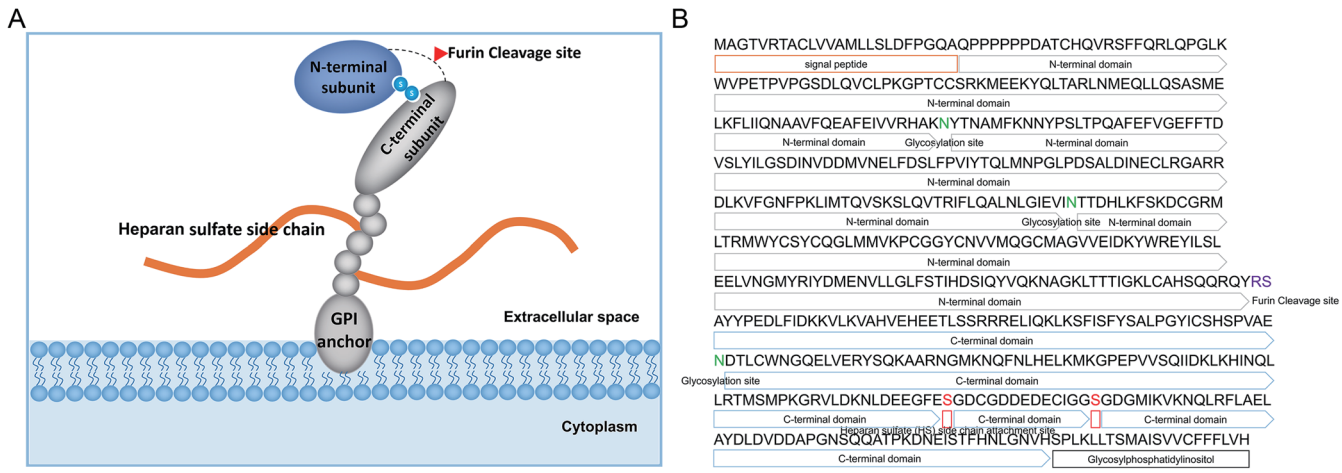

Fig. 2. Schematic diagram of the GPC3 protein structure on the cell membrane. (A) The membrane-bound GPC3 protein is composed of an N-terminal subunit and a C-terminal subunit. The C-terminal subunit also comprises two heparan sulfate side chains and is embedded in the cell membrane through GPI. (B) The amino acid sequences corresponding to each domain of the GPC3 protein. GPC3, glypican-3; GPI, Glycosylphosphatidylinositol.

cleavage has not been fully elucidated.

Studies indicate that varying expression levels of the GPC3 protein are associated with poor prognosis in HCC patients. Importantly, GPC3 knockout significantly inhibits HCC invasion and metastasis.[44] GPC3 promotes HCC progression through various signaling pathways (Fig. 3). The Wnt signaling pathway is a primary route by which GPC3 promotes HCC progression.[45,46] Upon binding of GPC3's HS and core protein to FRIZZLED and LRP5/6, Wnt signaling is activated and relayed into the cytoplasm, leading to β-catenin accumulation, which is subsequently transported to the cell nucleus to initiate the expression of genes associated with cell proliferation and survival, thereby facilitating HCC development.[47–49] In this context, elevated levels of GPC3 function as coreceptor storage proteins that enhance the interaction between Wnt and FZD/LRP5/6. Furthermore, GPC3 expression is closely correlated with β-catenin nuclear localization, indicating that GPC3 activates the Wnt signaling cascade.[45,50] Similarly, growth factor-related pathways promote HCC cell metastasis through GPC3 interaction. Mechanistically, HS chains facilitate binding of hepatocyte growth factor and fibroblast growth factor, which subsequently activate MAPK or PI3K/AKT/mTOR signaling pathways to enhance HCC cell migration and invasion.[51–53]

On the one hand, macrophages are prevalent immune cells within the microenvironment of solid tumors and are commonly referred to as tumor-associated macrophages (TAMs).[54,55] Studies indicate that GPC3 expression significantly enhances the migration of TAMs to HCC tissues;[24,56,57] however, a predominant proportion of these macrophages are M2-polarized TAMs (Fig. 3). Elevated GPC3 levels are hypothesized to facilitate the aggregation of M2-polarized TAMs, thereby promoting HCC invasion and metastasis.[58,59] Moreover, microarray and quantitative PCR analyses have demonstrated that GPC3 overexpression in HCC cells induces upregulation of chemokines CCL3, CCL5, and CSF1, which have been shown to recruit macrophages and promote their polarization towards the M2 phenotype.[56] GPC3 interacts with pyruvate kinase M2, a mechanism that drives enhanced glycolysis, oxidative phosphorylation, and ATP generation. Such metabolic reprogramming fosters a favorable microenvironment within tumors, supporting both the persistence of M2 macrophages and their differentiation into a pro-tumorigenic phenotype (Fig. 3).[60] On the other hand, epithelial-mesenchymal transition (EMT) represents a critical event in mediating the invasiveness of HCC.[61] The downregulation of E-cadherin is a significant feature of EMT-driven HCC migration.[62,63] In the HepG2 cell line, E-cadherin expression has been reported to be negatively correlated with GPC3 levels,[32,51] further supporting the association between elevated GPC3 expression and poor prognosis in HCC. A study focusing on HepG2 cells revealed that GPC3 expression inversely correlates with E-cadherin levels. Silencing GPC3 resulted in decreased expression of Snail, Slug, MMP-2, and MMP-9—proteins associated with EMT—suggesting that GPC3 overexpression facilitates EMT, as well as metastasis and invasion.[64]

Although alpha-fetoprotein (AFP) has been utilized for screening HCC patients through routine serum testing since the 1980s,[65] elevated levels of GPC3 can be detected in both liver cancer tissues and the serum of AFP-negative primary liver cancer patients.[66–68] Notably, studies have demonstrated that when HCC tumors are less than 3 cm in size, the expression frequency of GPC3 in HCC tissues is significantly higher than the increased frequency of serum AFP and the expression frequency of AFP in HCC.[69,70] Consequently, GPC3 can serve as a valuable biomarker for early screening and/or combined diagnosis of primary HCC.

Immunotherapy targeting GPC3 in clinical application

Innovative therapeutics targeting GPC3 have been developed across various research domains as therapeutic strategies for the treatment of advanced HCC. In this article, we categorize GPC3-targeted therapies into two groups based on perspectives from new drug development and clinical research: noncellular therapeutics and cellular therapeutics. We conducted a comprehensive search of clinical trial registration databases and publicly available data to summarize noncellular therapeutics targeting the GPC3 antigen, which include therapeutic vaccines, monoclonal antibodies/bispecific antibodies, tumor vaccines, and nucleic acid-based therapies, from 2008 to 2025 (Table 1). Additionally, we provide an overview of cell-based therapies targeting the GPC3 antigen, primarily CAR-T cells, with summarized clinical research information from 2015 to 2025 presented in Table 2.

