Annals of Medical and Clinical Oncology
Iravani O, et al. Ann med clin Oncol 3: 138.
www.doi.org/10.29011/AMCO-137.000138
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Review Article

Glypican 3 (GPC 3) In Cancer Pathogenesis and Progression

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Citation: Iravani O, Yazdani R, Forootan FS (2022) Glypican 3 (GPC 3) In Cancer Pathogenesis and Progression. Ann med clin Oncol 3: 138. DOI: 10.29011/AMCO-130.000138

Received Date: 05 June, 2022; **Accepted Date:** 11 July, 2022; **Published Date:** 14 July, 2022

Abstract

Glypican 3 (GPC 3) is one of six glypicans identified in humans. Glypicans are external cell surface membrane-bound Heparan Sulfate Proteoglycans (HSPGs), anchored by a Glycosyl-Phosphatidylinositol (GPI). GPC 3 is a regulator of morphogens and growth factors in cell maturation during development. GPC 3 plays diverse roles in cancer and modulates various aspects of tumour biology. Its effects on cancer progression depend on the type of tissue involved. GPC 3 may serve as a tumour marker for cancer diagnosis and may also be a viable target for cancer therapy. Here, we review the expression, localization, mutations, and functional roles of GPC 3 during the pathogenesis and progression of various human cancers.

Keywords: Biomarker; Cancer; Expression; Glypican 3 (GPC 3); Heparan Sulphate; Molecular target

Introduction

Glypicans are external cell plasma membrane-bound Heparan Sulfate Proteoglycans (HSPGs) anchored by a Glycosyl-Phosphatidylinositol (GPI) [1]. At present, six glypicans (GPC1 to GPC6) have been characterized in humans [2-4] as depicted in Figure 1. GPC 3 (also known as OCI-5 or MXR-7) [5] has diverse functions in many aspects of cell behaviour, such as regulating development and cell maturation [6,7]. Through its heparan sulfate chains (Figure 2), GPC 3 regulates cellular growth during development, by interacting with several growth factors and morphogens to mediate their signalling at the level of ligand-receptor interaction [8-10]. This modulation involves mediating the interaction between the growth factors and their respective signalling receptors [3]. GPC 3 is a co-receptor for several Heparan-Binding Growth Factors (HBGFs), such as Insulin-Like Growth Factors (IGF), Fibroblast Growth Factors (FGF), and Wingless-related integration sites (Wnts) [11,12]. By acting as co-receptors, GPC 3 regulates the interaction of these HBGFs with their receptors and, consequently, modulates their biological activities [9]. The specific function of GPC 3 depends on growth factors and their receptors which are expressed by a particular cell type [3].

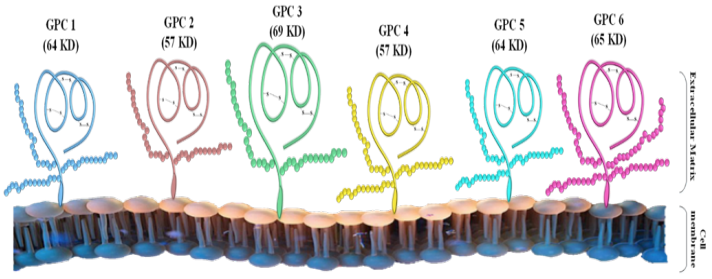

Figure 1: Cartoon of the Glypicans (GLCs) in association with cell membrane showing the six members of GLC family (as described by Bandtlow and Zimmerman [13]. The bars represent extended core protein and are adjusted based on the number of amino acids. Core proteins attach to the cell membrane by glycosylphosphatidylinositol (GPI) anchors and are completely located in the extracellular matrix. Glycosaminoglycan (GAG) chains bound to the core proteins.

