Therapeutic Potential of Targeting Glypican-3 in Hepatocellular Carcinoma

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Abstract

Glypican-3 (GPC3) is a developmentally-regulated oncofetal protein that has been established as a clinically-relevant biomarker for early hepatocellular carcinoma (HCC). It is one of the first transcripts to appear during malignant hepatocyte transformation, and is expressed at the protein level in approximately half of high-grade dysplastic macronodules in cirrhotic liver. Several studies show it is expressed in most (75 to 100%) of HCCs confirmed by histopathology. The protein is anchored to the hepatocyte membrane by a glycosyl-phosphatidylinositol (GPI) anchor and shows consistent membrane immunostaining pattern, making it a viable target for immunotherapeutic approaches. Targeting GPC3 for therapeutic intervention is a promising approach for the clinical management of HCC and selected other tumors that express the marker.

Keywords

Glypican-3; Hepatocellular Carcinoma; therapeutic antibody; peptide vaccine; immunohistochemistry; oncofetal protein; Wnt signaling

INTRODUCTION

Hepatocellular carcinoma is the fifth most common neoplasm in the world and the third most common cause of cancer-related death, responsible for more than 600,000 new cases and nearly as many deaths per year [1–3]. Agents that contribute to chronic liver injury and eventually cirrhosis promote oncogenesis. The most relevant are hepatitis B (HBV) and hepatitis C (HCV) viruses and alcohol, although other causes of hepatocyte damage such as iron or copper deposition, non-alcoholic steatohepatitis, or primary biliary cirrhosis, are also risk factors [2]. The American Cancer Society reports that HCC accounts for about 75% of liver cancer cases. Hepatocellular carcinoma is usually asymptomatic until later stages of disease. Patients with advanced HCC have considerably lower 5-year survival rates than those with early-stage disease. In the US between 1993 and 2002, the 5-year survival for early, localized HCC was 19.8%, whereas for advanced HCC it was 3.3% [3]. HCC does not respond to most chemotherapeutic agents. Surgical resection is one of the few therapeutic

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options, but only 10–20% of primary HCCs are found to be resectable at the time of diagnosis. Recently, sorafenib (Nexavar®) has been approved for treatment of patients with unresectable tumors [4], but the response of most patients to this treatment is very limited. Antibody or T-cell-directed immunotherapies represent alternative approaches, but the primary challenge has been identifying predominant and stable tumor-specific targets. Since GPC3 is a cell surface protein that is highly expressed predominantly in HCC, and it is not expressed in normal adult liver, it is a promising target.

Glypicans are a family of heparan sulfate proteoglycans that are linked to the exocyttoplasmic surface of the plasma membrane by a GPI anchor. To date six glypicans have been identified in mammals (GPC1 to GPC6). Glypican expression levels change in a developmental stage- and tissue-specific manner, suggesting that they are involved in morphogenesis [5]. Filmus and colleagues identified the gene called OCI- 5 in a rat undifferentiated epithelial cell line in 1998 [6]. The OCI-5 gene was later named GPC3 based on homology with other glypicans. The GPC3 gene is located on human X chromosome (Xq26) encoding a 70-kDa core protein which can be cleaved by furin to generate a 40-kDa amino (N) terminal protein and a 30-kDa carboxyl (C) terminal protein containing two heparan sulfate (HS) glycan chains [7]. Three variants have been detected that encode alternate splice forms (Isoforms 1, 3 and 4). The functional significance and differential expression of these GPC3 iso-forms are unknown. Most studies suggest the majority of GPC3 protein is attached to the cell membrane by a GPI anchor, however, GPC3 has been found to be mutated in patients with Simpson-Golabi-Behmel syndrome (SGBS), and these mutations sometimes affect the ability of GPC3 to be GPI-anchored [8]. SGBS is an X-linked disorder characterized by pre- and postnatal overgrowth with visceral and skeletal anomalies and a broad spectrum of clinical manifestations that vary from a mild phenotype in carrier females, to infantile lethal forms in some males [9]. The role of GPC3 in SGBS has been confirmed by studying the phenotype of GPC3-null mice. These mice display many of the abnormalities observed in SGBS patients, including overgrowth, congenital heart defects and kidney dysplasia due to increased uterine budding [10]. Recent studies point to a role in development of cardiac vasculature [11]. All glypicans share a structure characterized by a conserved pattern of 14 cysteine residues which may form intramolecular disulphide linkages [12]. In addition, the location of the HS chains in the C-terminal region near the cell membrane is conserved in all glypicans [13]. Serine 560 is predicted as a cleavage site in GPC3 for GPI anchorage [14]. GPC3 binds Wnt, Hedgehog (Hh) [15, 16] and fibroblast growth factor-2 (FGF-2) [17] through its core protein and/or the HS chains. The furin cleavage between Arg358 and Ser359 is required for GPC3 modulation of cell survival and Wnt signaling in zebrafish but is not required for HCC cell growth.

