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Review

Glypican-3: A new target for cancer immunotherapy

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ABSTRACT

Hepatocellular carcinoma (HCC) remains a common malignant cancer worldwide. There is an urgent need to identify new molecular targets for the development of novel therapeutic approaches. Herein, we review the structure, function and biology of glypican-3 (GPC3) and its role in human cancer with a focus on its potential as a therapeutic target for immunotherapy. GPC3 is a cell-surface protein that is over-expressed in HCC. Loss-of-function mutations of GPC3 cause Simpson–Golabi–Behmel syndrome (SGBS), a rare X-linked overgrowth condition. GPC3 binds Wnt and Hedgehog (Hh) signalling proteins. GPC3 is also able to bind basic growth factors such as fibroblast growth factor 2 through its heparan sulphate glycan chains. GPC3 is a promising candidate for liver cancer therapy given that it shows high expression in HCC. An anti-GPC3 monoclonal antibody has shown anti-cancer activity in mice and its humanised IgG molecule is currently undergoing clinical evaluation in patients with HCC. There is also evidence that soluble GPC3 may be a useful serum biomarker for HCC.

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1. Introduction

Liver cancer is the fifth most common malignant cancer worldwide. According to the American Cancer Society (www.cancer.org), hepatocellular carcinoma (HCC) accounts for about 75% of liver cancer cases. Cholangiocarcinoma (CCA) is the second most common primary liver malignant tumour arising from cholangiocytes and CCA accounts for about 10% of primary liver cancer cases. In 2009, 22,620 new cases of primary liver cancer were found and 18,160 of these patients died in the United States. There are often no symptoms of liver cancer until the later stages. Currently, surgery is the standard treatment for liver cancer. Liver cancer does not respond to most chemotherapy drugs. There is an urgent need to develop new drugs with different mechanisms of action. Immunotherapy represents one new approach, but it remains a challenge mainly due to lack of good tumour-specific targets.

Glypican-3 (GPC3, also called DGSX, GTR2-2, MXR7, OCI-5, SDYS, SGB, SGBS, and SGBS1) is a cell surface protein that is highly expressed in HCC and some other human cancers including melanoma. The *GPC3* gene encodes a 70-kDa precursor core protein, which can be cleaved by furin to generate a 40-kDa amino (N) terminal protein and a 30-kDa membrane-bound carboxyl (C) terminal protein, which has two heparan sulphate (HS) glycan chains. The GPC3 protein is attached to the cell membrane by a glycosyl-phosphatidylinositol (GPI) anchor (Fig. 1). The C terminal membrane-bound

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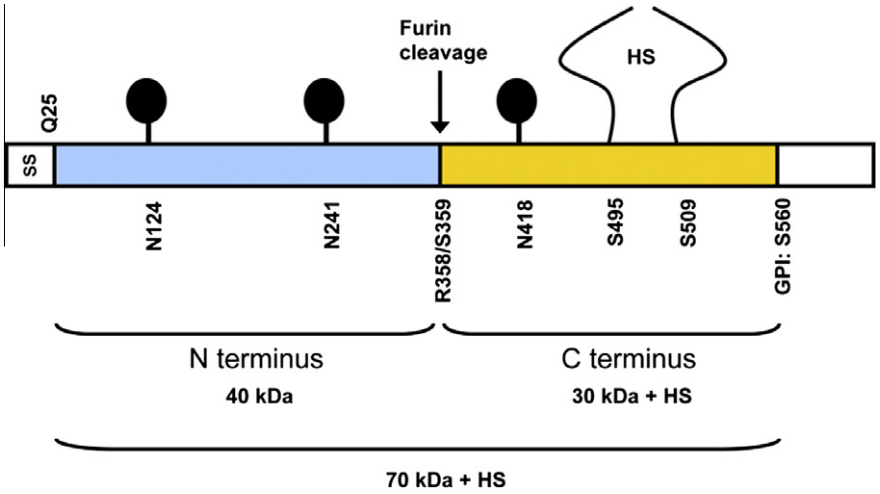

Fig. 1 – Schematic of the glypican-3 (GPC3) protein. The human GPC3 gene encodes a 70 kDa precursor protein of 580 amino acids. Upon translocation into the endoplasmic reticulum, the N-terminal signal peptide (SS; residues 1–24) and the C-terminal glycosyl-phosphatidylinositol (GPI) anchor addition signal (a predicted cleavage site: S560) are removed and the latter is replaced with a GPI anchor. The GPC3 precursor is cleaved into two subunits, NH_2 terminus (residues Q25 – R358) and the GPI-anchored membrane-bound COOH terminus starting from S359. The three N-linked glycans (black lollipops; N124, N241 and N418) are indicated.