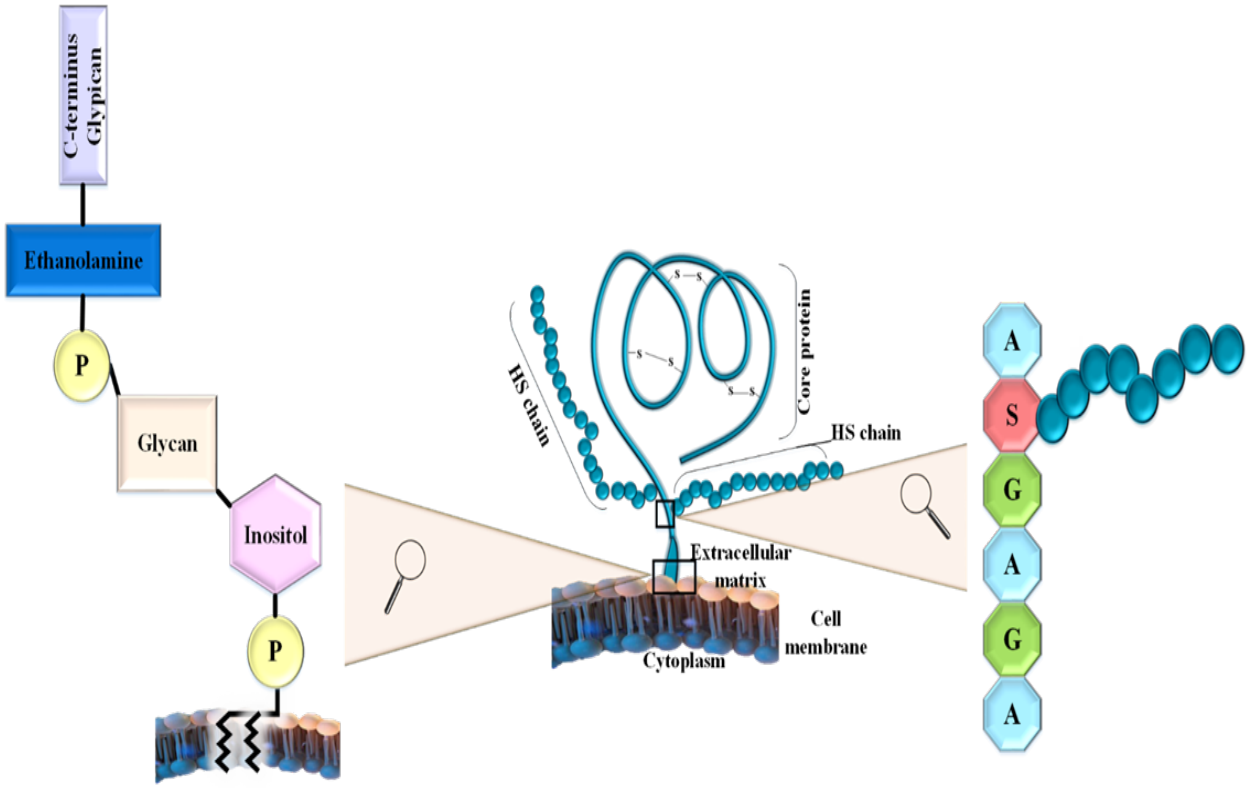

Figure 2: Cartoon of the Glypican 3 (according to De Cat and David [14]). Several disulphide bonds within the core protein keep the C-terminal highly compacted. Heparan sulphate links to seria ne residue of the core protein via the linker sequence (Xyl-Gal-Gal-GlcA). P: Phosphate; G: Glycine; A: Amino acid; S: Serine; HS: Heparan Sulfate chain; Xyl: Xylose; Gal: Galactose; GlcA: Glucuronic acid.

GPC 3 is crucial in modulating cell growth, programmed cell death, and drug resistance by triggering apoptosis in malignant tumours [15,16]. Given the ability of GPC 3 to regulate growth factors, [17] many of which have been implicated in angiogenesis and thus cancer progression, changes in GPC 3 expression have been associated with tumour pathogenesis, growth and development of various human cancers [18,19]. Within the glypican family, GPC 3 has been studied most extensively in the context of human malignancies and cancer biology [20] GPC 3 has been linked to malignancies through mutations and aberrant protein expression [21-23]. GPC 3 expression is specific to the cell, tissue, or development stage [16] and its function is tissue-dependentnt. [6,11] GPC 3 expression varies during tumour progression depending on the tissue type. It is posited that these tissue-specific differences are due to differential regulation of growth factors and signalling pathways in each tissue [2]. Since there is a correlation between GPC 3 protein expression and the tumour type, analysis of GPC 3 may be useful in the diagnosis of various cancers [20]. Depending on the tissue, GPC 3 may act as a tumour suppressor gene or as an oncofetal protein [11,24]. GPC 3 expression appears to be inhibited in tumours arising from normal tissues that typically express it, where as in tumours derived from tissues where GPC 3 is normally silenced, its expression is enhanced [21,24]. For precise description of its different actions in various contexts, it is important to understand the role of GPC 3 in promoting or protecting against carcinogenesis in various situations [9].