GLYPICAN 3 EXPRESSION IN MALIGNANCY

Early studies showed that GPC3 can be downregulated in several types of tumors, including breast cancer [18], ovarian cancer [19], lung cancer [20], and mesotheliomas [21]. The mechanism involved in GPC3 downregulation seems to be hypermethylation of its promoter, and treatment of tumor cell lines with a demethylating agent induces GPC3 re-expression. Thus, original data led to the speculation that GPC3 was a tumor suppressor. Recent work suggests that GPC3 acts as an inhibitor of cell proliferation by activating the non-canonical

Wnt signaling pathway [22]. This pathway is known to antagonize canonical Wnt/3-catenin signaling, which is a potent stimulator of cell proliferation [23]. Interactions with the Wnt pathway, whose primary ligands are differentially-expressed in a tissue-specific manner, may account for these opposed observations. Zynger *et al.*, reported GPC3 expression in testicular germ cell tumors [24]. All 24 yolk sac tumors (YST) and all 7 choriocarcinoma components were immunoreactive for GPC3, whereas only 38% of teratomas with immature elements and 8% of embryonal carcinomas expressed GPC3. There was no immunoreactivity in benign testicular tissue, intratubular germ cell neoplasia, seminomas, or teratomas with mature elements. Therefore the predominant expression of GPC3 in testicular germ cell tumors was useful for defining histologic subtypes. GPC3 was evaluated in a study of ovarian extragonadal Germ cell tumors (GCTs) from 63 patients (57 children and 6 adults), including 14 ovarian and 20 extragonadal primary GCTs and 8 metastases along with 21 primary testicular GCTs for comparison. All 33 yolk sac tumors and both choriocarcinomas were immunoreactive for GPC3. In contrast, a minority of immature (4/10) and mature (4/35) teratomas were positive. No positivity was seen in 6 embryonal carcinomas or 5 germinomas. GPC3 is differentially expressed in ovarian and extragonadal GCTs, with expression predominantly observed in YSTs and choriocarcinoma.

A comprehensive tissue microarray analysis with the 1G12 monoclonal antibody (mAb) to GPC3 shows the pattern of expression in pre-neoplastic, neoplastic and non-neoplastic tissues [25]. 4387 tissue samples from 139 tumor classifications and 36 pre- and non-neoplastic tissues were studied. Expression of GPC3 was determined semiquantitatively using a 10% cutoff score and was observed in 9.2% of nonneoplastic liver samples (11/119), 16% of preneoplastic nodular liver lesions (6/38), and 63.6% of HCCs (140/220). Furthermore, several other tumors revealed consistent expression of GPC3, including squamous cell carcinoma of the lung (54%), testicular nonseminomatous germ cell tumors (52%), and liposarcoma (52%).