protein is recognised by the monoclonal antibody (mAb) 1G12. Loss-of-function mutations of GPC3 cause Simpson–Golabi–Behmel syndrome (SGBS), a rare X chromosome-linked overgrowth disorder typically associated with coarse faces with protruding jaw and tongue, widened nasal bridge and upturned nasal tip.[1] The patients are usually quite tall. The mice with GPC3 knockout show similar symptoms as seen in SGBS.[2] GPC3 binds Wnt and Hedgehog (Hh) proteins.[3,4] GPC3 is also able to bind fibroblast growth factor-2 (FGF-2) through its HS chains.[5] Since it shows high expression in HCC, GPC3 has a potential as a promising target for tumour-specific therapy. Also, because small amounts of GPC3 can be detected in the blood of some patients with GPC3-positive cancers,[6,7] measurement of GPC3 in the blood may be a useful diagnostic to follow the course of these patients. This review will give a brief overview of the structure, function and biology of GPC3 and its role in human cancer with a focus on its potential as a therapeutic target for immunotherapy.

2. GPC3 structure

In 1988, Filmus and colleagues identified the gene called *OCI-5* in a rat undifferentiated epithelial cell line.[8] The *OCI-5* gene was later named *GPC3* based on its homology with other known members of the glypican family.[1] The *GPC3* gene is located on human X chromosome (Xq26) where the most common gene (Isoform 2, GenBank Accession No.: NP_004475) encodes a 70-kDa core protein with 580 amino acids. Three variants have been detected that encode alternatively spliced forms termed Isoforms 1 (NP_001158089), Isoform 3 (NP_001158090) and Isoform 4 (NP_001158091). The distribution and functional significance of GPC3 isoforms are unknown. The protein core of GPC3 consists of two subunits, where the N-terminal subunit has a size of ~40 kDa and the C-terminal subunit is ~30 kDa (Fig. 1). Furin cleavage between Arg358 and Ser359 is required for GPC3 modulation of cell survival and Wnt signalling in zebrafish,[9] but is not required for HCC cell growth.[10]

Six glypicans (GPC1–6) have been identified in mammals. All glypicans share a characteristic structure. First, they all have a conserved pattern of 14 cysteine residues, which may form intramolecular disulphide linkages.[11] Second, they all consist of the HS chains in the C-terminal region close to the cell membrane.[12] Third, glypicans are anchored to the cell surface via a GPI linkage. Using the big-PI Predictor (http://mendel.imp.ac.at/sat/gpi/gpi_server.html), serine 560 is predicted as a cleavage site in GPC3 for GPI anchorage (Fig. 1). These common features suggest that glypicans may share a similar three-dimensional (3D) structure.

3. GPC3 biology

Mutations in GPC3 result in SGBS, an X-linked condition characterised by pre- and postnatal overgrowth with visceral and skeletal anomalies.[1] GPC3-deficient mice are able to exhibit the clinical hallmarks of SGBS patients.[2] However, the biological functions of GPC3 and its role in tumourigenesis remain elusive.

As suggested by the knockout phenotype, GPC3 is involved in the control of cell proliferation and/or survival due possibly to its interaction with insulin-like growth factor (IGF)-2. However, many biochemical and genetic studies,[2,5,13–15] except one,[16] showing that GPC3 does not regulate IGF signalling. Song et al. mated GPC3 knockout mice with insulin receptor substrate-1 (IRS-1) nullizygous mice and showed that GPC3 regulated organism growth independent of IGF signalling.[15]

Interestingly, GPC3 knockout mice exhibit alterations in Wnt signalling. GPC3 can form a complex with Wnt molecules and promote the growth of HCC by stimulating canonical Wnt signalling.[4] It has also been established in genetic

experiments that GPC3 regulates developmental growth by interacting with the Hh signalling pathway.[3,17] Additionally, the HS chains of GPC3 are not required for its interaction with either Wnt or Hh proteins. While the HS chains are not essential for Wnt and Hh signalling, they may interact with other basic growth factors for cell growth. There is evidence showing that the HS chains can interact with FGF-2 and modulate growth factor activity.[5] An interaction between GPC3 and FGF-2 has also been found in HCC cells.[18]

