Biology and function of glypican-3 as a candidate for early cancerous transformation of hepatocytes in hepatocellular carcinoma (Review)

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Abstract. Glypican-3 (GPC-3), a transmembrane heparan sulfate proteoglycan (HSPG), has recently been investigated as a player in tissue-dependent cellular signaling, specifically as a regulator of growth. Noteworthy, the regulatory protein has been implicated in both stimulatory and inhibitory pathways involving cell growth. Initially, GPC-3 was thought to act as a cell cycle regulator, as a loss-of-function mutation in the gene caused a hyper-proliferative state known as Simpson-Golabi-Behmel (SGB) overgrowth syndrome. Additionally, certain cancer types have displayed a downregulation of GPC-3 expression. More recently, the protein has been evaluated as a useful marker for hepatocellular carcinoma (HCC) due to its increased expression in the liver during times of growth. In contrast, the GPC-3 marker is not detectable in normal adult liver. Immunotherapy that targets GPC-3 and its affiliated proteins is under investigation as these new biomarkers may hold potential for the detection and treatment of HCC and other diseases in which GPC-3 may be overexpressed. Studies have reported that an overexpression of GPC-3 in HCC predicts a poorer prognosis. This prognostic value further pushes the question regarding GPC-3's role in the regulation and progression of HCC. This review will summarize the current knowledge regarding the clinical aspects of GPC-3, while also synthesizing the current literature with the aim to better understand this molecule's biological interactions at a molecular level, not only in the liver, but in the rest of the body as well. Due to the existing gap in the literature surrounding GPC-3, we believe further investigation of function, structure and domains, cellular localization, and other subfields is warranted to evaluate the protein as a whole, as well as its part in the study of HCC.

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Abbreviations: CK19, cytokeratin 19; FGF2, fibroblast growth factor 2; GPC-3, glypican-3; GPI, glycosylphosphatidylinositol; HCC, hepatocellular carcinoma; HPC, hepatic progenitor cell; HSC, heparin sulfate chain; HSPG, heparan sulfate proteoglycan; Hh, hedgehog; IHC, immunohistochemistry; ISH, *in situ* hybridization; PH, partial hepatectomy; SGB, Simpson-Golabi-Behmel; SULF2, sulfatase 2; sGPC-3N, serum glypican-3 N-terminal; miRNA, microRNA; SCC, squamous cell carcinoma

Key words: glypican-3, hepatocarcinoma, liver, cancer

Contents

1. Introduction and structure of GPC-3

The glypicans, a family of proteins classified as HSPGs, have been shown to interact with a number of growth factors and modulate growth factor activity (1) and are linked to the extracellular side of cell membrane by a glycosylphosphatidylinositol (GPI) anchor (2). Members of this large family of transmembrane proteins have been identified in both mammals and drosophila: 6 glypicans (GPC-1 through GPC-6) in mammals, and two others in the fly (2). Glypicans have a core protein size of ~60-70 kDa and express an N-terminal secretory signal peptide along with a hydrophobic domain that is used for the addition of the GPI anchor at the C-terminus. Another characteristic that is shared by all glypicans is the location of the insertion sites for the heparan sulfate chains (HSC), which seems to be restricted to the last 50 amino acids in the C-terminus, placing the chains close to the cell membrane. Additionally, the position of 14 cysteine residues is conserved, further strengthening the structural relationship of the proteins within the family (2).

Much of the literature on GPC-3 stems from its proposed role *in vivo* as well as *in vitro* in cancer models. Multiple

studies have shown a correlation between GPC-3 and hepatocellular carcinoma (HCC) due to the nature of the protein being expressed during normal cellular growth and movement in hepatocytes (1,3-6). In patients who received surgical treatment for HCC, a decrease in GPC-3 protein was observed (3). In addition, in cells derived from hepatocellular carcinoma GPC-3 has been observed to be secreted into culture media (7). GPC-3 does not seems to be expressed by other liver cell types (hepatic stellate cells, kupffer cells, bile duct cells, endothelial cells and fibroblasts) but it appears to be expressed exclusively by hepatocytes. It is well known only that its expression from hepatocytes influences the activity and function of non-parenchymal liver cells.