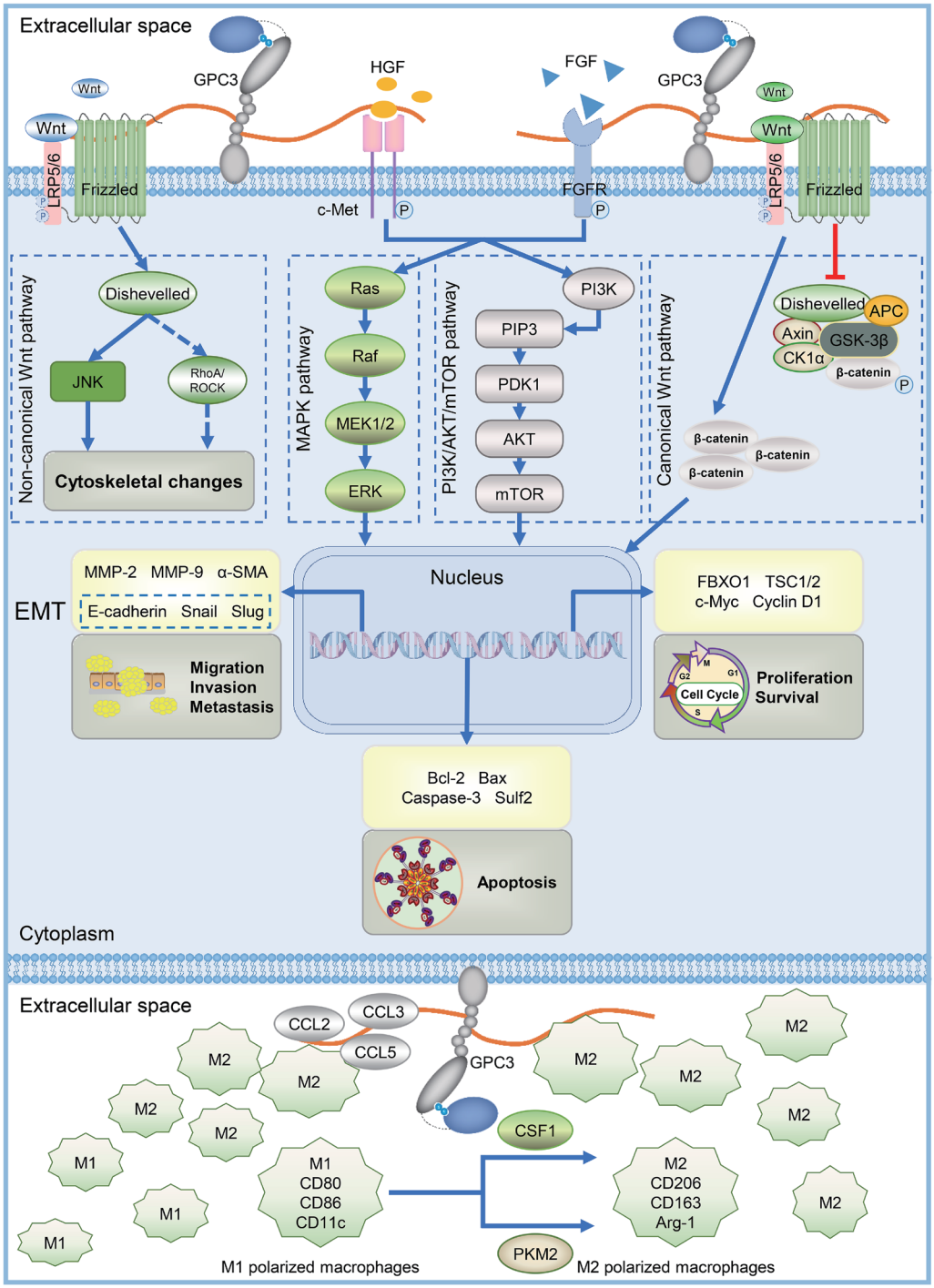

Fig. 3. The signaling pathways initiated by GPC3 in liver cancer. GPC3 may interact with Frizzled, LRP5/6, and growth factor receptors to initiate multiple signaling pathways that promote cancer cell proliferation, survival, invasion, metastasis, and apoptosis, as well as facilitate the aggregation of M2 tumor-associated macrophages. AKT, protein kinase B (PKB); APC, adenomatous polyposis coli; Arg-1, arginase-1; α-SMA, alpha-smooth muscle actin; Bax, bcl-2-associated X protein; Bcl-2, B-cell lymphoma 2; Caspase-3, cysteine-aspartic protease 3; CCL2/3/5, chemokine (C-C motif) ligand 2/3/5; CD80/86/11c/206/163, cluster of differentiation 80/86/11c/206/163; CK1α, casein kinase 1 alpha; c-Met, Met proto-oncogene; CSF1, colony-stimulating factor 1; E-cadherin, epithelial cadherin; EMT, epithelial-mesenchymal transition; ERK, extracellular signal-regulated kinase; FBXO1, F-box protein 1; FGF, fibroblast growth factor; FGFR, fibroblast growth factor receptor; GPC3, glypican-3; GSK-3β, glycogen synthase kinase-3 beta; HGF, hepatocyte growth factor; JNK, c-Jun N-terminal kinase; LRP5/6, low-density lipoprotein receptor-related protein 5/6; M1/2, macrophage M1 or M2 type; MEK1/2, mitogen-activated protein kinase kinase 1/2; MMP-2/9, matrix metalloproteinase-2/9; mTOR, mechanistic target of rapamycin; PDK1, 3-phosphoinositide-dependent protein kinase-1; PI3K, phosphatidylinositol 3-kinase; PIP3, phosphatidylinositol (3,4,5)-trisphosphate; PKM2, pyruvate kinase M2; Raf, v-Raf-1 murine leukemia viral oncogene homolog 1; Ras, rat sarcoma virus; RhoA, Ras homolog family member A; ROCK, rho-associated protein kinase; Slug, snail family transcriptional repressor 2; Snail, snail family transcriptional repressor; Surf2, surfeit locus protein 2; TSC1/2, tuberous sclerosis complex 1/2; Wnt, wingless-related integration site.