It has been hypothesised that a mutated GPC3 lacking the GPI anchoring domain can block Wnt signalling and inhibit the growth of Wnt-dependent tumours. Zittermann et al. recently showed that HCC cells infected with lentivirus expressing soluble GPC3 (sGPC3) have a lower cell proliferation rate possibly due to the sGPC3 protein secreted by infected cells.[19] We have recently made recombinant sGPC3 (GPC3ΔGPI, amino acid residues Q25–H559) that lacks the GPI-anchoring domain in human HEK-293 cells and found that recombinant sGPC3 protein can inhibit the growth of HCC.[20] However, we cannot rule out the possibility that inhibition of HCC growth may be due to the activity of other factors such as heparin-binding growth factors modulated by the HS chains.

4. GPC3 expression in human malignancies

HCC and CCA are the two major forms of primary liver cancer. There is a growing body of evidence to support that GPC3 is a new tumour marker for HCC. GPC3 is highly expressed in HCC,[21] but not in CCA or normal liver tissue (Fig. 2). GPC3 is also expressed to a lesser degree in melanoma,[22] ovarian clear-cell carcinomas,[23] yolk sac tumours (YST),[24] neuroblastoma, hepatoblastoma, Wilms' tumour cells and other tumours.[25,26] However, the significance of GPC3 as a diagnostic tool for human tumours other than HCC is unclear. On the other hand, GPC3 is silenced in breast cancer, mesothelioma, epithelial ovarian cancer and lung adenocarcinoma.[27–29]

4.1. *GPC3 expression in HCC*

GPC3 is highly expressed in HCC. In 1997, Hsu and colleagues first reported expression of GPC3 mRNA in 75% of HCC and 10% of gastric adenocarcinoma but not in CCA.[21] GPC3 is highly expressed in the HCC cell lines, HepG2, Hep3B, HT17, HuH6, HuH7 and PLC/PRF/5.[18] GPC3 protein expression is found in more than 70% of HCC tumours but not in normal liver tissue when using a rabbit polyclonal antibody raised against human GPC3 (residues 303–464; Catalogue No.: sc-11395, Santa Cruz Biotechnology, California)[30] or with mAb 1G12.[7] Due to the finding that GPC3-positive HCC patients have a significantly lower 5-year survival rate than GPC3-negative HCC patients, GPC3 expression is correlated with poor prognosis in HCC.[31]

4.2. *GPC3 expression in melanoma*

GPC3 has also been proposed as a novel tumour marker for the diagnosis of melanoma, especially in the early stages of the disease.[32] GPC3 mRNA and protein have been found in >80% of melanomas and melanocytic naevus. Using a goat polyclonal antibody (W-18) raised against a NH_2-terminal peptide (sc-10455, Santa Cruz Biotechnology, CA), an ELISA shows that sGPC3 protein is detected in the sera of 40% of melanoma patients. Surprisingly, serum GPC3 can even be detected in patients with stage 0 *in situ* melanoma. GPC3 protein in sera from patients dramatically decreases after surgical removal of the melanoma.[32] However, the role of GPC3 in melanomas is controversial. Kandil et al. could not detect the expression of GPC3 in melanoma using the 1G12 mAb specific for the C terminus.[33] Further studies are necessary to validate GPC3 as a new tumour marker in early detection and follow-up of patients with melanoma.

4.3. *GPC3 expression in ovarian cancer and other tumours*

Early studies have found that GPC3 expression is undetectable in a significant proportion of ovarian cancer cell lines.[34] However, using the 1G12 mAb, Stadlmann et al. recently examined tissues from 308 patients and found that GPC3 was expressed in a total of 18% of ovarian carcinomas.[23] Interestingly, more than 60% of ovarian clear-cell carcinomas express GPC3. The results suggest that GPC3 may represent a potential biomarker for ovarian clear-cell carcinomas.[23] However, using the same 1G12 mAb, Esheba et al. showed that most ovarian clear-cell carcinomas were negative for GPC3.[35] Because the histological distinction between clear cell adenocarcinoma