GPC-3 was initially hypothesized to interact with insulin-like growth factor in selected cell types, though this notion has been challenged as this may not be the case in all cell types (8). In a study done on the loss-of-function mutation of GPC-3 in embryonic development, researchers observed an overgrowth of organs in fetal and postnatal development, known as Simpson-Golabi-Behmel overgrowth syndrome (9). This evidence cast GPC-3 into a strict role as a tumor suppressor. In another study looking at the metastatic adenocarcinoma mammary LM3 cell line, GPC-3 was shown to inhibit the canonical Wnt signals involved in cell proliferation and survival. In the same study, GPC-3 was also observed to play a role in activating the non-canonical Wnt pathway, which directs cell morphology and migration (8). Furthermore, GPC-3 was also found to act as a potential tumor suppressor in lung tissue (10). This has re-opened the case of GPC-3 as a strict suppressor of cell growth and introduced the possibility of its role in stimulating growth and even tumorigenesis in a tissue-dependent manner in some cell lineages.

This being said, certain key elements have been observed which give reason for upregulation of GPC-3 in certain cell types and not others. GPC-3 is able to bind Wnt and Hedgehog (Hh) signaling proteins and was also shown to have the ability to bind basic growth factors such as fibroblast growth factor 2 (FGF2) through its heparan-sulfate glycan chains (11). Additionally, expression of sulfatase 2 (SULF2), another tumor marker expressed in hepatocytes, was found to be correlated with increased expression of GPC-3. When GPC-3 was decreased, FGF2 binding was decreased in SULF2 expressing hepatocellular carcinoma cells. SULF2 expression in resected HCC tissue was also shown to have a worse prognosis and a higher rate of recurrence after surgery (12).

Herein we synthesize the literature on GPC-3, including its structure, expression in normal tissues, as well its role as a serum marker as well as possible therapeutic target. The review also includes current knowledge on GPC-3 expression as a prognostic marker in HCC and its role in liver regeneration post partial hepatectomy (PH). In addition, we discuss embryonic and adult tissue expression of GPC-3. Lastly, we report the interactions of GPC-3 with CD81 as CD81 has been shown to interact with GPC-3 as a binding partner (13,14).

2. Expression pattern of GPC-3 in human and mouse

Embryonic expression of GPC-3. Embryonic expression of GPC-3 differs greatly from the expression map seen in health adult tissues. In most embryonic tissues and organs, there is positive expression of GPC-3 as illustrated by immunohistochemistry (IHC) in humans and *in situ* hybridization (ISH) in mice. Organs or tissues that display positive expression in human embryonic tissues include: tissues of the digestive tract, the gonads, kidney, limb buds, liver, nerve tissue, oral cavity, pancreas, respiratory syndrome, tongue, and vertebrae (15). In pancreatic tissue, positive expression was seen in exocrine glands, while endocrine glands showed negative expression. In contrast, IHC revealed negative expression of GPC-3 in human embryonic skin tissue (15). Additional detail regarding location and description of GPC-3 expression in human embryonic tissue can be seen in Table I.

Organs or tissues that displayed positive GPC-3 expression in mouse embryonic tissues include: digestive tract, gonads, kidney, limb buds, liver, oral cavity, respiratory system, skin, tongue, and vertebrae (9,15). In contrast, ISH revealed negative expression of GPC-3 in mouse nerve tissue. Pancreas expression in mice was not reported in this study. While GPC-3 expression across mouse and human embryonic tissue appears consistent in most organs, there are some tissues that differ. While nerve tissue shows positive expression in humans, in mice it is not expressed. On the other hand, human tissue is negative for expression of GPC-3 in skin, but this same tissue is positive for the protein in mice (9). Additional detail regarding location and description of GPC-3 expression in mice embryonic tissue can be seen in Table I.

Studies have also shown decreased GPC-3 expression in the SGB overgrowth syndrome, causing a spectrum of congenital defects such as macrosomia, congenital heart disease, conduction defects, supernumerary nipples, diastasis recti/umbilical hernia, diaphragmatic hernia, renal dysplasia/nephromegaly, cryptorchidism/hypospadias, and hand anomalies (brachydactyly, cutaneous syndactyly, polydactyly) (16). This change in expression and resulting defects are due to a mutation that causes sequence variants as well as multiexon/whole gene deletion in 37-70% of male cases (16,17). Table I gives a summary of the SGB syndrome abnormalities found in various organs and tissues as a result of the GPC-3 loss of function mutation (15,16,18). In these cases, GPC-3 acts as a regulator for normal embryonic development and is only found to cause problems in embryonic tissue when it is mutated (Table I).