In the adult, GPC 3 is only expressed in a limited number of tissues, including the breast, lung, ovary, mesothelium and kidney (Figure 3) [8,25,26]. In cancers originating from these tissues, the GPC 3 expression is often diminished or downregulated during tumour progression [2], suggesting that GPC 3 may inhibit cell proliferation, induce apoptosis and act as a tumour suppressor gene in such tissues [7,21]. Downregulation of GPC 3 could enhance cell growth by indirectly upregulating cell signalling through competition with Patched (the receptor for Hedgehog (Hh) for binding to the Hh growth factor [20]. GPC 3 expression is also upregulated in most embryonic tissue, such as placenta and liver, but downregulated in the adult tissue of corresponding organ (Figure 3) [11]. In these tissues, GPC 3 expression is predominantly observed during development, with expression peaking in-utero before downregulation after birth [16,21,27]. Hence, this suggests

the important role of GPC 3 in developmental morphogenesis [28]. Interestingly, in tumours originating from embryonic tissues that express GPC 3, its expression reappears during malignant transformation of these tissues [11,29]. These tumours overexpress GPC 3 while in normal tissues, its expression is silenced. [21] This can be observed in cancers such as hepatocellular carcinoma (HCC), testicular germ-cell tumours, yolk sac tumours as well as in embryonal tumours such as neuroblastoma, hepatoblastoma and Wilms' tumour [17,21,30,31], where GPC 3 expression is upregulated in the tumour while it is not expressed in the adjacent normal tissue [17]. This suggests that GPC 3 acts as an oncofetal protein in these organs [11]. In this regard, GPC 3 expression can be used as a specific diagnostic serum and immunohistochemical tumour marker [16,21,30,32,33], and a potential target for immunotherapy in GPC 3-positive cancers [16,26,34,35]. In this review, we discuss the functional roles of GPC 3 during tumourigenesis and progression of various human cancers (Figure 4).

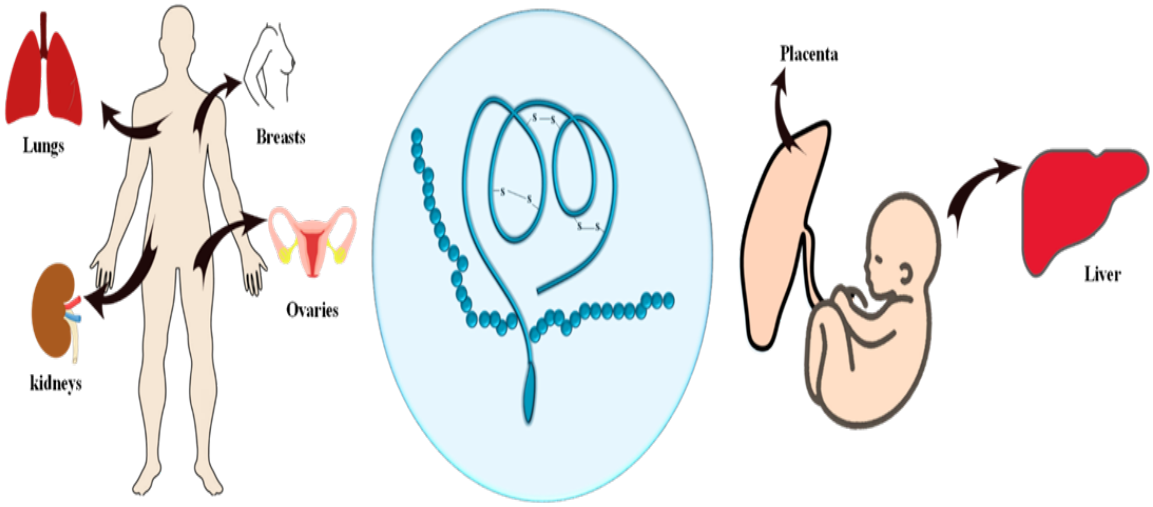

Figure 3: Schematic representation of the organs that express GPC 3 during the fetal period and in adulthood.