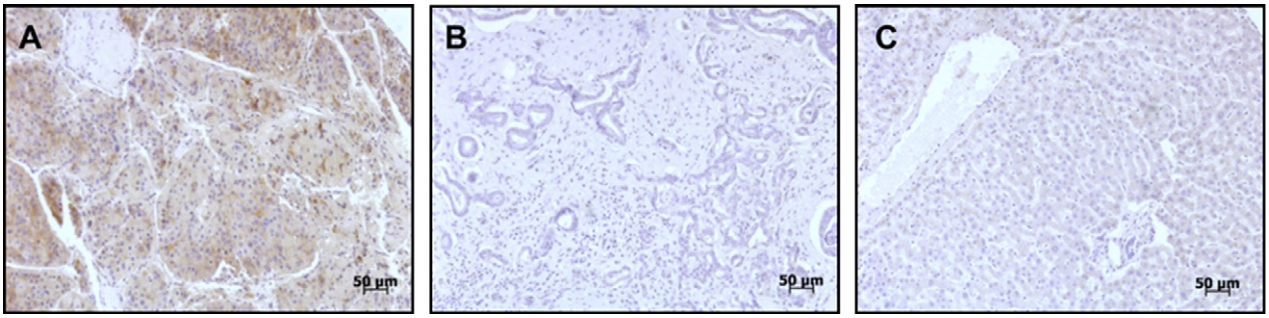

Fig. 2 – GPC3 expression in human primary liver tumours. GPC3 expression was detected by immunohistochemistry using mAb 1G12 in tissue specimens of patients with hepatocellular carcinoma (HCC) (A); cholangiocarcinoma (CCA) (B); and normal liver tissue (C). Brown staining indicates GPC3 protein expression.

and yolk sac tumour is difficult, it has been suggested that GPC3 immunostaining in ovarian tumours should be used in combination with other tumour markers.[36]

GPC3 immunostaining may be useful in the diagnosis of YST and choriocarcinoma,[24,35] since there is evidence showing that YST and choriocarcinoma are immunoreactive for GPC3. Expression of GPC3 is also found in neuroblastoma, hepatoblastoma, Wilms' tumour cells,[26,37] testicular non-seminomatous germ cell tumours,[25] liposarcoma[25] and a distinct group of gastric carcinomas called GPC3-GC.[38] Further studies are needed to determine the role of GPC3 as a tumour marker in these tumours.

5. GPC3-targeted immunotherapy

Given the high expression of GPC3 in HCC, melanoma and clear cell carcinomas of the ovary, GPC3 has been evaluated as a potential candidate for cancer immunotherapy. The usefulness of GPC3 as a therapeutic target for both antibody- and cell-based immunotherapies has been explored.[32,39]

5.1. *Anti-GPC3 antibody therapy in HCC*

In 2003, a mAb against a GPC3 peptide consisting of 17 residues (355–371) was reported as a research tool to study the interaction of GPC3 and FGF-2.[18] Also in 2003, Capurro et al. described a mAb called 1G12 (IgG1, κ) specific for the last 70 amino acids of the C terminus of the GPC3 core protein[7] and used it to detect serum GPC3 in HCC patients. Hippo et al. developed two mAbs, M18D04 and M19B11, specific for the N terminus of GPC3 (residues: 25–358).[6] Although both laboratories used the mAbs with two different terminal groups, they found a similar proportion of GPC3-positive sera in HCC patients. In addition, Yamauchi et al. made a form of GPC3 protein lacking the GPI anchor (GPC3GPI) region in CHO cells and used the recombinant protein as an immunogen to obtain two mAbs, A1836A for the N terminus of GPC3 and GPC3-C02 for the C terminus. These mAbs were used as reagents for immunohistochemistry analysis of cancer.[40]