GLYPICAN 3 EXPRESSION IN HCC

In 1997 Hsu *et al.*, reported the identification of a transcript that was upregulated in HCC [26]. This transcript, which they named MXR7, turned out to be GPC3. These investigators found that GPC3 mRNA was expressed in 143 out of 191 (74.8%) primary and recurrent HCCs, but in only 5 of 154 (3.2%) normal livers. In addition, no GPC3 expression was detected in 3 hepatocellular adenomas, and in 6 cholangiosarcomas. These findings have been corroborated by Zhu *et al.*, where they show that Northern blot analysis indicated that expression of GPC3 mRNA was low or absent in normal liver, focal nodular hyperplasia and in liver cirrhosis. In contrast, expression of GPC3 mRNA was markedly increased in 20 of 30 HCC samples, and was moderately increased in five of 30 HCC samples [27]. In a genomics study, investigators used cDNA microarrays containing 17,000 unique human genes to study the gene expression profiles in over 200 liver tissue samples, including 102 primary HCC (from 82 patients) and 74 nontumor liver tissues [28]. GPC3 showed the highest magnitude increase for the 'Membrane protein' category, and was second only to alpha-fetoprotein (AFP) in the 'Secretory or extracellular proteins' category. These microarray findings have been corroborated by Luo *et al.*, [29]. In a study designed to identify a molecular signature for early HCC, Llovet *et al.*, tested the transcriptional profiles

of 55 genes previously implicated as biomarkers of HCC or hepatocarcinogenesis in early HCC compared to dysplastic nodules [30]. A 3-gene signature including GPC3 (18-fold increase in early HCC vs. dysplastic nodules), survivin (2.2-fold increase) and LYVE-1 (12-fold decrease) was identified with a discriminatory accuracy of 94%.

These efforts with microarray analysis provide the basis for proceeding with immunohistochemical studies. At the protein level, a study by Paradis *et al.*, reported that out of 54 cases of HCC, 38 (70%) expressed GPC3, showing both membranous and diffuse cytoplasmic staining [31]. In a few cases, GPC3 immunostaining was granular and prominently localized in areas adjacent to canaliculi. GPC3 staining was significantly more often observed in HCC from cirrhotic livers (90% of 21 cases) compared with HCC in normal liver (64% of 28 cases). Among the 5 fibrolamellar HCCs present in normal liver, only 1 case (20%) displayed significant positive staining. In the group of HCC in cirrhotic livers, GPC3 expression was expressed in 13 (93%) of 14 moderately differentiated and in 6 (86%) of 7 poorly differentiated HCC. This tendency for expression in moderately and poorly differentiated HCC has been observed by others [32]. Given that the GPC3 gene is located on the X chromosome, one plausible mechanism for absence of GPC3 expression in well-differentiated HCC is functional loss of heterozygosity. Size of the tumor and any other clinicopathologic factors (etiology of cirrhosis, age of the patient, presence of vascular invasion) were not significantly related to GPC3 expression. Because GPC3 appeared to be a relevant marker of fully developed HCC, they also analyzed 94 cirrhotic macronodules with various grades of dysplasia from 27 cirrhotic specimens, 8 of them with overt HCC. Sixty-five nodules were benign or had low-grade dysplasia, whereas 9 had high-grade dysplasia and 20 were early HCC. In the group of benign and low-grade dysplastic macronodules, only 2 low-grade dysplastic macronodules (3%) displayed significant GPC3 expression. Among the high-grade dysplastic and early HCC, 14 (48%) of 29 of them were immunostained with GPC3. Similarly Shafizadeh *et al.*, observed GPC3 positive immunostaining in 43% of high grade dysplastic nodules.