Expression of GPC-3 in normal adult tissue. Table II summarizes GPC-3 expression in adult tissues normal versus tumor, while Table I summarizes expression of GPC-3 in embryonic tissue in human and mice. As is shown in Table II, the current literature has some contradictions which must be further evaluated to better understand the expression of GPC-3 in tissue types. The tissues represented as having two or more sources contradicting one another in normal tissue (expressed/not expressed) are: gastric glands, kidney tubules, testicular germ cells, breast tissue, gall bladder, and ovary (19-27). In addition, there are also sources reporting no expression, trace amounts, or scarce expression in normal tissue. These tissues are: liver, brain, colon, esophagus, fat tissue, heart, lung, lymph, mouth and associated glandular tissues, pancreas, prostate, skeletal and smooth muscle, small intestine, thyroid and parathyroid, thymus, bladder, and uterus (2,19,21,22,28-33). In Fig. 1, expression of GPC-3 in normal tissue was constructed based

Table I. GPC-3 expression in human and mouse embryonic tissue.

Organ	Embryonic human staining (IHC) (15)	Embryonic mouse tissue (ISH) (9)	Additional description/location of embryonic expression of GPC-3 (15)	SGBS abnormalities (15,16,18)
Digestive tract	Positive expression	Positive expression	Human: Smooth muscle and gastric parietal cells; Mice: Epithelium only at 8.5 days, submucosal layer	Diastasis recti, omphalocele and hernias
Gonads	Positive in male embryos	Positive expression	Human: Leydig cells, seminiferous epithelium, urethra, deferent duct	Cryptorchidism, reduced penile length, risk for testicular gonadoblastoma
Kidney	Positive expression	Positive expression	Human: Cortex convoluted tubules and Bowman's capsule; Mice: Mesenchymal	Renal dysplasia, nephromegaly, risk for Wilms tumors
Limb buds	Positive expression	Positive expression	Human: Mesenchymal in P1; Mice: Mesenchymal from 9.5 to 11.5 days post-coitum. After, only cartilage precursor of limb bones	Polydactyly, syndactyly
Liver	Positive expression	Positive expression	-	Hepatomegaly
Nervous system	Positive expression	Negative expression	Human: Only spinal cord motoneurons and dorsal root ganglia neurons	Hypotony, developmental delay, CNS malformations, and high risk for neuroblastoma and medulloblastoma
Oral cavity	Positive expression	Positive expression	Human: Palate; Mice: Epithelium of oral cavity	Cleft palate
Pancreas	Positive/Negative expression	Not reported	Human: Positive exocrine in P1 and P2, negative in endocrine	Hyperplastic islets of Langerhans, hypoglycemia
Respiratory system	Positive expression	Positive expression	Human: Only mesenchymal cells of the lungs in P1 and P2; Mice: Cartilage of trachea, larynx and major bronchi, mesenchymal of lungs	Pneumonia
Skin	Negative expression	Positive expression	Mouse: Dermis and hair follicles	None reported
Tongue	Positive expression	Positive expression	Human: Striated muscle, epithelium, and connective tissue of tongue; Mouse: Only connective tissue	Macroglossia and midline groove
Vertebrae	Positive expression	Positive expression	Human: Mesenchymal between caudal vertebrae; Mice: Mesenchymal cells, cartilage and intervertebral discs	Vertebral abnormalities