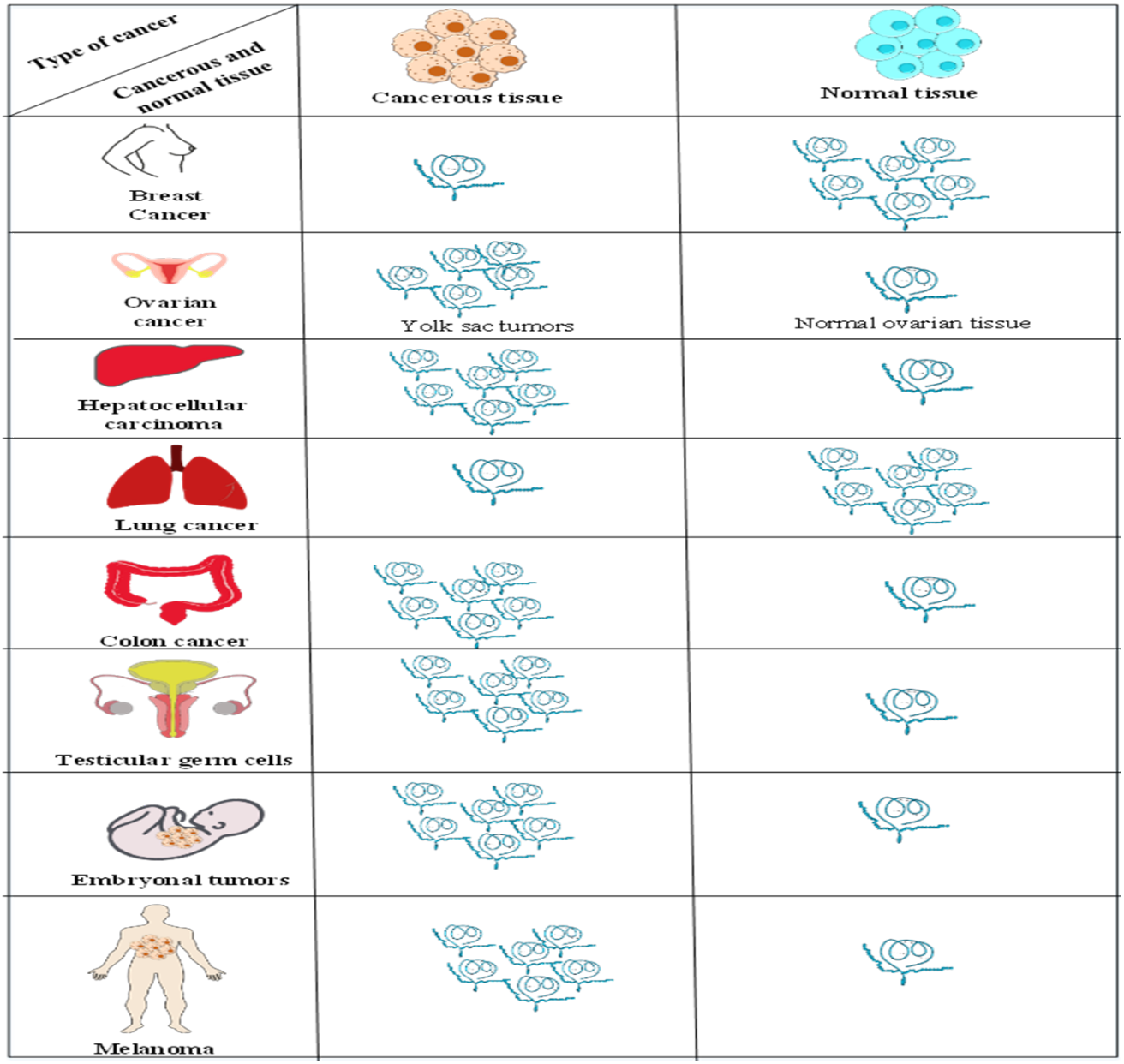

Figure 4: Graphical Abstract.

GPC 3 in Breast Cancer

Gonzalez et al. reported that enhanced GPC 3 expression induced apoptosis in MCF-7 breast cancers but not in colorectal tumours, without requirement for heparan sulfate [36,37]. Xiang et al. reported the downregulation of the GPC 3 gene in human breast cancer. Assessment of GPC 3 expression in normal epithelium and breast cancer was performed by in situ hybridisation of tissue sections containing both normal and cancerous tissue [31]. In all 12 patients, expression of GPC 3 was downregulated in the malignant cells as compared to the adjacent normal epithelium. Furthermore, GPC 3 expression was undetectable in the cancer cells in seven of these patients. In addition, in 8 out of 10 breast cancer cell lines, upregulated expression of GPC 3 suppressed cell proliferation. This suggests that GPC 3 may inhibit breast cancer progression [38].Yan et al. found that GPC 3 silencing in human breast cancer was a result of hypermethylation of the GPC 3 promoter, particularly in tumours not expressing the hormone receptor [39]. In a study by Peters et

al., GPC 3 expression was found to inhibit breast cancer invasion and metastasis in a syngeneic murine model, and observed to be associated with reduced growth and survival of tumour cells, impairment of tumour cell motility, and increased cell adhesion. With an ectopic expression of GPC 3 in the LM3 breast tumour cell line, cell sensitivity to apoptosis triggered by serum depletion was increased. These data support the perception that GPC 3 has a protective role as a suppressor of breast cancer progression [16]. A single known case of a GPC 3 mutation in breast ductal carcinoma has been described, resulting in a premature stop codon. Because GPC 3 is known to influence proliferation in other cancers, this mutation may be important for further study [19].