Libbrecht *et al.*, [33] evaluated GPC3 for the ability to distinguish between small HCC (less than 3 cm) from cirrhosis, dysplastic nodules, and focal nodular hyperplasia-like lesions. They studied resected lesions and those tested by needle biopsy and observed sensitivity and specificity for diagnosis of HCC of 0.77 and .096 respectively for resected tissues and 0.83 and 1 respectively for needle biopsies. While most studies show that cirrhotic liver is negative for GPC3, care should be taken in using GPC3 as a sole criteria for HCC diagnosis, since positive immunostaining of liver tissue sections with active hepatitis C has been observed [34]. To improve the accuracy of immunohistochemical diagnosis of HCC, Di Tomasso and colleagues included glutamine synthetase and heat shock protein 70 to generate an algorithm. Inclusion of positive immunostaining for two of the three markers was strongly suggestive of malignancy. The overall accuracy for detecting HCC was 78.4 percent (for any 2 markers) with 100% specificity. GPC3 had the best performance for discriminating between high-grade dysplastic macronodules and very well-differentiated HCC, with sensitivity of 61.4% and specificity of 92%. Additional studies using fine needle aspirates (FNAs) and GPC3 immunocytochemistry shows similar results, with sensitivities of 90% [35], 83% [36] and 81% [37]. Because many studies have confirmed the validity of GPC3 immunohistochemistry in HCC diagnosis, recent revisions to the clinical practice

guidelines for management of HCC support performing immunohistochemical evaluation with GPC3 and other markers (including CD34, CK7, HSP-70, and glutamine synthetase) to improve diagnostic accuracy on tissue biopsies of lesions that are not clearly confirmed as HCC by two radiological procedures (American Association for the Study of Liver Disease; Revised Practice Guidelines for HCC; Management of Hepatocellular Carcinoma: An Update; July 2010).

GLYPICAN 3 TARGETED THERAPY; ANTIBODY-BASED APPROACH

A therapeutic mAb (GC33) has been described that recognizes an epitope in the C-terminal portion of GPC3 (524-563). Generated in MRL/lpr mice against a GST-fusion protein fragment of C-terminal GPC3 (amino acids 524-563), GC33 is an IgG2a isotype capable of inducing antibody-dependent cellular cytotoxicity (ADCC), and has been shown to inhibit tumor growth of subcutaneous HepG2 and HuH-7 cell line xenografts transplanted into mice. The chimeric antibody in which the mouse variable regions were linked to the human IgG1 and kappa constant regions was derived by cloning the VH region of GC33 into pBluescript KS (+) that contained the constant region of human IgG1. VL regions were cloned into pBluescript KS (+) harboring the constant region of the human kappa chain. The resulting plasmid DNA was transfected into CHO cells by electroporation for further characterization [38].

GC33 has been humanized (hGC33) for clinical trials by complementarity-determining region grafting. Comparative studies with the parental GC33 antibody show similar results in the xenograft model. Ishiguro *et al.*, show that, in addition to inhibiting tumor growth in an orthotopic Hep G2 xenograft model, they could dramatically reduce the expression of AFP after treatment with GC33 at a dose of 5 mg/kg [39]. Animals were inoculated with 5×10^6 Hep G2 cells and after 21 days were administered anti-GC33 antibody or vehicle. Serum AFP levels in the antibody-treated group were reduced to less than 1 ng/ml whereas the control vehicle-treated group showed an increase in AFP to a mean of over 100 ng/ml. Concomitant with the decrease in AFP expression and inhibition of tumor growth was a stabilization in weight loss in treated animals. The ability of GC33 to synergize with chemotherapeutic agents was also evaluated. In the same Hep G2 xenograft model, administration of GC33 at 1 mg/kg or sorafenib at a maximum tolerated dose of 80 mg/kg inhibited tumor growth, and the additive effect of both agents was statistically significant. Collectively these results show significant therapeutic effect of GC33, both alone as a single agent or in combination with other chemotherapeutic drugs.