The first therapeutic mAb against GPC3 has recently been described.[39,41] Its epitope is located at the C terminus (residues: 524–563). The mAb, designated GC33 (IgG2a, κ), induced antibody-dependent cellular cytotoxicity (ADCC) and exhibited tumour growth inhibition of subcutaneous transplanted HepG2 and HuH-7 ectopic xenografts in mice. GC33 also reduced the blood α-fetoprotein levels of mice intrahepatically transplanted with HepG2 cells in an orthotopic model. Humanised GC33 (hGC33) has been generated by CDR grafting and is as effective as GC33 against the HepG2 xenograft.[42] The ADCC anti-tumour activity of GC33 is mainly due to natural killer cells.[39] On the other hand, Takai et al. investigated the relationship between membrane expression of GPC3 and recruitment of tumour-associated macrophage (TAM).[43,44] Based on these evidences, they observed the involvements of infiltrated TAM in anti-GPC3 immunotherapy model using GC33, showing macrophages may play an important role in the anti-tumour activity of GC33 by non-ADCC mechanisms such as modulation of GPC3 functions.[45] In addition, GC33 does not directly inhibit the proliferation of GPC3-positive tumour cells.[45] To fully evaluate GPC3-targeted antibody therapy, the mAbs that target different functional domains (including the HS chain) of GPC3 would be useful. It would be interesting to investigate the anti-tumour activity of the anti-GPC3 mAbs that are able to directly inhibit cancer cell proliferation and/or survival by blocking Wnt and/or other signalling pathways. It has been suggested that an anti-GPC3 antibody may increase the susceptibility of HCC to chemotherapeutic agents.[46] The anti-tumour activity of hGC33 combined with standard chemotherapy agents has recently been evaluated. The combination of hGC33 and sorafenib is more potent in inhibiting tumour growth than sorafenib alone in the HepG2 xenograft model. The combination regimen may be clinically useful as an anti-liver cancer therapy. hGC33 is currently being evaluated in phase I clinical trials for liver cancer (Chugai Pharmaceutical Development Pipeline Report, April 23, 2010; http://www.chugai-pharm.co.jp/english/ir/pdf/100423ePipeline.pdf).

5.2. *GPC3 cancer vaccines*

There is an increasing evidence that GPC3 is an effective target in HCC and melanoma for cancer vaccines. In subcutaneous ectopic xenografts in mice using the Colon26 cell line transfected with mouse *GPC3* gene, the transfer of GPC3-reactive cytotoxic T lymphocytes (CTL) or GPC3 peptide pulsed dendritic cells elicit a protective effect in mice.[32] This work indicates that GPC3 is highly immunogenic in mice and can induce effective antitumour immune response with no evidence of autoimmunity. Transgenic mice have been used to identify the HLA-A2-restricted GPC3 epitopes in order to expand the applications of GPC3-based immunotherapy to HLA-A2+ HCC patients.[47] The GPC3 (residues: 144–152) peptide can induce peptide-reactive CTLs in transgenic mice. A phase I clinical trial of a GPC3-derived peptide vaccine for advanced HCC is now underway.[48] In another study, stem cell-derived dendritic cells expressing GPC3 can elicit protective immunity against mouse B16-F10 melanoma, naturally expressing GPC3 and GPC3-transfectant MCA205 sarcoma.[49] Both CD8+ and CD4+ T cells may contribute to the antitumour effect. Therefore, it is possible that dendritic cells expressing GPC3 may be useful for cancer therapy.

6. Soluble GPC3 as an HCC diagnostic tumour marker

Filmus and colleagues developed the 1G12 mAb specific for the C terminus of GPC3 and established an ELISA method to measure serum GPC3 in HCC patients.[7] In this study, GPC3 was significantly increased in the serum of 53% of patients with HCC but undetectable in healthy donors and patients with hepatitis. On the other hand, Hippo et al. developed mAbs specific for the N terminus (residues: 25–358) of GPC3 and found GPC3 was significantly increased in the serum of 51% of HCC patients.[6] In a recent study done in Thailand using 1G12, serum GPC3 levels were increased in 53% patients with HCC.[50] The percentage was in agreement with previous reports.[6,7] However, it is not clear what form of the GPC3 protein is actually present in the circulating blood of cancer

patients. As such, this is an important question for GPC3 as a serological marker of HCC and other tumours. The published studies coincide that the N-terminus and C-terminus of GPC3 are linked by disulphide bonds despite the cleavage of GPC3 by furin. Future studies are necessary to reveal the biochemistry of serum GPC3.

7. GPC3 immunohistochemistry for HCC tumour diagnosis

GPC3 has been proposed as an immunohistochemistry marker to differentiate HCC from benign hepatocyte nodules.[40] It remains an essential problem to differentiate between HCC and CCA, particularly intrahepatic CCA (ICC). GPC3 expression cannot be found in either ICC or gallbladder cancer. Thus, these studies have suggested a diagnostic potential of GPC3 in primary liver cancer, particularly HCC.[50,51] As shown in Fig. 2, most of the GPC3 expression patterns in HCC show the cytoplasmic pattern. Shirakawa and colleagues previously found that almost half of the HCC cases showed the mixed pattern (cytoplasm and membrane) and the other half showed only the cytoplasmic pattern.[31] A recent study was performed using HepG2 xenograft tissues to optimise the tissue processing method for GPC3 immunohistochemistry.[52] Further studies are needed to clarify whether a different localisation of GPC3 has a different functional significance and a diagnostic value. An examination of additional patient studies from various human risk populations including specific clinicopathological features and prognostic values is also necessary to provide further insight into GPC3 detection as a general diagnostic tool.