Table 1. Non-cellular therapeutics for GPC3-targeted clinical trials

Name	For-mate	Phase	ID	Indication	Status/Results	Identifica-tion meth-ods for GPC3	Register	Source
GC-33	mAb	II	NCT01507168	HCC	Completed/No clinical benefit after administration of 1600 mg	IHC	2012-01-06	Chugai Pharmaceutical
Codrituzumab	mAb	I	NCT04928677	Extra-cranial solid tumor	Recruiting/Being collected	IHC	2021-06-10	Memorial Sloan Kettering Cancer Center
RO-5137382	mAb	I	NCT00746317	Advanced or metastatic HCC	Completed/Undisclosed	IHC	2008-09-01	Chugai Pharmaceutical
ERY974	BsAb	I	NCT02748837	Solid tumor	Completed/Undisclosed	Undisclosed	2016-03-29	Chugai Pharmaceutical
CM350	BsAb	I/II	NCT05263960	Advanced HCC	Recruiting/Being collected	IHC	2022-02-10	Keymed Biosciences Co.Ltd
SAR444200	BsAb	I/II	NCT05450562	Neoplasm	Recruiting/Being collected	IHC	2022-07-05	Sanofi
BGB-B2033	BsAb	I	NCT06427941	Metastatic HCC; Gastric cancer; EYST; SNCLC	Not yet recruiting/ Being collected	IHC	2024-05-20	BeiGene
AZD9793	TsAb	I/II	NCT06795022	HCC	Recruiting/Being collected	IHC	2025-01-08	AstraZeneca
GPC3 Peptide Vaccine	Vaccine	I	UMIN000001395	HCC	Completed/Well-tolerated; Induced a GPC3-specific CTL response in 91% patients (30/33)	IHC	2007-2	National Cancer Center Hospital East, Japan
GPC3 Peptide Vaccine	Vaccine	II	UMIN000002614	HCC	Completed/1- and 2-y recurrence rates: 24.4%, 53.7%. The primary endpoint was not reached	IHC	2009-9	National Cancer Center Hospital East, Japan
RYZ811	RDC	I	NCT06726161	HCC	Recruiting/Being collected	H/C	2024-11-05	RayzeBio, Inc.
AST-201	ADC	I	NCT06687941	NC, HCC, NSLC, LN	Recruiting/Being collected	IHC	2024-11-05	Aptamer Sciences, Inc.
MTS105	mRNA	I	NCT06689540	LC, AM-LC, HCC	Recruiting/Being collected	IHC	2024-11-08	Shen Lin
MT-303	mRNA	I	NCT06478693	HCC	Recruiting/Being collected	Histological diagnosis	2024-06-24	Myeloid Therapeutics
NWRD-06	DNA	I	CXSL2300150	HCC	Recruiting/Being collected	IHC	2023-02-23	NEWISH

ADC, aptamer-drug conjugate; AM-LC, adult metastatic liver cancer; BsAb, bispecific monoclonal antibody; CTL, cytotoxic T lymphocyte; DNA, deoxyribonucleic acid; EYST, extra-gonadal yolk sac tumor; GPC3, glypican-3; H/C, histologically/cytologically diagnosed; HCC, hepatocellular carcinoma; IHC, immunohistochemistry; LC, liver cancer; LN, liver neoplasms; mAb, monoclonal antibody; mRNA, messenger ribonucleic acid; NC, neoplasms carcinoma; NSLC, non-small-cell lung cancer; RDC, radionuclide drug conjugates; SNCLC, squamous non-small cell lung cancer; TsAb, triple-specific antibody.

Table 2. Cellular therapeutics for GPC3-targeted clinical trials

Name	Phase	ID	Indication	Status/Results	Identification methods for GPC3	First submission	Source
GPC3 CAR-T	N/A	NCT03146234	HCC	Completed/Patient number: 13; CRS (9/13): Grade 1/2 (8/13), Grade 5 (1/13); overall survival rates at 3 years, 1 year, and 6 months: 10.5%, 42.0%, and 50.3%	IHC	2017-04-24	RenJi Hospital
CD8+T cell	I	NCT03175705	HCC	Unknown/Undisclosed	HE	2017-05-24	Beijing YouAn Hospital
GPC3 CAR-T; CT017	I	NCT03198546	HCC; SCLC	Recruiting/(Preliminary experimental results) Patient number: 6; No grade 2-4 adverse events or major complications; 1/6 (16.7%) pancreatic carcinoma patient achieved complete response; 1/6 (16.7%) HCC patient achieved partial response; 2/6 (33.3%) achieved steady disease	Undisclosed	2017-06-21	Second Affiliated Hospital of Guangzhou Medical University
GPC3 CAR-T	I/II	NCT03130712	HCC	Unknown/Undisclosed	IHC/FCM	2017-04-22	Shanghai GeneChem Co., Ltd.
GPC3 CAR-T	I/II	NCT03084380	HCC	Unknown/Undisclosed	IHC	2017-03-05	Xinqiao Hospital of Chongqing
GPC3 CAR-T	I/II	NCT02959151	HCC	Unknown/Undisclosed	HE	2016-11-06	Shanghai GeneChem Co., Ltd.
GPC3 CAR-T	I	NCT02932956	Liver cancer	Completed/Patient number: 12; HCC continues to progress or remains stable; no objective antitumour responses	IHC	2016-10-12	Baylor College of Medicine
GLYCAR	I	NCT02905188	HCC	Completed/Patient number: 12; HCC continues to progress or remains stable; no objective antitumour responses	IHC	2016-09-14	Baylor College of Medicine
GPC3 CAR-T	I	NCT02876978	SCLC	Unknown/Undisclosed	IHC	2016-08-09	CARsgen Therapeutics Co., Ltd.
GPC3 CAR-T	I/II	NCT02723942	HCC	Withdrawn/No available outcomes	Undisclosed	2016-03-20	Fuda Cancer Hospital, Guangzhou
GPC3 CAR-T	I/II	NCT02715362	HCC	Unknown/Undisclosed	IHC	2016-03-11	Shanghai GeneChem Co., Ltd.
GPC3 CAR-T	I	NCT02395250	HCC	Completed/Patient number: 13; CRS (9/13): Grade 1/2 (8/13), Grade 5 (1/13); overall survival rates at 3 years, 1 year, and 6 months: 10.5%, 42.0%, and 50.3%	IHC	2015-03-17	RenJi Hospital

(continued)

Table 2. *(continued)*

Name	Phase	ID	Indication	Status/Results	Identification methods for GPC3	First submission	Source
GPC3 CAR-T	I	NCT03198052	Lung Cancer	Recruiting/Being collected	Undisclosed	2017-06-22	Second Affiliated Hospital of Guangzhou Medical University
GPC3 CAR-T; CT011	I	NCT03884751	HCC	Completed/Undisclosed	IHC	2019-02-25	CARsgen Therapeutics Co., Ltd.
4th GPC3 CAR-T	I	NCT03980288	Advanced HCC	Completed/ (Preliminary experimental results) Patient number: 6; All CRS (100%): 3/6 (50%) Grade 2, 3/6 (50%) Grade 3, resolved after treatment; Objective response rate: 1/6 (16.7%); Disease control rate: 3/6 (50%); Median progression-free survival: 3.5 month; Median duration of disease control: 3.2 month; Median overall survival: 7.9 months (range: X-18.2 months).	IHC	2019-06-02	Zhejiang University
Interleukin-21 and 15 CAR-T	I	NCT04093648	HCC	Withdrawn/No available outcomes	undisclosed	2019-09-16	Baylor College of Medicine
GPC3 CAR-T	I	NCT04121273	HCC	Unknown/Undisclosed	IHC	2019-10-08	The Affiliated Nanjing Drum Tower Hospital of Nanjing University Medical School
Interleukin-15 Armored GPC3 CAR-T	I	NCT04377932	Liver Cancer; Rhabdomyosarcoma; Malignant rhabdoid tumor; Liposarcoma; Wilms tumor; Yolk sac tumor	Completed/Patient number: 12; Increased CRS (compared with the GPC3 CAR-T group): relative risk 3.3; Disease control rate: 8/12 (66%); Antitumor response rate: 4/12 (33%)	IHC	2020-05-04	Baylor College of Medicine
GPC3 CAR-T	I	NCT04506983	HCC	Suspended/No available outcomes	IHC	2020-08-06	Beijing Tsinghua Chang Gung Hospital
CT0180	I	NCT04756648	Advanced HCC	Recruiting/Being collected	IHC	2021-02-11	Zhejiang University
TILs/CAR TILs	I	NCT04842812	ST	Recruiting/Being collected	Tumor biopsy	2021-04-04	Second Affiliated Hospital of Guangzhou Medical University
ECT204	II	NCT04864054	Liver cancer	Recruiting/Being collected	IHC	2021-04-14	Eureka Therapeutics Inc.
GPC3 CAR-T	I	NCT04951141	HCC; Cholangiocarcinoma	Unknown/Undisclosed	HE	2021-06-24	Beijing Immunochina Medical Science & Technology Co., Ltd.
CT0181	I	NCT04973098	HCC	Unknown/Undisclosed	IHC	2021-07-13	Peking University