The mode of action of GC33 was evaluated considering the observation of ADCC in GPC3-positive hepatoma cells. A mutant antibody was generated that lacked carbohydrates, thereby preventing the ability to induce ADCC. This was achieved by amino acid substitution of alanine for asparagine at position 297 within the human IgG Fc region of the antibody. While the aglycosylated mutant humanized GC33 had the same antigen-binding affinity for GPC3 ($K_d = 0.67$ nmol/L), it was not capable of inducing ADCC in the presence of mouse effector cells. In these studies, the antitumor effect was essentially completely abolished by the absence of the carbohydrate moiety, suggesting that most if not all of the antitumor effect of GC33 is mediated by ADCC.

By using human peripheral blood mononuclear cells (PBMCs) depleted of either CD56⁺ or CD14⁺ cells, they show that the ADCC is carried out primarily by CD56⁺ natural killer cells, while additional studies suggest tumor-associated macrophages play a role in the antitumor effect [40]. They show tumor infiltration by macrophages, but also consider mechanisms other than ADCC such as GPC3 modulation. A substantial increase in the number of macrophages was seen in the outer tumor margins, and macrophage depletion dramatically reduced efficacy of anti-tumor effect by GC33 treatment. Histopathological time course analysis after a single injection of GC33 showed peak effect at days 3 through 5. Observed morphological changes included increased tumor cell death, tumor cells with rounded and/or multinucleated morphology and infiltration of small spindle cells (F4/80-positive macrophages). There was no demonstration of a direct effect on proliferation of tumor cells by treatment with GC33 as assessed by Ki-67 immunostaining, and this is supported by the absence of inhibition of SK03 or Hep G2 growth *in vitro* by GC33. An interesting aspect of GC33 treatment is its effect on the extracellular matrix (ECM). After administration of GC33, investigators observed a disappearance of Periodic acid-Schiff positive thin fibers, and a detachment of round cells from the remaining fiber associated with tumor cell and mononuclear cell fusion. It is known that multinucleated hepatocytes occur in the surrounding disrupted architecture in HCC, and the adhesion of HCC cells to type I collagen and fibronectin is decreased by GPC3 expression [41]. Therefore macrophage-mediated changes to the ECM in HCC may contribute to tumorigenicity and invasiveness. GC33 is currently in clinical trials in humans as a safety and tolerability study (A Phase I, Open-Label, Multi-center, Dose-escalation Study of the Safety, Tolerability, and Pharmacokinetics of GC33 Administered Weekly in Patients With Advanced or Metastatic Hepatocellular Carcinoma (HCC); ClinicalTrials.gov Identifier: NCT00746317) and as a treatment study in combination with sorafenib (A Phase I, Open-Label, Multi-center, Dose-escalation Study of the Safety, Tolerability, and Pharmacokinetics of GC33 in Combination With Sorafenib (Nexavar®) in Patients With Advanced or Metastatic Hepatocellular Carcinoma (HCC); ClinicalTrials.gov Identifier: NCT00976170).

IMMUNOTHERAPEUTIC GPC3 VACCINE

Nakatsura was first to identify a mouse GPC3 epitope for cytotoxic T-lymphocytes (CTLs) in BALB/c mice and to initiate a pre-clinical study to investigate the usefulness of GPC3 as a target for cancer immunotherapy [42]. Mouse K^d is similar to the common human class I HLA-A24 histocompatibility antigen, so they identified portions of GPC3 that constituted binding motifs for each molecule. Twelve peptides were identified and used to generate CTLs. One K^d-restricted peptide (GPC3-8 EYILSLEEL; GPC3₂₉₈₋₃₀₆) induced CTLs with specific cytotoxicity for Colon26 cancer cells transfected with the mouse GPC3 gene (C26/GPC3). Inoculation of these CTLs by intra-venous route into sublethally irradiated mice inhibited *in vivo* growth of established C26/GPC3 subcutaneous tumor. In addition, inoculation of bone marrow-derived dendritic cells (DCs) pulsed with this CTL peptide prevented the growth of subcutaneous and splenic C26/GPC3 cells and was associated with significant infiltration of CD8⁺ T cells into tumors. For these experiments, mice were placed in 5 groups; 1) C26/GPC3 cells + DCs +GPC3₂₉₈₋₃₀₆; 2) group 1 minus GPC3₂₉₈₋₃₀₆; 3) C26/GPC3 cells alone; 4) parental C26 cells + DCs +GPC3₂₉₈₋₃₀₆; and 5) parental C26 cells