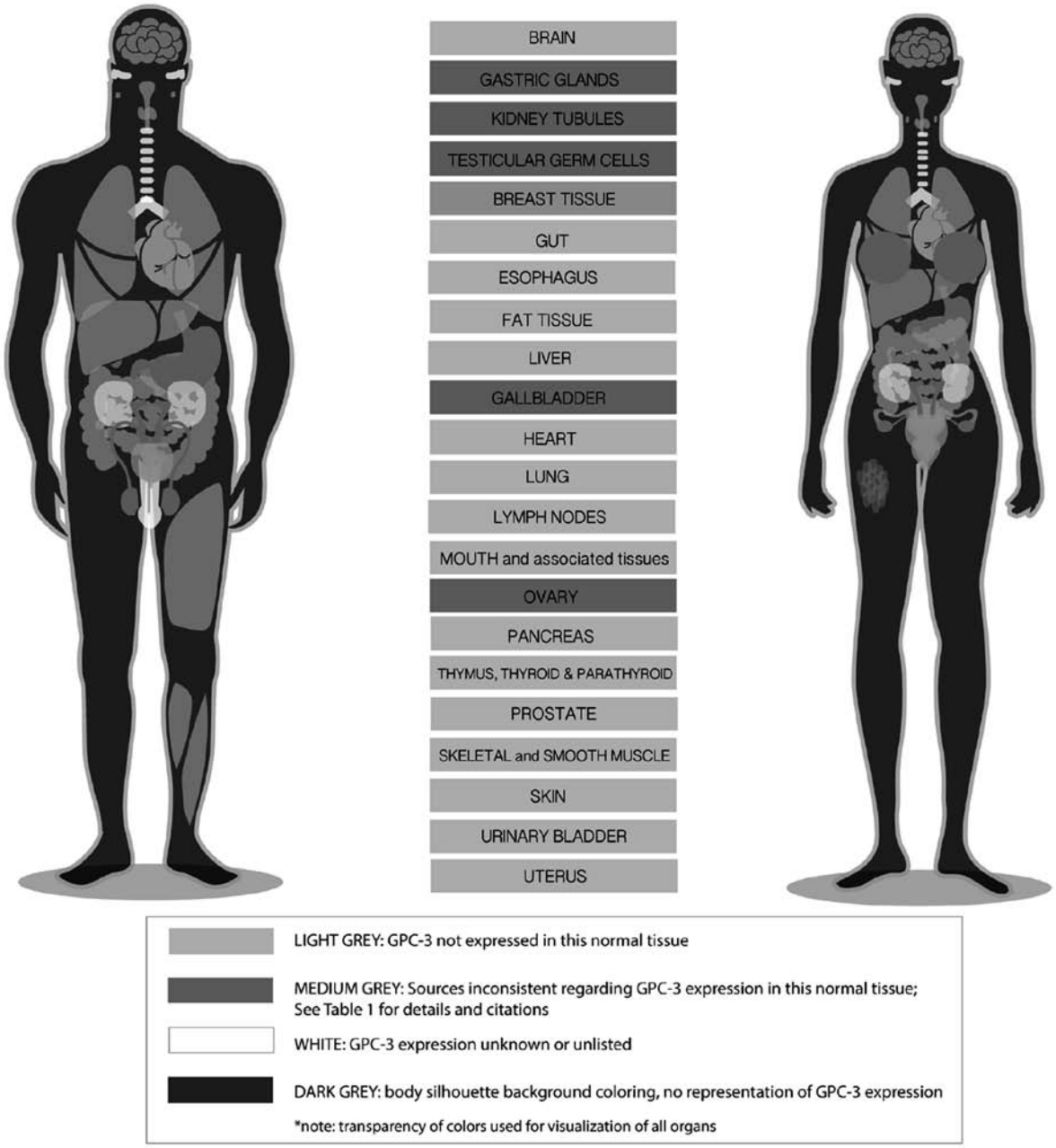

Figure 1. Expression of GPC-3 in normal tissue. The above image was constructed based on Table II. Different grey shades were used to differentiate GPC-3 expression in various tissues. Opacity and transparency of tissues or organs were adjusted for maximized visualization of all organs and their coloring, while retaining anatomical positioning. In organs or tissues colored in light grey (opaque and transparent), GPC-3 is not expressed. In organs or tissues colored in medium grey (opaque and transparent), we found the literature inconsistent regarding GPC-3 expression in those tissues. Table II and its citations for each organ should be referenced for further detail on the nuances of GPC-3 expression in these tissues. Organs or tissues in white (opaque and transparent), expression of GPC-3 was unknown or unfound in the literature. Lastly, the dark grey coloring of the body is simply intended to show the silhouette of body and does not represent any expression of GPC-3.

on Table II. Different grey shades were used to differentiate GPC-3 expression in various tissues.

Expression of GPC-3 in cancer tissue. GPC-3 expression in various types of cancer is not fully understood, though the variance may be due to the specific type of cancer being studied. Table II lists the reported expression of GPC-3 in cancer in various tissue types. Expression of GPC-3 was found in the following cancers: liver, gastric carcinoma, melanoma, high-grade urothelial carcinoma, testicular, and some uterine and vaginal cancers (1,3-6,23,28,33-35). GPC-3 expression was also reported in some non-CNS tumors of the brain (36). In various other tissue types such as colon, lung, anal, esophageal, mouth mucosa, pancreas, skin, thyroid, bladder, uterus, and vulvar tissue GPC-3 expression varied based on the type, and origin, and grade of cancer [i.e. squamous cell carcinoma (SCC) versus mesothelioma of the lung] (1,10,11,19,28,31-33,37-40).