(continued)

Table 2. *(continued)*

Name	Phase	ID	Indication	Status/Results	Identification methods for GPC3	First submission	Source
GPC3 CAR-T	I	NCT05003895	HCC	Recruiting/Being collected	IHC	2021-08-12	National Cancer Institute
B010-A	I	NCT05070156	Advanced HCC	Active, not recruiting/ Being collected	IHC	2021-09-10	Tongji University
IL15-GPC3-CAR-T	I	NCT05103631	HCC; ST; Wilms tumor; Malignant rhabdoid tumor; Yolk sac tumor; Rhabdomyosarcoma; Liposarcoma; Liver embryonal sarcoma	Completed/Patient number: 12; Increased CRS (compared with the GPC3 CAR-T group): relative risk 3.3; Disease control rate: 8/12 (66%); Antitumor response rate: 4/12 (33%)	IHC	2021-10-21	Baylor College of Medicine
BOXR1030	I/II	NCT05120271	HCC; Lung squamous cell carcinoma; Merkel cell carcinoma; Myxoid/round cell liposarcoma	Recruiting/Being collected	IHC	2021-11-02	Sotio Biotech Inc.
C-CAR031	I	NCT05155189	HCC	Recruiting/Being collected	Undisclosed	2021-12-07	Zhejiang University
LCAR-H93T	I	NCT05352542	HCC	Recruiting/Being collected	IHC	2022-04-01	Jianming Xu
GPC3 CAR-T	I	NCT05344664	HCC	Unknown/Undisclosed	IHC	2022-04-19	Peking University
GPC3 CAR-T	N/A	NCT05620706	HCC	Recruiting/Being collected	IHC	2022-11-11	Shenzhen University General Hospital
Ori-C101	I/II	NCT05652920	HCC	Recruiting/Being collected	IHC	2022-11-27	OriCell Therapeutics Co., Ltd.
EU307	I	NCT05783570	HCC	Recruiting/Being collected	IHC	2023-02-27	Eutilex
Mesothelin/ GPC3/ GUCY2C-CAR-T Cells	I	NCT05779917	ST	Recruiting/Being collected	Undisclosed	2023-03-10	Second Affiliated Hospital of Guangzhou Medical University
CAR-NK Cell	I	NCT05410717	Stage IV ovarian cancer; Testis cancer; Endometrial cancer	Recruiting/Being collected	Undisclosed	2022-06-05	Second Affiliated Hospital of Guangzhou Medical University
AZD5851	I/II	NCT06084884	HCC	Recruiting/Being collected	IHC	2023-10-03	AstraZeneca

(continued)

Table 2. *(continued)*

Name	Phase	ID	Indication	Status/Results	Identification methods for GPC3	First submission	Source
GPC3-directed CAR-T	N/A	NCT05926726	HCC	Recruiting/Being collected	IHC	2024-04-04	RenJi Hospital
JWATM204	I	NCT06144385	HCC; Lung cancer	Recruiting/Being collected	IHC	2023-11-15	Shanghai Ming Ju Biotechnology Co., Ltd.
GPC3/ Mesothelin-CAR-γδT Cells	I	NCT06196294	Pancreas cancer; Lung cancer; Liver cancer; Mesothelioma; ST	Recruiting/Being collected	Undisclosed	2023-12-22	Second Affiliated Hospital of Guangzhou Medical University
IL-15 and IL-21 Armored GPC3-CAR-T	I	NCT06198296	HCC; Wilms tumor; Malignant rhabdoid tumor; Yolk sac tumor; Rhabdomyosarcoma; Liposarcoma; Liver embryonal sarcoma	Not yet recruiting/Being collected	IHC	2023-12-27	Baylor College of Medicine
CBG166	I	NCT06461624	Advanced HCC	Recruiting/Being collected	IHC	2024-06-05	Zhejiang University

CAR-T, chimeric antigen receptor T-cell; CD8, cluster of differentiation 8; CRS, cytokine release syndrome; FCM, flow cytometry; GPC3, glypican-3; HCC, hepatocellular carcinoma; HE, hematoxylin and eosin; IHC, immunohistochemistry; N/A, not applicable; PC, pancreatic carcinoma; SCLC, squamous cell lung cancer; ST, solid tumor.

Noncellular therapeutics

Monoclonal antibodies constitute a significant class of noncellular immunotherapies that target GPC3. Codrituzumab, also known as RO-5137382 or GC33, is among the earliest humanized monoclonal antibodies targeting GPC3, developed by Chugai Pharmaceutical. This antibody demonstrated favorable tolerability and antitumor efficacy in phase I clinical trials involving GPC3-positive HCC patients.[71,72] However, it did not yield significant benefits in the subsequent randomized phase II trial.[73] In 2021, Chugai Pharmaceuticals reinitiated the phase I clinical trial for codrituzumab (NCT04928677) to further investigate its potential in solid tumors; it is currently still recruiting participants.

Advancements in antibody technology have enabled engineered antibodies to achieve therapeutic effects unattainable by traditional antibodies. A notable representative of next-generation therapeutic antibodies is bispecific antibodies.[74–76] ERY974, derived from codrituzumab, represents one of the earliest bispecific antibodies targeting both GPC3 and CD3; it facilitates the formation of an immune synapse between T cells and tumor cells, thereby mediating T-cell-mediated cytotoxicity against GPC3-overexpressing tumor cells.[77] In 2024, Chugai Pharmaceuticals launched a phase I clinical study of ERY974 (NCT05022927), aimed at evaluating its safety, tolerability, efficacy, and preliminary effectiveness when combined with atezolizumab and bevacizumab for treating local or metastatic HCC. CM350 is a GPC3×CD3 bispecific antibody developed by Keymed Biosciences Co., Ltd. (Chengdu, China), marking it as the second bispecific antibody to enter clinical trials following ERY974; it is currently undergoing evaluation in phase II trials (NCT05263960).

According to the official website of the Center for Drug Evaluation under the National Medical Products Administration of China, Sanofi's first-in-class new drug SAR444200 has received implied authorization for clinical trials, targeting GPC3-positive advanced solid tumors. SAR444200 is a GPC3/TCR nanobody developed by Sanofi and is currently undergoing phase I clinical trials in the United States. BGB-B2033, developed by BeiGene (Guangzhou, China), has also obtained implied authorization from the Center for Drug Evaluation for clinical use (CXSL2400253); however, no public information regarding its target or indications is currently available.