alone. Peptide-pulsed or unpulsed DCs were injected intraperitoneally twice at 7-day intervals and subcutaneous inoculation of C26 or C26/GPC3 cells occurred 7 days after the last vaccination. In all groups except group 1 (C26/GPC3 cells + DCs + GPC3₂₉₈₋₃₀₆), tumor appeared 13 days after inoculation and continued at least through 38 days. All mice in group 1 rejected C26/GPC3 cells, and no tumor was observed at 150 days after inoculation. Additional studies with the GPC3₂₉₈₋₃₀₆ peptide showed that proper adjuvants are needed to induce CTLs in vaccine studies. GPC3₂₉₈₋₃₀₆ peptide alone was insufficient to induce an immune response; at least two vaccinations with a single dose greater than 10 µg were needed, and repeated vaccination with lower doses of GPC3₂₉₈₋₃₀₆ did not induce peptide-specific CTL responses. The same dose-dependent immunization constraints were observed with a second HLA-A2 (A*0201)-restricted peptide (GPC3₁₄₄₋₁₅₂) [43].

Hayashi *et al.*, quantified the number of GPC3-specific CTLs in the peripheral blood of patients with either chronic hepatitis or liver cirrhosis [44]. They selected patients who were HCV-RNA (+) or HBs antigen (+) within 6 months prior to the study for which no HCC nodules were detected by radiologic imaging. A total of 56 patients with chronic hepatitis or liver cirrhosis, and 45 patients with HLA-A24+ or HLA-A2+ were enrolled in the study and PBMCs were tested by ELISPOT assay using HLA-A24- and HLA-A2-restricted GPC3 peptides. In the ELISPOT assay, GPC3-specific CTLs were detected in 22% of the patients with chronic hepatitis or cirrhosis. GPC3-specific CTLs were present not only in patients with HCC, but also in patients with chronic hepatitis or cirrhosis, suggesting not only the possibility of GPC3-specific CTLs serving as a potential therapeutic agent, but also as a marker for the early diagnosis of HCC.

Further study suggesting a role for CTL responses in HCC are shown by Hiroishi *et al.*, where they determined that a vigorous CD8+ T-cell response against tumor antigens is associated with a prolonged recurrence-free period after treatment with trans-arterial chemo-embolization or radiofrequency ablation [45]. They tested CTL response by interferon-γ ELISPOT to a pool of peptide antigens derived from GPC3, NY-ESO-1 and MAGE-1. In univariate analysis, the number of tumor antigen-specific CD8+ T-cells after treatment was significantly associated with the duration of tumor-free period and on multivariate analysis, only the magnitude of the tumor antigen-specific CD8+ T-cell response was associated with prolonged tumor-free interval. More work is needed to dissect the contribution of GPC3-specific T-cells in this setting. These findings show strong evidence for the ability of GPC3 peptides to generate vigorous *in vivo* CTL immunity and for those responses to associate with tumor progression.

SOLUBLE GLYPICAN 3 AS A THERAPEUTIC TOOL FOR THE TREATMENT OF HCC

Significantly, GPC3 is not only a marker for the early diagnosis of HCC, but also promotes the growth of HCC by stimulating canonical Wnt signaling [16, 46]. GPC3 binds to Wnt through its core protein, and needs to be attached to the cell membrane to be able to display its growth-promoting activity. Based on these findings, it was hypothesized that GPC3 stimulates Wnt signaling by facilitating and/or stabilizing the interaction of Wnt with