GPC 3 in Ovarian Cancer

The GPC 3 gene, which is located at Xq26, has frequent deletions of this region in advanced ovarian cancers [40]. Lin et al. reported frequent downregulation of the GPC 3 gene in human ovarian cancer. In their study, the same authors showed that while GPC 3 was expressed in normal ovaries, its expression was downregulated and not detectable in a high proportion of ovarian cancers. It was observed that wherever expression of GPC 3 was inhibited, there was associated hypermethylation of the GPC 3 promoter, with no mutations found in the coding region of the GPC 3 gene in the ovarian tumours. Expression of GPC 3 was restored after treating of cells with a demethylating agent. Moreover, restoration of ectopic GPC 3 expression inhibited colony-forming activity and cell growth in several ovarian cancers, suggesting that GPC 3 may act as a tumour suppressor gene in ovarian cancer. Accordingly, GPC 3 in the ovary is believed to either trigger apoptosis or regulate cell proliferation [41]. GPC 3 expression has been investigated in clear cell carcinomas of the ovary. Two earlier studies yielded inconsistent results regarding GPC 3 expression in ovarian clear cell adenocarcinoma. The discrepancy may be attributed to the small sample size and limited tissue area evaluated on microarray sections. One study was performed by Esheba et al. (2008) on a series of ovarian neoplasms that included 24 clear cell adenocarcinomas. Immunohistochemistry (IHC) was carried out on tissue microarray (TMA) tissue sections revealed that the GPC 3 protein was absent or undetectable in most clear cell, serous, endometrioid, and mucinous tumours. The authors reported more frequent expression of GPC 3 in ovarian yolk sac tumours (98%) as compared to clear cell adenocarcinomas (7%). While GPC 3 was expressed in the vast majority of yolk sac tumours, all normal ovarian tissue, teratomas, embryonal and mucinous carcinomas did not exhibit GPC 3 expression. 65% of endometrioid carcinomas and just 1% of serous carcinomas were GPC 3-positive [28].

On the other hand, another study found that about 13% of ovarian carcinomas were GPC 3-positive. GPC 3 expression was found to be related to tumour histotype. In this immunohistochemical study of 251 ovarian cancer sections using tissue microarrays, Stadlmann et al. reported that the clear cell histotype was strongly associated with expression of GPC 3 [10]. GPC 3 expression was observed by the same authors in a significant proportion of primary and corresponding ovarian carcinomas recurring after platinum-based chemotherapy. While serous and endometroid carcinomas were rarely positive for GPC 3, it was expressed in over 60% of clear cell ovarian carcinomas. It was suggested that GPC 3 is important in the early differentiation of ovarian carcinoma to the clear cell histotype rather than in progression of ovarian cancer. In addition, the same authors investigated GPC 3 expression and its relation to the response of advanced ovarian serous carcinomas to chemotherapy. No significant difference was found between the frequency of GPC 3 expression in platinum-sensitive and platinum-resistant tumours. Positive staining for GPC 3 was described in some recurrent carcinomas but there was no association with chemoresponse. These data suggests that GPC 3 does not have a major role in ovarian cancers developing platinum-based chemoresistance. The authors further suggested that GPC 3 could potentially represent a (second-line) therapy target in ovarian cancer [16].