In the development of GPC3-targeted vaccines, clinical applications of GPC3-derived peptide vaccines demonstrated favorable tolerability in phase I trials and successfully activated cytotoxic T lymphocytes to target HCC cells. However, no significant benefits were observed in phase II trials that included HCC patients who had undergone surgical resection or radiofrequency ablation.[78,79] The nucleic acid vaccine NWRD06 specifically targets GPC3 and enters muscle cells to express a fusion protein that engages antigen-presenting cells, thereby enhancing the processing and presentation of the target antigen GPC3, which further promotes the proliferation and differentiation of GPC3-matched T cells, ultimately leading to the destruction of GPC3-positive HCC cells. Preclinical data indicate that in a primary liver cancer mouse model, intramuscular administration of NWRD06 resulted in a significant reduction in both the number and size of liver tumors following three doses. While preclinical studies suggest promising prospects for therapeutic vaccines in treating HCC, there have yet to be successful clinical translations within this field.

In summary, while there are ongoing efforts and advancements in noncellular therapies targeted at GPC3 for HCC, including monoclonal and bispecific antibodies, nanobodies, and vaccines, the clinical efficacy remains to be fully established, with many candidates still in early trial phases.

Cellular therapeutics

Chimeric antigen receptor (CAR) T cells have demonstrated remarkable success in clinical applications for hematological malignancies and are rapidly being adapted for use against solid tumors.[80–82] Given the rapid advancements in monoclonal antibody technology, CAR cell therapy targeting GPC3 is considered a highly promising therapeutic strategy.[83–85] Furthermore, researchers have developed various T-cell subtypes (including $CD8^+$ T cells, tumor-infiltrating lymphocytes, and γδT cells), as well as natural killer cells, which have been engineered via CAR technology and have swiftly progressed into clinical trials.[86,87] As illustrated in Table 2, this article provides a summary of the clinical trials registered by June 2025.

The registered clinical trials investigating GPC3-targeted cellular therapeutics substantially outnumber those exploring noncellular therapeutic approaches. Preclinical and clinical evidence indicates that CAR-T cells engineered to target GPC3 exhibit potent cytotoxic activity against GPC3-positive HCC, with efficacy positively correlated to tumor antigen expression levels. Notably, GPC3 CAR-T therapy represents the first cellular intervention specifically designed for HCC (NCT02395250, NCT03146234).[88] As of July 24, 2019, data cutoff, 13 patients had received a median dose of 1.99×10^9 CAR-GPC3 T cells. Treatment-related adverse events included pyrexia (13/13), lymphopenia (12/13), and cytokine release syndrome (9/13). The severity of the cytokine release syndrome was predominantly graded as 1/2 (resolved in eight cases). Kaplan–Meier analysis revealed overall survival rates of 50.3% at six months, 42.0% at one year, and 10.5% at three years. Objective responses included two partial remissions and one case of sustained stable disease lasting 44.2 months, with CAR-T cell expansion dynamics suggesting a potential correlation with clinical outcomes.[88] These findings underscore the preliminary safety and antitumor efficacy of GPC3-targeted CAR-T therapy in advanced HCC, while providing a robust rationale for advancing next-generation immunotherapeutic strategies against this challenging malignancy.

In addition to the classical structural features of CAR-T cells, CAR-T cells are capable of secreting self-regulatory cytokines such as IL-15 and IL-21. To increase the safety and antitumor efficacy of CAR-T cells, structural optimizations are frequently employed, with typical modifications including the following: 1) Gene modification of the CAR structure utilizing proinflammatory cytokines.[80,81,89] For example, CT017, developed by CARsgen Therapeutics, coexpresses IL-7 and CCL21, which improves the survival and infiltration of CAR-T cells while inhibiting tumor angiogenesis. CT017 has superior antitumor effects compared to those of GPC3-7×19 CAR-T cells without necessitating lymphocyte depletion chemotherapy. This is particularly significant for patients with compromised T lymphocytes or those unable to tolerate lymphocyte depletion chemotherapy, and it has progressed into clinical phase I trials (NCT03198546). 2) Gene editing of immune checkpoints. Studies have indicated that employing CRISPR/Cas9 to knock out PD-1 expression in CAR-T cells can increase both the persistence and infiltration of GPC3-targeted CAR-T cells while increasing their antitumor activity.[90] However, within clinical cell transfer therapies targeting GPC3, only studies involving combinations with PD-1 antibodies have been reported thus far; no investigations on gene-edited immune cells have been conducted yet. 3) Other structural optimizations. G3-CAR-ori2—a second-generation CAR-T cell that incorporates a proprietary Ori2 element following 4-1BB and CD3ζ—significantly enhances its proliferation and persistence while demonstrating excellent potential in phase I clinical trials.[91] Furthermore, research findings suggest that low-affinity 8F8-BBz CAR-T cells exhibit comparable tumor-killing capabilities to their high-affinity counterparts but possess greater expansion capacity and durability, indicating enhanced therapeutic potential.[92]

Similar to other subtypes within the T lymphocyte lineage, such as GPC3-CAR-γδT cells, enhanced universality has been observed, with related clinical trials currently underway in China that have not yet initiated recruitment (NCT06196294). Previous studies have demonstrated that CARVδ1 T cells exhibit robust proliferation, cytokine production, and cytotoxicity against various GPC3-expressing HCC cells, with minimal sensitivity to sGPC. These cells effectively control tumors in the HepG2 mouse model without inducing graft-versus-host disease. Furthermore, their antitumor efficacy is significantly augmented when they are coexpressed with IL-15, without associated toxicity.[86] Additionally, targeting GPC3-CAR-natural killer cells also results in off-the-shelf versatility and is presently open for recruitment (NCT05410717).

Obviously, noncellular drugs typically possess stable pharmacokinetic attributes that can be utilized to more effectively assess their absorption, metabolism, distribution, and excretion *in vivo*. The dosage and potential toxicity or side effects of the drugs have been well-defined in preclinical studies, which is more conducive to expediting the application of targeted drugs in the treatment of HCC. Nevertheless, for cellular drugs, which are regarded as "living drugs", their pharmacokinetic characteristics are relatively less stable than those of noncellular drugs. Hence, in addition to emphasizing potency, greater attention should be given to the metabolism of the drug itself and its potential toxicity or side effects, such as reproductive toxicity, genetic toxicity, carcinogenicity, tumorigenicity, and cytokine storm. However, cellular drugs might be capable of better compensating for the drawbacks of noncellular drugs being prone to drug-fast *in vivo*, and safe cellular drugs may alleviate or even cure HCC.

Methods and applications of patient-specific GPC3 identification

As stated above, GPC3 is recognized as a promising specific biomarker for HCC that may guide targeted therapy. Various detection methods have been developed in recent years to increase the efficiency of HCC diagnosis and treatment. In this section, we summarize the GPC3 detection methods employed in clinical trials and preclinical studies, review clinical trial reports related to different detection techniques along with their respective advantages and disadvantages (Table 3),[93–112] and ultimately categorize these methods into three distinct types based on characteristics such as target samples, operational procedures, and analytical methods: chemical immunoassays, imaging methods, and biosensors.