8. Conclusions

GPC3 is highly expressed in HCC and currently being evaluated as a target for antibody- and cell-based therapies of HCC. Future work may lead to the generation of new anti-GPC3 mAbs that target various functional domains including the HS chain and examine whether an anti-GPC3 mAb can directly inhibit tumour growth by blocking the ligand–receptor interactions involved in Wnt and/or other signalling pathways. Also, recent studies have indicated that soluble GPC3 could be valuable as a tumour marker for diagnosis and follow-up of patients with GPC3-expressing malignancies. However, the biochemistry of serum GPC3 remains elusive. It would be interesting to investigate the 3D structure of GPC3 and the molecular mechanisms underlying the shedding of GPC3 from cancer cells. In addition, immunohistochemistry studies show GPC3 is valuable to the pathologist for tumour diagnosis. Further studies with a large number of patient studies from various human risk populations and with GPC3 alone or in combination with other tumour proteins would validate the use of GPC3 as a diagnostic marker for HCC and other GPC3-expressing human tumours.

Conflict of interest statement

None declared.

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REFERENCES

1. Pilia G, Hughes-Benzie RM, MacKenzie A, et al. Mutations in GPC3, a glypican gene, cause the Simpson–Golabi–Behmel overgrowth syndrome. *Nat Genet* 1996;**12**:241–7.
2. Cano-Gauci DF, Song HH, Yang H, et al. Glypican-3-deficient mice exhibit developmental overgrowth and some of the abnormalities typical of Simpson–Golabi–Behmel syndrome. *J Cell Biol* 1999;**146**:255–64.
3. Capurro MI, Xu P, Shi W, et al. Glypican-3 inhibits Hedgehog signaling during development by competing with patched for Hedgehog binding. *Dev Cell* 2008;**14**:700–11.
4. Capurro MI, Xiang YY, Lobe C, Filmus J. Glypican-3 promotes the growth of hepatocellular carcinoma by stimulating canonical Wnt signaling. *Cancer Res* 2005;**65**:6245–54.
5. Song HH, Shi W, Filmus J. OCI-5/rat glypican-3 binds to fibroblast growth factor-2 but not to insulin-like growth factor-2. *J Biol Chem* 1997;**272**:7574–7.
6. Hippo Y, Watanabe K, Watanabe A, et al. Identification of soluble NH_2-terminal fragment of glypican-3 as a serological marker for early-stage hepatocellular carcinoma. *Cancer Res* 2004;**64**:2418–23.
7. Capurro M, Wanless IR, Sherman M, et al. Glypican-3: a novel serum and histochemical marker for hepatocellular carcinoma. *Gastroenterology* 2003;**125**:89–97.
8. Filmus J, Church JG, Buick RN. Isolation of a cDNA corresponding to a developmentally regulated transcript in rat intestine. *Mol Cell Biol* 1988;**8**:4243–9.
9. De CB, Muyldermans SY, Coomans C, et al. Processing by proprotein convertases is required for glypican-3 modulation of cell survival, Wnt signaling, and gastrulation movements. *J Cell Biol* 2003;**163**:625–35.
10. Capurro MI, Shi W, Sandal S, Filmus J. Processing by convertases is not required for glypican-3-induced stimulation of hepatocellular carcinoma growth. *J Biol Chem* 2005;**280**:41201–6.
11. Veugelers M, Vermeesch J, Reekmans G, et al. Characterization of glypican-5 and chromosomal localization of human GPC5, a new member of the glypican gene family. *Genomics* 1997;**40**:24–30.
12. Filmus J. Glypicans in growth control and cancer. *Glycobiology* 2001;**11**:19R–23R.
13. Chiao E, Fisher P, Crisponi L, et al. Overgrowth of a mouse model of the Simpson–Golabi–Behmel syndrome is independent of IGF signaling. *Dev Biol* 2002;**243**:185–206.
14. Paine-Saunders S, Viviano BL, Zupicich J, Skarnes WC, Saunders S. Glypican-3 controls cellular responses to Bmp4 in limb patterning and skeletal development. *Dev Biol* 2000;**225**:179–87.
15. Song HH, Shi W, Xiang YY, Filmus J. The loss of glypican-3 induces alterations in Wnt signaling. *J Biol Chem* 2005;**280**:2116–25.