Clear cell carcinoma and yolk sac tumour of the ovary appear to show more overexpression of GPC 3, but not other ovarian cancer histotypes. A previous study found that among ovarian adenocarcinomas, GPC 3 is overexpressed in clear cell carcinoma only [42]. More recent studies from Japan, with a focus on clear cell carcinoma have attempted to elucidate the significance of GPC 3 expression in this subtype of ovarian trumors. Maeda et al. evaluated GPC 3 expression in nonneoplastic ovaries as well as ovarian carcinomas, particularly clear cell adenocarcinomas [43]. In order to surmount the limitation of insufficient tissue microarray studies, immunohistochemistry was conducted on full tissue sections of 94 clear cell adenocarcinoma patients. They reported that GPC 3 was overexpressed in 44% of clear cell adenocarcinomas of the ovary, while rarely expressed in other subtypes such as mucinous, endometrioid, or serous ovarian carcinomas. In addition, they showed a significant association between expression of GPC 3 and poor prognosis and overall survival in advanced (stage III/IV) clear cell adenocarcinoma, indicating that overexpression of GPC 3 could be possibly linked to the development and invasive behaviour of this subtype of ovarian cancer [43].Another study by Umezu et al. examined the immunohistochemical expression of GPC 3 in clear cell carcinoma tissues to determine if GPC 3 expression correlates with clinicopathological factors, such as the prognosis of clear cell carcinoma patients, particularly in early-stage disease. They also assessed if GPC 3 expression was associated with cell proliferation in clear cell carcinoma based on immunohistochemistry. In this study, GPC 3 was found to be expressed in 40.4% of clear cell carcinomas, which are consistent with the results obtained by Maeda et al. GPC 3 expression was also associated with worse progression-free survival in early-stage

(stage I) clear cell carcinoma patients. The results suggest that GPC 3 overexpression may be linked to low-level proliferation of carcinomas. GPC 3 expression may be related to development of taxane-based chemotherapy resistance and poor prognosis in early-stage ovarian clear cell carcinoma. The same authors suggest that GPC 3 is promising as a reliable prognostic indicator in early-stage clear cell carcinoma, and could be a novel molecular target in treating clear cell carcinoma. [42]. Additionally, a recent study by Liu et al. found that decreased expression of GPC 3 enhanced the development of human ovarian cancer cells in nude mice by upregulating matrix metalloproteinase (MMP)-2 and (MMP)-9 and downregulating tissue inhibitor of metalloproteinase-1. This increased cancer cell growth, division and motility, suggesting that GPC 3 may be a therapeutic target for ovarian cancer [44].

GPC 3 in Hepatocellular Carcinoma (HCC)

GPC 3 was introduced as a potential tumour marker for HCC by detecting high levels of GPC 3 protein in serum of HCC patients while it remains undetectable in normal individuals and in patients with benign liver conditions [11,24,32,45-49].
Hsu et al. conducted a study comparing differential GPC 3 mRNA levels in normal liver and human HCCs. They reported that while GPC 3 is not expressed in normal human adult liver, its expression is upregulated in most HCCs. In particular, GPC 3 mRNA levels were significantly raised in most HCCs as compared to normal liver and benign liver disease [29]. Specifically, GPC 3 mRNA expression was detected in 74.8% of HCC livers but in just 3.2% of normal livers [51].

In HCC tumours originating from tissues which express GPC 3 only in the embryo, GPC 3 expression tend to turn up with malignant transformation [52]. Because GPC 3 is strongly expressed in embryonic liver, downregulated in the normal adult liver and overexpressed/re-expressed in HCC, these results suggest that GPC 3 behaves as an oncofetal protein in these tissues [53]. Oncofetal proteins may be useful as tumour markers or as immunotherapy targets [2,54]. Regarding this, Hsu et al. found that GPC 3 mRNA levels are frequently higher in HCC than α-fetoprotein (AFP), [3,55] another oncofetal protein that has found widespread use as a tumour marker of HCC and which may be a potential immunotherapy target [2]. For example, Hirofumi et al explored the application of immunotherapy termed 'photoimmunotherapy', that is coupled with photosensitizing phthalocyanine dye IRDye700DX® (IR700) which responds to Near-Infrared (NIR) light to target HCC. This is also combined with nanoparticles which carry paclitaxel to achieve target-specific necrotic cell death both *in vitro* and *in vivo* [56].

Zhu et al. [7] evaluated expression of GPC 3 mRNA in HCC compared with normal liver and benign liver conditions. Consistent with the findings of Hsu et al. [51], they reported that GPC 3 is upregulated in HCC but not in healthy liver or benign hepatic disorders, suggesting the role of GPC 3 as a tumour promoter in this malignancy. The authors noted that GPC 3 may influence the local growth behaviour and characteristics of HCCs and may serve as a marker for differentiation of benign liver disease and HCC [7].