Chemical immunoassay

Chemical immunoassay integrates immunological and chemical principles for precise biomarker detection, with enzyme-linked immunosorbent assay (ELISA) standing out as the most clinically validated method for GPC3 in HCC. ELISA-based GPC3 detection has demonstrated significant diagnostic specificity, particularly in distinguishing HCC from liver cirrhosis. For instance, serum GPC3 levels in HCC patients (16.81±0.56 μg/L) markedly exceeded those in cirrhosis controls (7.41±0.25 μg/L), achieving 96.77% specificity.[113] Commercial ELISA kits further validated this differentiation, revealing median serum GPC3 levels of 924.8 pg/mL in HCC

Table 3. Detection methods for GPC3

Type	Name	Advantages	Disadvantages	Reference
Chemical immunoassay	Flow cytometry	High-throughput, multi-parameter, real-time, and rapid analysis	Sample pretreatment, complex operations and analyses, and a limited measurement range	93,94
Chemical immunoassay	Hematoxylin-eosin staining	Simple operation, low cost, can be utilized for rapid screening	Only presenting morphological information makes it difficult to accurately determine complex lesions and tiny foci	95
Chemical immunoassay	Immunohis-tochemistry	Accurate positioning, visualized outcomes, with high specificity and sensitivity	Cross-reaction, non-specific staining, cumbersome operation, relatively high costs	96
Chemical immunoassay	Immunofluo-rescence	High specificity, sensitivity, and rapidity	Non-specific staining, complicated procedures	97,98
Chemical immunoassay	Enzyme-linked immunosorbent assay	Diverse sample sources, high-throughput, easy to operate, high specificity, high sensitivity	Limited antigen information	99,100
Imaging method	Magnetic resonance imaging	High soft tissue resolution with no radiation, clinical applied widely	High level of noise, slow imaging speed	101,102
Imaging method	Single-photon emission computed tomography	High sensitivity, strong tissue penetration	Low spatial resolution, challenging to conduct quantitative analysis, radioactive	103–105
Imaging method	Immuno PET	Non-invasive, easy to operate, fast imaging, real-time dynamic detection	Requirement use radiolabeled nuclides, relatively high uptake and retention of agents might cause radiation harm and influence the detection of suspicious lesions	93,106,107
Imaging method	Near infrared spectrum	Convenient operation, analysis with fast speed and high efficiency, online analysis	Low sensitivity, poor selectivity, challenges in model establishment	108
Biosensor	Enzymes, microorganisms, cells, nanoparticles, aptamers, etc	Operation is simple, strong specificity, high selectivity, requiring only a small sample, capable of conducting continuous online detection	Need exorbitant equipment and technology, restricted application scope, prone to interference	109–112

etc, et cetera (and so on); GPC3, glypican-3; Immuno PET, immuno-positron emission tomography.

patients compared to 1161.6 pg/mL in chronic liver disease cohorts (n = 200 per group).[114]

Clinical correlations highlight ELISA's utility in multi-modal diagnostics. Among 38 HCC resection samples, 36.9% showed tissue GPC3 positivity via ELISA, paralleling serum elevation patterns.[114] Notably, in AFP-normal HCC patients (n = 74/147 post-hepatectomy cases), serum GPC3 levels (mean 5.1) significantly surpassed healthy controls ($p < 0.01$), while immunohistochemistry (IHC) results remained consistent across AFP subgroups.[115] ELISA also enabled simultaneous profiling of GPC3 with VEGF and GP73 in HCC/cirrhosis/healthy cohorts (n = 120), confirming GPC3's diagnostic superiority over conventional biomarkers.[116]

However, technical limitations constrain ELISA's standalone use. Serum GPC3 quantification alone exhibited insufficient diagnostic power, necessitating complementary IHC verification in moderately/poorly differentiated HCC tissues.[114] Additionally, no significant GPC3 expression differences emerged between AFP-normal and AFP-abnormal HCC groups via IHC.[115]

Supportive methodologies enhance GPC3 detection frameworks. Flow cytometry achieves 85% sensitivity and 95% specificity in detecting membrane-bound GPC3 on circulating tumor cells from HCC patients,[117] while IHC remains the gold standard for tissue-based GPC3 identification in clinical protocols (Table 3).

Imaging methods

In clinical practice, imaging detection technologies predominantly based on CT and PET employ radiolabeled tracers to noninvasively and dynamically evaluate the expression and variations of GPC3 in real-time, thereby effectively addressing discrepancies in detection results arising from tumor heterogeneity.[118] Wu *et al*. developed an imaging genomics model based on magnetic resonance (MR) images to predict the pathological grade of HCC. Their findings indicated that this MR imaging-based model outperformed models relying solely on clinical factors (areas under the curve (AUCs) of 0.74 vs. 0.60), whereas the AUC for the combined model reached 0.80 (95% CI: 0.65–0.90), demonstrating superior performance overall.[119] Gu *et al*. established a support vector machine imaging radiomic signature utilizing enhanced MR images of tumors to predict the GPC3 expression status in HCC, achieving AUC values of 0.879 and 0.871 in the training and validation cohorts, respectively. Furthermore, combining the imaging radiomic model with clinical risk factors significantly improved the predictive performance, yielding AUC values of 0.926 and 0.914 in both sets.[120] A recent

study involving 259 patients with HCC aimed to develop an imaging radiomic model for predicting the expression status of GPC3 in patients with solitary HCC measuring ≤5 cm prior to surgery. The findings indicated that the combined model, which integrates clinical risk factors with the optimal imaging radiomics model, exhibited superior predictive ability for GPC3 expression status, with AUCs of 0.931 and 0.943 in the training and validation sets, respectively.[121] In another study, Liu *et al*. utilized a SMOTE-LR model based on three features—AFP, apparent diffusion coefficient, and R2* map data—from 158 HCC patients to improve the prediction and identification of GPC3 in HCC, achieving AUC values of 0.909 and 0.829 for the training and validation sets, respectively, along with accuracy rates of 83.7% and 82.1%.[122]

Biosensors

Recent advances in aptamer-based biosensors demonstrate remarkable potential for GPC3 detection in HCC through innovative signal amplification and transduction strategies. Chen *et al*. engineered an enzyme-free catalytic hairpin assembly biosensor using N-methyl mesoporphyrin IX and quantum dots as dual signal reporters, enabling simultaneous AFP/GPC3 quantification in clinical samples with picomolar sensitivity. This system incorporated CdTe quantum dot strips for color-distance binary readouts, achieving visual GPC3 detection in serum through spectral shift patterns.[42] Addressing soluble GPC3 detection challenges, Duo *et al*. developed slow off-rate modified aptamers that specifically bind epitope-variable GPC3 fragments, overcoming antibody limitations through π-π stacking-enhanced binding kinetics (Kd < 1 nM).[123]