16. Cheng W, Tseng CJ, Lin TT, et al. Glypican-3-mediated oncogenesis involves the insulin-like growth factor-signaling pathway. *Carcinogenesis* 2008;**29**:1319–26.
17. Capurro MI, Li F, Filmus J. Overgrowth of a mouse model of Simpson–Golabi–Behmel syndrome is partly mediated by Indian Hedgehog. *EMBO Rep* 2009;**10**:901–7.
18. Midorikawa Y, Ishikawa S, Iwanari H, et al. Glypican-3, overexpressed in hepatocellular carcinoma, modulates FGF2 and BMP-7 signaling. *Int J Cancer* 2003;**103**:455–65.
19. Zittermann SI, Capurro MI, Shi W, Filmus J. Soluble glypican 3 inhibits the growth of hepatocellular carcinoma in vitro and in vivo. *Int J Cancer* 2010;**126**:1291–301.
20. Feng M, Kim H, Phung Y, Ho M. Recombinant soluble glypican 3 protein inhibits the growth of hepatocellular carcinoma in vitro. *Int J Cancer* 2010. doi:10.1002/ijc.25549 [Epub ahead of print].
21. Hsu HC, Cheng W, Lai PL. Cloning and expression of a developmentally regulated transcript MXR7 in hepatocellular carcinoma: biological significance and temporospatial distribution. *Cancer Res* 1997;**57**:5179–84.
22. Nakatsura T, Kageshita T, Ito S, et al. Identification of glypican-3 as a novel tumor marker for melanoma. *Clin Cancer Res* 2004;**10**:6612–21.
23. Stadlmann S, Gueth U, Baumhoer D, et al. Glypican-3 expression in primary and recurrent ovarian carcinomas. *Int J Gynecol Pathol* 2007;**26**:341–4.
24. Zynger DL, Dimov ND, Luan C, Teh BT, Yang XJ. Glypican 3: a novel marker in testicular germ cell tumors. *Am J Surg Pathol* 2006;**30**:1570–5.
25. Baumhoer D, Tornillo L, Stadlmann S, et al. Glypican 3 expression in human nonneoplastic, preneoplastic, and neoplastic tissues: a tissue microarray analysis of 4,387 tissue samples. *Am J Clin Pathol* 2008;**129**:899–906.
26. Saikali Z, Sinnett D. Expression of glypican 3 (GPC3) in embryonal tumors. *Int J Cancer* 2000;**89**:418–22.
27. Kim H, Xu GL, Borczuk AC, et al. The heparan sulfate proteoglycan GPC3 is a potential lung tumor suppressor. *Am J Respir Cell Mol Biol* 2003;**29**:694–701.
28. Murthy SS, Shen T, De RA, et al. Expression of GPC3, an X-linked recessive overgrowth gene, is silenced in malignant mesothelioma. *Oncogene* 2000;**19**:410–6.
29. Xiang YY, Ladeda V, Filmus J. Glypican-3 expression is silenced in human breast cancer. *Oncogene* 2001;**20**:7408–12.
30. Nakatsura T, Yoshitake Y, Senju S, et al. Glypican-3, overexpressed specifically in human hepatocellular carcinoma, is a novel tumor marker. *Biochem Biophys Res Commun* 2003;**306**:16–25.
31. Shirakawa H, Suzuki H, Shimomura M, et al. Glypican-3 expression is correlated with poor prognosis in hepatocellular carcinoma. *Cancer Sci* 2009;**100**:1403–7.
32. Nakatsura T, Komori H, Kubo T, et al. Mouse homologue of a novel human oncofetal antigen, glypican-3, evokes T-cell-mediated tumor rejection without autoimmune reactions in mice. *Clin Cancer Res* 2004;**10**:8630–40.
33. Kandil D, Leiman G, Allegretta M, Evans M. Glypican-3 protein expression in primary and metastatic melanoma: a combined immunohistochemistry and immunocytochemistry study. *Cancer Cytopathol* 2009;**117**:271–8.
34. Lin H, Huber R, Schlessinger D, Morin PJ. Frequent silencing of the GPC3 gene in ovarian cancer cell lines. *Cancer Res* 1999;**59**:807–10.
35. Esheba GE, Pate LL, Longacre TA. Oncofetal protein glypican-3 distinguishes yolk sac tumor from clear cell carcinoma of the ovary. *Am J Surg Pathol* 2008;**32**:600–7.