Later studies confirmed these results at the protein level [7,47,48,57-59]. Immunohistochemical investigations on surgically excised liver tumours found that GPC 3 is overexpressed in HCC but not in surrounding unaffected tissue or in benign liver lesions [1,11,60]. In general, the GPC 3 protein exhibited granular or dot-like cytoplasmic or diffuse cytoplasmic staining pattern, in the presence or absence of membranous enhancement [61]. In addition, GPC 3 staining was more profound in well-differentiated hepatocellular carcinomas; as opposed to atypical neoplasms and very well-differentiated HCC which shows negative GPC 3 staining [61]. Thus GPC 3 immunostaining may be helpful in distinguishing high-grade dysplastic nodule and HCC [48]. In South Korean HCC patients, the expression of GPC 3 is associated with high aggressiveness. [58] In a recent study by Soo et al., they found that GPC 3 is expressed only in the tumour tissue but not in the circulating tumour cells [60]. Nguyen et al. observed that GPC 3 showed high sensitivity in poorly differentiated HCC; and the detection efficiency for this type of HCC is greater when combined with arginase-1 [62]. Mounajjed et al. observed GPC 3 intense granular staining at intracytoplasmic pigment in 89% of the pigmented hepatocellular adenomas cases, without any diffuse cytoplasmic staining [63].

In an immunohistochemistry study using a monoclonal antibody, Capurro et al. observed that 57% of HCCs overexpress GPC 3, whereas it was undetectable in normal liver cells, liver cirrhosis or benign lesions such as focal nodular hyperplasia [55]. Staining was localised to the cytoplasm and/or the cell surface membrane. This study also reported that, while serum GPC 3 was detected in 53% of HCC cases, it was not found in normal serum. In addition, it was reported that only 1 of 12 hepatitis and cirrhosis patients was GPC 3-positive [55]. Using an enzyme-linked immunosorbent assay (ELISA), Nakatsura et al. detected GPC 3 protein in 80% of HCC cell line culture supernatants and in 40% of HCC patients' serum, suggesting that GPC 3 may be secreted [15]. Similar findings were reported by several other laboratories. A few studies used thin-core biopsy tissue sections and early stage HCCs [3]. As a result, the International Consensus Group for HCC agreed that GPC 3 positivity strongly suggests malignancy due to the high reported sensitivity and specificity of GPC 3 immunoreactivity in the early pathologic diagnosis of small HCC [64]. Immunohistochemical staining for GPC 3 has been observed to be highly sensitive and specific and can differentiate HCC from metastatic liver adenocarcinoma in liver fine-needle aspiration biopsies [65].

GPC 3 has been shown to be a useful marker for HCC. Its

sensitivity and specificity exceeds that of both AFP and hepatocyte-paraffin1 (HepPar-1) [11]. The expression of GPC 3 at high levels by HCC cells suggests that in HCC patients, GPC 3 is secreted in the serum [6,9]. It has now been clearly established that while most HCCs express GPC 3, it is not produced in normal liver, liver cirrhosis or benign hepatic diseases. Consequently, clinical pathologists currently use GPC 3 immunostaining of liver biopsies to confirm a diagnosis of HCC [3]. Zhou et al. identified an immunohistochemical staining panel combining quantification of GPC 3, β-catenin and claudin-1 protein expression that was useful in differentiating hepatoblastoma subtypes and distinguishing paediatric hepatoblastoma from HCC [66]. In a more detailed study, they found that GPC 3 showed a distinct immuno-staining profile which may aid in differentiating hepatoblastoma subtypes from HCC and providing a subclassification of malignant paediatric liver tumours. [66]. Studies on hepatoblastomas revealed positive GPC 3 expression in all patients, [67,68] with GPC 3 cytoplasmic immunoreactivity in over 90% of cases [68]. Zhang et al. confirmed the overexpression of GPC 3 in HCCs as a useful diagnostic marker and its high specificity in the differentiation of primary liver neoplasms. GPC 3 can distinguish preneoplastic from hepatocellular neoplasms and HCCs, and is a useful biomarker in differentiating HCCs from benign liver diseases [18,69]. The authors noted that GPC 3 may be useful in the early diagnosis of HCC in clinical practice. This notion is further supported by a recent meta-analysis conducted by combining 17 studies which concluded that GPC 3 is of high diagnostic efficacy to improve the early diagnosis for HCC, [55] which has also been verified by another meta- analysis study. [70].