Frizzled (Fz), its signaling receptor, and that a secreted version of GPC3 would inhibit autocrine and/or paracrine Wnt signaling by removing this growth factor from the cell surface [47]. Furthermore, it was proposed that because sGPC3 displays heparan sulfate chains, this soluble glypican could also block the activity of other heparin-binding growth factors that are produced by HCC tumors. To test this hypothesis Zittermann *et al.*, generated a mutated GPC3 lacking the GPI anchoring domain (sGPC3), and transfected various HCC lines with a sGPC3-expressing vector. The *in vitro* and *in vivo* growth of the sGPC3-expressing cell lines was then compared with that of the corresponding parental cell lines. The results clearly showed that sGPC3 can significantly inhibit the growth of HCC both *in vitro* and *in vivo*. The mechanism by which sGPC3 acts as an inhibitory agent was also investigated. As expected, sGPC3 inhibited Wnt signaling in some of the studied HCC cell lines. However, inhibition of Erk1/2- and Akt-associated signaling pathways was also observed in some cell lines, providing experimental support to the idea that sGPC3 could inhibit tumor progression by simultaneously blocking the activity of several growth factors. Furthermore, analysis of tissue sections of the sGPC3-expressing tumors showed that this secreted glypican can also inhibit angiogenesis. In summary, this study demonstrated that sGPC3 can inhibit HCC growth by acting at various levels of the pro-tumorigenic phenotype. Given the high heterogeneity of the molecular pathology of HCC, the use of a therapeutic tool like sGPC3, which can simultaneously block several pro-tumorigenic mechanisms, is highly promising.

Recently, Feng *et al.*, provided additional experimental evidence to support the use of sGPC3 as a therapeutic tool for HCC. These authors demonstrated that purified recombinant sGPC3 can inhibit the *in vitro* growth of the HepG2 HCC cell line [48]. Whether recombinant sGPC3 can also inhibit the *in vivo* growth of HCC cells remains to be investigated

POTENTIAL ASSOCIATION WITH WNT/3-CATENIN THERAPEUTICS

Activation of the canonical Wnt pathway has been found to be one of the most frequent events associated with the malignant transformation of liver cells [49–52]. Because GPC3 promotes HCC growth by stimulating canonical Wnt signaling, treatments that target this pathway downstream of this glypican could offer alternative possibilities for blocking its pro-tumorigenic activity.

Experimental therapeutic studies have already established that the *in vitro* and *in vivo* growth of HCC can be reduced by the inhibition of the canonical Wnt pathway [53, 54]. For example, Hu *et al.*, showed that adenoviral vectors encoding two extracellular inhibitors of Wnt signaling (WIF1 and sFRP1) can inhibit HCC growth in mice. Recently, the identification of small molecule inhibitors of 3-catenin, the key transcription factor that mediates canonical Wnt signaling, has been reported [55]. Likewise, a number of existing drugs and natural compounds have been shown to block this signaling pathway. In principle, therefore, these drugs and compounds could also be used to block GPC3-induced activation of Wnt signaling in HCC.

CONCLUSION

Glypican-3 has progressed from being a promising candidate biomarker for early HCC to a validated target for several therapeutic approaches. While much remains to be discovered about the mechanism of GPC3's function in HCC, there are clear associations with molecular pathways broadly implicated in oncogenesis. Antibody-based therapy is most clinically advanced, and investigators will watch for results as ongoing trials proceed. Given the appearance of GPC3 in early, high-grade dysplastic macronodules, plus the significant proportion of overt HCCs that are immunoreactive with anti-GPC3 antibodies, and the promising *in vitro* and *in vivo* animal model therapeutic studies, GPC3 represents a unique opportunity in cancer therapy where the approach is directly targeted on a molecular marker that is expressed very early in the course of disease, and remains expressed on a high proportion of intermediate and advanced tumors. Therefore it is reasonable to be optimistic about the prospects for therapies targeting GPC3 to dramatically improve clinical outcome in HCC.

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