Electrochemical aptasensors demonstrate rapid response capabilities through their high sensitivity and target affinity, offering significant potential for disease diagnostics. As depicted in Figure 4A, hollow gold nanoparticles (HGNs) were first synthesized via a one-step reduction method using hydrazine hydrate as the reducing agent, followed by functionalization with GPC3-specific aptamers through π-π interactions to create HGNs-Apt signal probes. Concurrently, a screen-printed electrode or screen-printed carbon electrode (SPCE) surface was activated and electrodeposited with Au nanoparticles (AuNPs) to enhance conductivity. GPC3-targeting aptamers (GPC3-Apt) were then physically adsorbed onto the AuNPs/screen-printed electrode or AuNPs/SPCE surface. Upon introducing GPC3, specific recognition between GPC3-Apt and the antigen formed aptamer-antigen complexes on the electrode, which subsequently anchored HGNs-Apt probes via π-π stacking and electrostatic interactions, establishing a sandwich architecture. Leveraging the intrinsic peroxidase-like activity of HGNs, hydrogen peroxide (H_2O_2) catalyzed the reduction of Ag^+ ions to metallic Ag, which deposited onto the Au NPs/SPCE surface. The accumulated Ag was quantified through differential pulse voltammetry, where the current intensity exhibited linear proportionality to GPC3 concentrations across 10.0–100.0 mg/mL, achieving a detection limit of 3.16 mg/mL.[110] This integrated approach synergizes aptamer selectivity with nanomaterial-amplified signaling, enabling precise electrochemical detection of GPC3 in complex biological matrices.

In addition, Li *et al*. developed a highly sensitive homogeneous detection GPC3 aptasensor utilizing the GPC3 aptamer-labelled gold carbon dots (AuCDs-GPC3-Apt) as the donor and magnetic graphene oxide (Fe_3O_4-GO) nanosheets as the acceptor on the basis of fluorescence resonance energy transfer (FRET) (Fig. 4B).[124] The AuCDs were synthesized via a one-step hydrothermal method to ensure adequate fluorescence emission. The FRET interaction between AuCDs-GPC3-Apt and the Fe_3O_4-GO nanosheets led to a reduction in the overall fluorescence intensity of the system. Upon addition of the target GPC3 into the FRET system, fluorescent AuCDs-GPC3-Apt bound to GPC3, forming a folded structure that resulted in separation from the Fe_3O_4-GO nanosheets. Subsequently, magnetic separation of Fe_3O_4-GO allowed the recovery of fluorescence from free-labelled AuCDs-GPC3-Apt. Under optimal conditions, the fluorescence recovery rate exhibited a linear correlation with GPC3 concentrations ranging from 5 to 100 ng/mL, achieving a detection limit of 3.01 ng/mL (S/N = 3). This strategy demonstrated a recovery rate ranging from 98.76% to 101.29% in real human serum samples, providing an immediate and effective method for the quantitative detection of GPC3 with significant potential for the early diagnosis of HCC.[124] Collectively, these biosensors leverage aptamer-programmable recognition paired with nanomaterial-enhanced signal conversion, establishing robust platforms for multiplexed GPC3 detection in complex biological matrices.

In summary, chemical immunoassays can detect a variety of samples, including tissues, cells, and serum, and have high sensitivity. The time required for detection varies from a few hours (flow cytometry) to several days (hematoxylin and eosin, IHC). The detection time of imaging methods is shorter, generally within tens of minutes. All of the above methods can be used for rapid screening and diagnosis of GPC3 in early HCC patients. However, for advanced patients, one of these methods may first be used to determine whether GPC3 expression is upregulated (serum detection is recommended), and then the concentration of GPC3 on the cell surface can be measured to determine whether it is suitable for targeted therapy. Although the biosensor detection method requires fewer samples and may be more suitable for early screening and diagnosis, unfortunately, it can only be expected that biosensor detection will be popularized in clinical use as soon as possible.

Achievements and limitations

Recent studies indicate that the concurrent utilization of two or three specific biomarkers may increase the accuracy of HCC diagnosis.[42,68,125,126] At present, preclinical studies targeting GPC3 have yielded systematic results, and data from multiple clinical trials have also shown positive efficacy. We also look forward to breakthrough progress in GPC3-targeted treatment strategies in subsequent clinical trials. It is worth noting that GPC3 can not only serve as a key biomarker for the early diagnosis of HCC, but also has the dual potential of becoming a therapeutic target. Based on the significant potential of GPC3 in both diagnosis and treatment, we suggest that medical institutions include serum GPC3 protein detection in the routine diagnostic protocol for liver cancer patients to improve the accuracy of screening for patients with AFP-negative liver cancer or early-stage liver cancer. It should be coordinated with AFP serum marker protein detection and imaging examinations, such as CT, to enable early detection and treatment of HCC patients.

It is crucial to routinely conduct GPC3 immunohistochemical testing on tumor specimens before treatment for newly diagnosed HCC patients to evaluate their suitability for GPC3-targeted treatment strategies. Consequently, specific inclusion criteria have been established in clinical trials of GPC3-targeted immunotherapy to exclude patients with low or absent expression of GPC3 on tumor cells. For example, in many clinical studies targeting GPC3, when enrolling HCC patients, the IHC test is first used to detect whether the tumor tissue sections show strong GPC3 positivity (≥ ++), with