36. Maeda D, Ota S, Takazawa Y, et al. Glypican-3 expression in clear cell adenocarcinoma of the ovary. *Mod Pathol* 2009;**22**:824–32.
37. Zynger DL, Gupta A, Luan C, et al. Expression of glypican 3 in hepatoblastoma: an immunohistochemical study of 65 cases. *Hum Pathol* 2008;**39**:224–30.
38. Ushiku T, Uozaki H, Shinozaki A, et al. Glypican 3-expressing gastric carcinoma: distinct subgroup unifying hepatoid, clear-cell, and alpha-fetoprotein-producing gastric carcinomas. *Cancer Sci* 2009;**100**:626–32.
39. Ishiguro T, Sugimoto M, Kinoshita Y, et al. Anti-glypican 3 antibody as a potential antitumor agent for human liver cancer. *Cancer Res* 2008;**68**:9832–8.
40. Yamauchi N, Watanabe A, Hishinuma M, et al. The glypican 3 oncofetal protein is a promising diagnostic marker for hepatocellular carcinoma. *Mod Pathol* 2005;**18**:1591–8.
41. Nakano K, Orita T, Nezu J, et al. Anti-glypican 3 antibodies cause ADCC against human hepatocellular carcinoma cells. *Biochem Biophys Res Commun* 2009;**378**:279–84.
42. Nakano K, Ishiguro T, Konishi H, et al. Generation of a humanized anti-glypican 3 antibody by CDR grafting and stability optimization. *Anticancer Drugs* 2010;**21**:907–16.
43. Takai H, Kato A, Kato C, et al. The expression profile of glypican-3 and its relation to macrophage population in human hepatocellular carcinoma. *Liver Int* 2009;**29**:1056–64.
44. Takai H, Ashihara M, Ishiguro T, et al. Involvement of glypican-3 in the recruitment of M2-polarized tumor-associated macrophages in hepatocellular carcinoma. *Cancer Biol Ther* 2009;**8**:2329–38.
45. Takai H, Kato A, Kinoshita Y, et al. Histopathological analyses of the antitumor activity of anti-glypican-3 antibody (GC33) in human liver cancer xenograft models: the contribution of macrophages. *Cancer Biol Ther* 2009;**8**:930–8.
46. Ishiguro T, Kinoshita Y, Sugimoto M, et al. Anti-glypican3 antibody for treatment of human liver cancer. In: Proceedings of the 101st annual meeting of the AACR. Washington, DC; 2010 April 17–21 [Abstract #A2426].
47. Komori H, Nakatsura T, Senju S, et al. Identification of HLA-A2- or HLA-A24-restricted CTL epitopes possibly useful for glypican-3-specific immunotherapy of hepatocellular carcinoma. *Clin Cancer Res* 2006;**12**:2689–97.
48. Motomura Y, Ikuta Y, Kuronuma T, et al. HLA-A2 and -A24-restricted glypican-3-derived peptide vaccine induces specific CTLs: preclinical study using mice. *Int J Oncol* 2008;**32**:985–90.
49. Motomura Y, Senju S, Nakatsura T, et al. Embryonic stem cell-derived dendritic cells expressing glypican-3, a recently identified oncofetal antigen, induce protective immunity against highly metastatic mouse melanoma, B16-F10. *Cancer Res* 2006;**66**:2414–22.
50. Tangkijvanich P, Chanmee T, Komtong S, et al. Diagnostic role of serum glypican-3 in differentiating hepatocellular carcinoma from non-malignant chronic liver disease and other liver cancers. *J Gastroenterol Hepatol* 2010;**25**:129–37.
51. Man XB, Tang L, Zhang BH, et al. Upregulation of glypican-3 expression in hepatocellular carcinoma but downregulation in cholangiocarcinoma indicates its differential diagnosis value in primary liver cancers. *Liver Int* 2005;**25**:962–6.
52. Takai H, Kato A, Ishiguro T, et al. Optimization of tissue processing for immunohistochemistry for the detection of human glypican-3. *Acta Histochem* 2010;**112**:240–50.