In some cancerous tissues, reduced or even silenced expression of GPC-3 was found; namely renal cell carcinomas, as well as cancer of the breast, ovaries, penis, prostate, and gallbladder (10,11,24-26,33,38,41,42). Similarly, infrequent expression of GPC-3 was reported in some cancers of the

Table II. GPC-3 expression in adult human tissue types.

Tissue	Normal tissue	Cancer tissue	References
Liver	Not expressed (28-30)	Expressed (1,3-6)	1,3-6,28,29,30
Gastric glands	Expressed, small subset of gastric tissues normally express GPC-3 (19); Not expressed (20)	Expressed in gastric carcinoma (34)	19,20,34
Kidney tubules	Expressed (19); Not expressed (20); Low expression (21,22)	Reduced expression in renal cell carcinoma (41 mentions renal cell carcinomas exposed to GPC-3 caused reduced proliferation); Infrequent expression in clear cell carcinoma, oncocytoma, and papillary carcinoma of the kidney (19)	19-22,41
Testicular germ cells	Expressed (19); Not expressed (23)	Expressed (23)	19,23
Anus	Unknown	Expressed (33); Infrequent expression in SCC (19)	19,33
Breast tissue	Not expressed (19); Expressed (24); Expressed (25)	Reduced expression (24) Silenced (25); Infrequent expression in invasive ductal and tubular carcinoma (19)	19,24,25
Brain	Not expressed (19)	Expressed in some non-CNS tumors and restricted expression in atypical teratoid rhabdoid tumors and in some craniopharyngiomas (36)	19,36
Colon	Not expressed (2,19); Trace amounts expressed (21,22)	Expressed (1,37); Infrequent expression in adenocarcinoma and adenoma with high-grade dysplasia (19)	1,2,19,21,22,37
Esophagus	Not expressed (31,19)	Expressed (31,33); Infrequent expression in squamous cell carcinoma (19)	19,31,33
Fat tissue	Not expressed (19)	Unknown	19
Gallbladder	Not expressed (19); Expressed (26)	Reduced expression in gallbladder cancer (26); Infrequent expression in adenocarcinoma (19)	19,26
Heart	Not expressed (19); Low expression (21,22)	Unknown	19,21,22
Lung	Not expressed (19); Low expression (21,22)	Silenced in mesothelioma and lung adenocarcinoma (10,11,38); Expressed in lung squamous cell carcinoma (39); Expressed (33); Infrequent expression in adenocarcinoma and large cell carcinoma (19)	10,11,19,21,22,33,38,39
Lymph nodes	Not expressed (19)	Unknown	19
Mouth (mucosa)	Not expressed (19)	Expressed on some tongue, base/tonsil, ventral tongue/ floor of mouth (33); Infrequent expression in squamous cell carcinoma (19)	19,33

Table II. Continued.

Tissue	Normal tissue	Cancer tissue	References
Ovary	Not expressed (19); Low expression (21,22); Expressed (27)	Silenced in epithelial ovarian cancer (10,11,25,38); Infrequent expression in Brenner tumor, endometrioid carcinoma, mucinous carcinoma, serous carcinoma, and yolk sac tumor (19); No expression (27)	10,11,19,21,22,25,27,38
Pancreas	Not expressed (19); Trace amounts expressed (21,22)	Expressed (31); No expression in pancreatic adenocarcinoma (40)	19,21,22,31,40
Parathyroid	Not expressed (19)	Unknown	19
Parotid gland	Not expressed (19)	Unknown	19
Penis	Unknown	No expression in squamous cell carcinoma (33); Infrequent expression in SCC (19)	19,33
Prostate	Not expressed (19)	Infrequent expression in adenocarcinoma (19); No expression (42)	19,42
Skeletal muscle	Not expressed (19); Trace amounts expressed (21,22)	Infrequent expression in rhabdomyosarcoma (19)	19,21,22
Skin	Not expressed (19)	No expression in squamous cell carcinoma (33); Expression in melanoma (28)	19,33
Small intestine (mucosa)	Not expressed (19); Trace amounts expressed (21,22)	Infrequent expression in adenocarcinoma and gastrointestinal stromal tumor (19)	19,21,22
Smooth muscle	Not expressed (19)	Infrequent expression in leiomyosarcoma (19)	19
Submandibular gland	Not expressed (19)	Infrequent expression in acinar cell carcinoma, adenoid cystic carcinoma, and small cell carcinoma (19)	19
Thymus	Not expressed (19)	Unknown	19
Thyroid	Not expressed (19); Scarcely expressed (32)	Infrequent expression in papillary carcinoma (19); Expressed in early papillary carcinoma (32)	19,32
Urinary bladder	Not expressed (19,33)	Expressed (33); Expressed in urothelial carcinoma, mostly in high grade tumors (35); Infrequent expression in noninvasive transitional cell carcinoma, small cell carcinoma, and squamous cell carcinoma (19)	19,33,35
Uterus (cervix, endometrium, myometrium)	Not expressed (19)	Expressed (33); Infrequent expression in cervix as SCC and endometrium as endometrioid and serous carcinoma (19)	19,33
Vagina	Unknown	Expressed (33)	33
Vulva	Unknown	No expression in squamous cell carcinoma (33); Expression in some tumors analyzed in SCC (19)	19,33