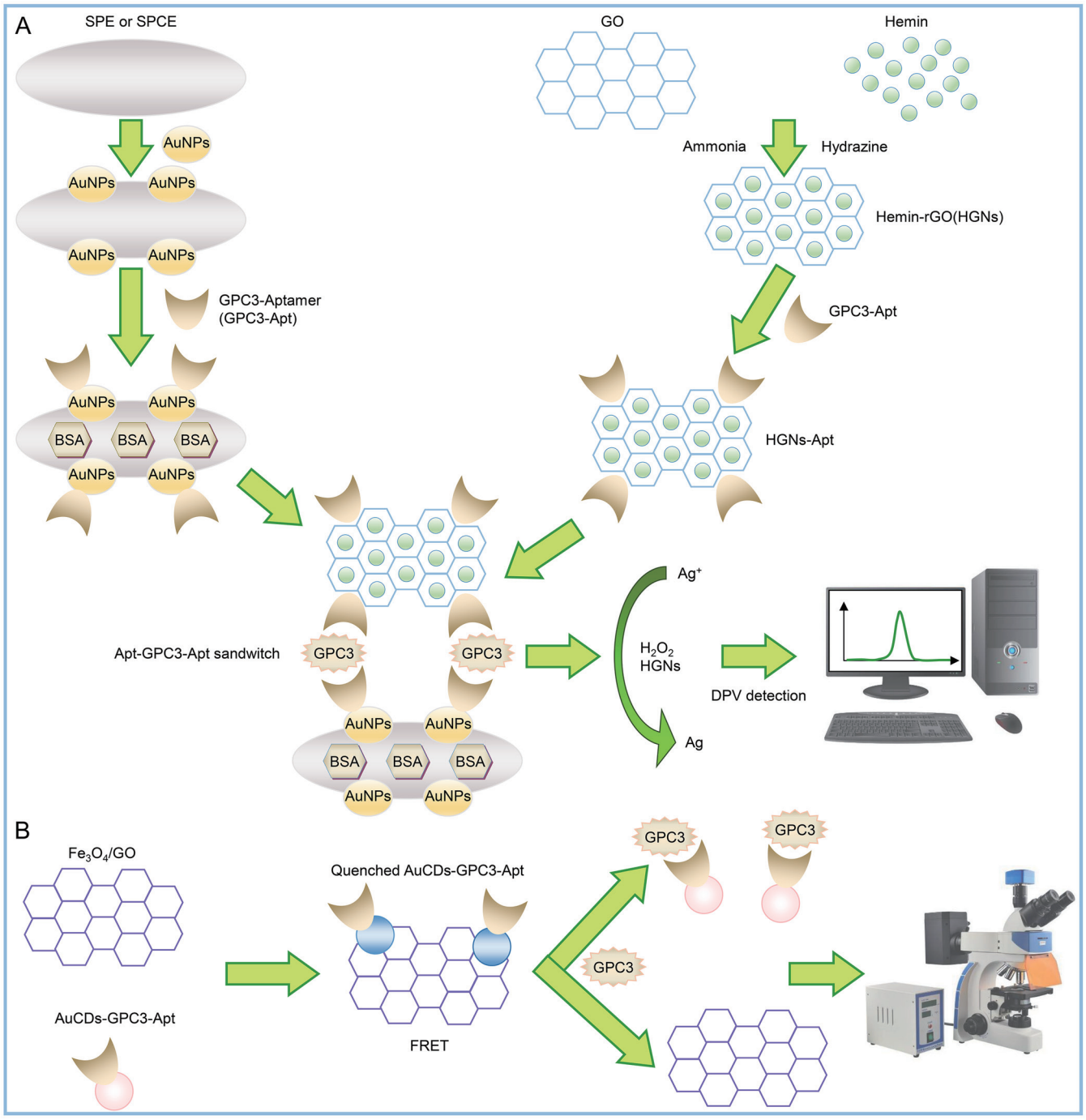

Fig. 4. Schematic diagram of electrochemical GPC3 aptasensors. (A) Aptamer biosensor based on hemin/GO nanocomposite for the determination of GPC3. (B) FRET sensor for analyzing GPC3 based on the Fe_3O_4/GO-GPC3-Apt system. Ag, silver; Apt-GPC3-Apt sandwich, aptamer-glypican-3-aptamer sandwich; AuCDs, gold carbon dots; AuNPs, gold nanoparticles; DPV detection, differential pulse voltammetry detection; Fe_3O_4, iron(III) oxide; FRET, förster resonance energy transfer; GO, graphene oxide; GPC3, glypican-3; GPC3-Apt, glypican-3 aptamer; H_2O_2, hydrogen peroxide; HGNS, hemin-rGO nanocomposites; HGNs-Apt, hemin-rGO nanocomposites-aptamer; SPCE, screen-printed carbon electrode; SPE, screen-printed electrode.

patients exhibiting strong GPC3 expression preferentially enrolled for subsequent trials. For patients with negative (-) or weakly positive (+) results (i.e., no more than 30% of HCC patients exhibit low GPC3 expression[127,128]), non-GPC3-dependent treatment regimens such as lenvatinib, sorafenib, and anti-PD-L1 monoclonal antibodies are preferentially recommended. Such strict enrollment criteria can not only ensure the efficacy of GPC3-targeted strategies in the GPC3-positive population but also avoid delays in disease progression caused by ineffective treatments, providing a high-quality, evidence-based foundation for subsequent drug research and development.

In terms of GPC3 detection methods, classical chemical immunoassays, such as ELISA, have demonstrated a limit of detection reaching pg/mL levels in tumor cell lysates.[129] Due to its advantages, such as high throughput, low cost, convenient operation, and high specificity, ELISA has become a routine method in medical institutions for detecting antibodies against infectious pathogens (such as bacteria), hormone levels, and tumor markers (such as AFP and carcinoembryonic antigen). Based on its technical maturity, we recommend using ELISA as the preferred method for detecting serum GPC3 protein in patients with HCC. Similarly, biosensors can achieve a sensitivity level with a detection limit of 0.07 ng/mL for GPC3 detection.[130] However, despite their advantages, such as ease of operation and minimal sample requirements, the application of biosensors in clinical settings has been hindered by the absence of industry standards and inherent limitations.[131] Imaging techniques allow for noninvasive and dynamic assessment of GPC3 expression *in vivo*; however, their use requires consideration of the heterogeneous distribution of target antigen proteins within the body, and the implications for result interpretation, alongside potential harm caused by radioactive isotopes used in imaging procedures. Moreover, clinical treatment must also account for variations in GPC3 distribution between serum and the cell surface, as well as the implications of membrane-targeted GPC3 therapy.

As previously mentioned, both membrane-bound and soluble GPC3 serve as valuable biomarkers for the diagnosis and treatment of HCC.[25,132] Nevertheless, numerous questions regarding the expression and function of GPC3 remain unanswered. For example, investigations are needed to elucidate the correlation between membrane expression density and soluble protein concentration, as well as to explore how morphological changes from membrane-bound to soluble proteins in circulation relate to HCC progression. In clinical tests, clarifying the correlation between serum soluble GPC3 and the progression of HCC has dual significance. On the one hand, it can provide data to support the formulation of diagnostic criteria for HCC based on serum GPC3 concentration thresholds (pg/mL or μg/L). On the other hand, dynamic monitoring of changes in GPC3 levels can facilitate the assessment of patients' treatment responses and disease outcomes. Additionally, GPC3 is primarily mediated by the Wnt pathway to increase tumor cell invasion and proliferation.[45] Studies indicate that elevated GPC3 expression is closely associated with EMT in HCC cells.[15,32,51] The planar cell polarity pathway, a component of the Wnt signaling cascade, regulates cytoskeletal reorganization through its activation. This mechanistic link suggests that GPC3 may promote EMT progression by modulating Wnt signaling activity. Furthermore, elevated levels of GPC3 can recruit a greater number of M2 macrophages;[56] however, whether this recruitment contributes to the establishment of a tumor immune-suppressive microenvironment requires further investigation. Elucidating the expression mode and functional characteristics of GPC3 can further enhance diagnostic accuracy and therapeutic strategies for HCC.

Conclusions

Consequently, assessing GPC3 expression and distribution in HCC patients is essential for targeted treatment. For early-stage patients, various detection methods can be used to evaluate the GPC3 concentration in serum and on cell surfaces, as well as its tissue distribution, thereby enhancing diagnostic accuracy. Furthermore, rapid sampling and diagnosis using sensitive biosensors may reduce patient burden. Subsequently, targeted therapy can be initiated promptly based on GPC3 localization and expression levels to improve disease control rates. In contrast, advanced-stage patients should utilize combined methods, such as serum ELISA or flow cytometry, in combination with imaging methods, to accurately assess GPC3 expression levels and distribution. This approach facilitates the implementation of targeted therapies while maximizing clinical benefits.

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Conflict of interest

JZ and CW are employee of Chengdu Yunce Medical Biotechnology Co., Ltd. The other authors have no conflict of interests related to this publication.

Author contributions

Conceptualization (JZ, CW), writing - original draft (JZ), writing - review & editing (RL, XT, CW), and supervision (CW). The work reported in the paper has been performed by the authors, unless clearly specified in the text. All authors have approved the final version and publication of the manuscript.

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