kidney, invasive ductal and tubular carcinomas of the breast, some brain tumors, adenocarcinomas of the gall bladder, prostate, and small intestine, squamous cell carcinoma of the penis, rhabdosarcomas and leimyosarcomas of the skeletal and smooth muscle, adeno- and small cell carcinomas of the submandibular glands, among other cancerous tissues depending upon grade and location within the tissue (19,33,36). For more detail regarding GPC-3 expression in these tissues, refer to Table II.

In human breast cancer, GPC-3 has been shown to be silenced due to hypermethylation of its promoter region. The data also suggest that GPC-3 can act as a negative regulator of breast cancer growth as well a downregulator in mesotheliomas and ovarian cancer (25). Xq26 is a region frequently deleted in advanced ovarian cancers and is where the GPC-3 gene is located.

In ovarian cancer, GPC-3 expression was lost in cell lines analyzed which was also due to hypermethylation of the promoter region. Expression of GPC-3 was restored with the treatment of 5-aza-2'-deoxycytidinem, a demethylating agent. Ectopic GPC-3 expression inhibited the growth of ovarian cancer cell lines and results show that GPC-3 is frequently inactivated in a subset of ovarian cancers which may suggest that it functions as a tumor suppressor in the ovary (27).

In 15% or more of studied cases, GPC-3 was expressed in tumor types of hepatocellular carcinoma, squamous cell carcinoma of the lung, liposarcoma, testicular nonseminomatous germ cell tumor, cervical intraepithelial neoplasia (grade 3), malignant melanoma, adenoma of the adrenal gland, schwannoma, malignant fibrous histiocytoma, adenocarcinoma of the stomach (intestinal subtype), chromophobe renal cell carcinoma, invasive lobular carcinoma of the breast, medullary carcinoma of the breast, squamous cell carcinoma of the larynx, small cell carcinoma of the lung, invasive transitional cell carcinoma of the urinary bladder, mucinous carcinoma of the breast, and squamous cell carcinoma of the cervix (19).

3. GPC-3 as a serum marker for early detection of HCC

GPC-3 may be a potential serum marker in diseases such as HCC in which expression of GPC-3 is markedly increased (20,21). Capurro *et al* examined GPC-3 protein expression and serum levels using immunohistochemistry and ELISA in HCC patients, as well as serum levels in healthy donors and patients with hepatitis and liver cirrhosis. Results revealed increased GPC-3 expression levels in patients with HCC, but not in healthy hepatocytes. GPC-3 serum levels were significantly elevated in HCC patients, but undetectable in the serum of healthy and hepatitis-infected patients (22).

Discussing where this protein is cleaved, the cleaved portion of the NH(2)-terminal was found to be between Arg(358) and Ser(359) of GPC-3. In addition to this, it was observed that soluble GPC-3 can be specifically detected in the sera of patients with HCC using N-mAbs (23). Unfortunately, the cleavage events and other post-translational modifications are understudied. It is necessary to investigate in terms of function and cellular localization the cleavage events on GPC-3. Focusing on serum marker it is necessary to understand if the protein is cleaved and which portion is secreted improving the knowledge of secretory pathways involved in its sorting in and out of the cells, with major attention on exosomal cargos.

GPC-3 was also identified as a novel diagnostic marker for human melanoma it its early stages. In patients with melanoma, GPC was detected. However, the protein was not found in the sera of healthy patients, or those with benign skin lesions (large congenital melanocytic nevus). It was also detected in the serum of patients with stage 0, *in situ* melanoma (28).

In another study carried out to observe biomarkers for HCC, preoperative serum GPC-3-N (sGPC-3N) levels were measured alongside with serum AFP in patients with HCC. It was observed that high serum AFP corresponded with a high sGPC-3N level. The study states that sGPC-3N may serve as an independent prognostic biomarker in HCC patients (24).

Serum α-fetoprotein is the common diagnostic marker to detect HCC. Several studies showed that the combination of α-fetoprotein and GPC-3 increase significantly the sensitivity of diagnostic value compared to their sensitivity analyzed independently (43). Several markers are under investigation to ameliorate the sensitivity and specificity of HCC detection such as: des-γ carboxyprothrombin, lens culinaris agglutinin-reactive α-fetoprotein, Golgi protein 73 (43,44). However, GPC-3 seems to be more specific due to its peculiar expression in hepatocytes as showed before, and this represents the main advantage for its use in HCC detection even if its mechanism of transport in the serum is still unknown. For its peculiar localization and post-translational modifications GPC-3 could represent an ideal target for antibody therapeutic approach with the possibility to bind its membrane form thus inhibiting its activity. Moreover, a chemical approach could obtain the cleavage events that determine GPC-3 functional modulation and cellular localization. Consequent to its expression in pre-neoplastic hepatocytes, this offers the unique advantage of allowing an early and specific therapeutic approach.

4. GPC-3 in resection, regeneration and liver diseases

Few studies have demonstrated the expression of GPC-3 pre- and post-hepatic resection. Due to hepatic progenitor cells expressing cytokeratin 19 (CK19) and GPC-3 at varying phenotypes, cell samples were analyzed from patients who had a liver resection and were statistically compared with each phenotype of expression in Table III. It was found that $CK19^+$/$GPC\text{-}3^+$ HCC was the most aggressive subtype, followed by the $CK19^-$/$GPC\text{-}3^+$ HCC and finally with the $CK19^-$/$GPC\text{-}3^-$ HCC subtype being the least aggressive from the subtypes observed (45). Therefore, a poorer prognosis may be associated with patients undergoing a hepatectomy who have the expression of both CK19 and GPC-3.

Liu *et al*, on the other hand, described transgenic GPC-3 mice, which were under the control of the albumin promoter gene, overexpressed GPC-3 and actually had a suppression of hepatocyte proliferation and liver regeneration following partial hepatectomy (30,46). These mice developed normally and the authors claim that GPC-3 may play a negative regulatory role in hepatocyte proliferation, showing a contrasting role of GPC-3 in the hepatocytes. Further investigation using models post-PH is needed to uncover various important aspects of GPC-3 expression such as the density and location

Table III. Phenotype of expression (25).

Order of severity	Cell marker phenotype CK19/GPC-3 HCC	Cell marker description
1	+ +	HCC subtype transformed from hepatic progenitor cells (HPC)
2	- +	Immature hepatocyte
3	- -	Terminal differentiated hepatocyte

of GPC-3 expression (i.e. the cut margin or other location). This research may be instrumental in further elucidation of the role and mechanisms of GPC-3 in liver growth and regeneration post-resection and even injury (mechanically, inflammatory, or otherwise). However, limited studies have been conducted on the regeneration of the liver.

At this time there is no evidence on a possible function and presence of GPC-3 in other liver diseases such as fibrosis, fatty liver disease or liver cirrhosis. The peculiarity of its expression in neoplastic hepatocytes makes GPC-3 not only a unique HCC marker but also a possible therapeutic target. However, studies in cirrhotic livers without HCC are necessary to rule out the role of GPC-3 or the presence in these circumstances.

5. GPC-3 as a therapeutic target for HCC

Therapeutic targets are especially important in the treatment of various types of cancer. GC33, which is a humanized monoclonal antibody, was shown to bind human GPC-3. GC33 has been observed to contain antitumor properties and targets GPC-3 specifically. It was noted that GC33 was well tolerated in advanced HCC and provided some benefit in the clinical treatment (47). In addition, human (MDX-1414 and HN3) and humanized mouse (GC33 and YP7) antibodies that also target GPC-3 are under different stages of clinical development and could also aid in the treatment of HCC (21). miR-219-5p is a microRNA (miRNA) which could exert tumor suppression on hepatic cells expressing GPC-3. miR-219-5p reduced both the mRNA and protein levels of GPC-3 and exerted tumor-suppressive effects in HCC (48). Finally, nanoparticles are also under development to target and bind GPC-3 in cells that could provide useful for further imaging and targeting of GPC-3 (49).

Research has also been conducted in regards to peptide sequences which bind GPC-3. Feng *et al* described a 12-mer peptide sequence (DHLASLWWGTEL) which was able to act as a probe and bind GPC-3 for HCC detection (21). The peptide allows visualization of the specific sequence via near-infrared fluorescence (50). Another therapeutic target on GPC-3 is mir717, a miRNA, which is located on intron 3 of GPC-3 on the X chromosome. It has been shown to play a regulatory role in renal osmoregulation. mir717 might be connected in some way with obesity regulation, one of the risk factors of HCC (51,52).

It has been proposed that GPC-3 acts as a negative regulator in the Hedgehog signaling pathway during development and as a result, a non-functional GPC-3 protein could be the cause of overgrowth or a GPC3 modified by post-translational modifications. GPC-3 has also been associated with the binding of Wnt to its signaling receptor, Frizzled (53-56). Both the Hh and canonical/non-canonical pathways of Wnt could be potential therapeutic targets as GPC-3 may play a role in proliferation and/or growth suppression, depending on the type of tissue and the stage of development (8,57). In addition, the peptide sequences Arg-Leu-Asn-Val-Gly-Gly-Thr-Tyr-Phe-Leu-Thr-Thr-Arg-Gln and Tyr-Phe-Leu,Thr-Thr-Arg-Gln showed selective binding of GPC-3 (58) which could provide useful for a target that is needing to bind onto GPC-3 for regulation.

6. GPC-3 and co-receptors

GPC-3 has been observed to interact with a number of co-receptors that further modulate cellular expression. Liu *et al* showed that GPC-3 and CD81 levels were significantly upregulated in general in the transgenic mice following partial hepatectomy (30,46). They showed that the negative regulatory role was somehow associated with GPC-3 and CD81 and that there is enhanced association between GPC-3 and CD81. GPC-3 and CD81 have both been associated as binding partners in which expression could influence the Hh (13). Co-localization of GPC-3 and CD81 was also shown to occur 2-6 days after hepatectomy (30). In contrast, another study observed that GPC-3 binds to members of the Hh pathway and prevents their interactions with the patched-1 receptor. There was decreased binding of GPC-3 with Hh and CD81 following PH and GPC-3/CD81 may play a role in the termination of liver growth following liver regeneration (14,46). Dysregulation of the association between GPC-3/CD81, which may occur during hepatitis C infection can result in dysregulated signaling and proliferation in infected hepatocytes (59). The structure and character of GPC-3 as a transmembrane protein, in conjunction with the current literature, lends itself to the strong possibility of many associations or interactions with other co-receptors and proteins, not only in hepatocytes, but possible in other normal or cancerous tissue types. It is important to emphasize again the paucity in the current literature in regard to this, and many other aspects of GPC-3.

7. Conclusion

GPC-3 is a molecule that is still not fully understood in its role in the proliferation and suppression of cell growth in normal and abnormal or cancerous tissue and also in structural and post-translational modifications. The question still remains as to why it is normally expressed in some tissues, while remaining silenced in others. Future research that looks into the upstream and downstream cell signaling pathways and how GPC-3 may be involved could provide further answers. In addition, studies investigating a more complete picture and

analysis of GPC-3 structure are warranted, as glycosylation, sulfonation, or other structural components of the protein may be important in understanding its regulatory function within differing tissue types. In this same vein, continued insight into the cleavage of GPC-3 and the function of the cleaved versus non-cleaved form of the molecule could lead to a more complete comprehension of function. Other research paths may include developing the theory of GPC-3 as a dual tumor suppressor and oncogene, as dependent upon its structure. With exosome research brightening the horizons, more attention and detail must be paid to uncover the importance of exosomal GPC-3 as a serum marker or as possible therapeutic target in HCC. GPC-3 is a molecule that could further connect the missing links in liver cancer research and lead to an abundance of new intercellular relationships to reveal important aspects of the biology of this disease.

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