While GPC 3 expression is upregulated in HCC, it has been found to be downregulated in cholangiocarcinoma, both intrahepatic and extrahepatic [67,71]. Man et al. used RT-PCR to quantify GPC 3 expression in HCC and intrahepatic chanlangiocarcinoma (ICC). Their results showed that GPC 3 is differentially expressed at mRNA level in HCC and cholangiocarcinoma, which develop from various cell types. The authors suggested that GPC 3 could have different functions in these tumours and therefore could be used as a potential diagnostic marker for distinguishing HCC from cholangiocarcinoma [71]. Kandil et al. investigated GPC 3 expression in hepatic fine-needle aspiration (FNA). 90% of HCC individuals showed strong cytoplasmic expression for GPC 3, with 70% exhibiting membranous expression. All benign liver lesions and other non-HCC metastatic liver carcinomas were nonreactive for GPC3, indicating the utility of this protein in distinguishing HCC from other metastatic liver tumours and benign liver lesions. [72]. Other than conventional use of GPC 3 in detecting HCC, Li et al has explored the "one-pot method" using magnetic resonance probes that target GPC 3 magnetic resonance imaging for HCC. [73]. Another study by Gao et al. used Pseudomonas exotoxin A (PE38) fused with antibodies targeting glypican-3, HN3 and YP7 to create immunotoxins for liver tumour in mice. Tumour regression was achieved by targeting both inactivation of Wnt signalling through the antibody and blockage of protein synthesis via the toxin [74].

In addition to being a diagnostic marker, GPC 3 promotes progression of HCC. In a study of the effect of GPC 3 on HCC, Capurro et al. found that GPC 3 promotes the *in vivo* and *in vitro* growth of HCC cells by activating the canonical Wnt growth factor signalling pathway. This stimulation may be based on the potential ability of GPC 3 to facilitate the interaction and stabilise the binding of Wnt to its signalling receptor, Frizzled [75,76]. This interaction is important in cell growth and differentiation [11]. Midorikawa et al. GPC 3 interacts with fibroblast growth factor 2 (FGF2) and modulates the signalling activity of both growth factors, FGF2 and BMP-7 in HCC. In particular, GPC 3 also acts as a suppressor of the inhibitory growth factor bone morphogenetic protein 7 (BMP-7), regulating its transcriptional activation. These findings suggest that GPC 3 is important in promoting hepatocarcinogenesis [9]. Recently, several studies have shown that GPC 3 can enhance the growth, migration and adhesion of HCC cells by upregulating autocrine/paracrine canonical Wnt signalling [24,77-79] Cheng et al. [80] found that GPC 3 promotes Wnt signalling and oncogenesis via the Insulin-like growth factor (IGF) signalling pathway in HCC [20,80]. Interestingly, a recent study by Zittermann et al. demonstrated that overexpression of soluble GPC 3 inhibits HCC growth by blocking Wnt signalling [43,81]. Subsequently, it was found that mutated glypican-3 which lacks the GPI anchor domain inhibit tumour growth by blocking Wnt signalling too [78]. Moreover, Ho et al. reported GPC 3 to be expressed in liver CD90+ cancer stem cells, which are important in tumour progression and metastasis [82]. Thus, GPC 3 expression may function as a prognostic marker for HCC patients as explained by Xiao et al. They conducted a meta-analysis to show the prognostic value of GPC 3 in HCC regarding its ability to predict relapse and patients' outcomes. They concluded that current available evidence supports a strong prognostic effect of GPC 3 overexpression in HCC patients. High GPC 3 expression is a worse prognostic sign HCC cases, and it may also predict HCC invasion and metastasis [79]. Similarly, Yukihiro et al. observed high level of serum GPC 3 which is associated with poor prognosis in HCC patients after partial hepatectomy [83].

Haruyama et al. reported that measurement of serum N-terminal subunit containing-GPC 3 is useful for identifying GPC 3 expression in HCC, and high preoperative levels are associated with poor prognosis in HCC patients after curative hepatectomy [83]. GPC 3 has been reported to promote epithelial-mesenchymal transition and thus development and migration of HCC cells via activation of extracellular signal-regulated kinase signalling [84]. Liu et al. has demonstrated that upregulation of GPC 3 enhances HCC development-

related mRNA expression and was associated with dysplasia in liver cirrhosis, where GPC 3 may serve as a premalignant molecular marker [85]. Qi et al. showed that GPC 3 silencing using RNA interference led to a reduction in HCC cell proliferation and a rise in apoptosis and demonstrated that GPC 3 regulates HCC migration and metastasis through epithelial-mesenchymal transition [86]. A GPC 3 targeted human heavy chain antibody has been designed as a drug carrier for HCC therapy.[87] In summary, GPC 3 has been shown to be overexpressed in HCC [71]. However, it is absent in the hepatocytes of healthy individuals and in benign liver diseases [7,48]. Thus, it is used as a serum and immunohistochemical molecular marker that is highly sensitive and specific for HCC [6,11,88,89]. As a tumour biomarker for the screening and diagnosis of early HCC, [8,77] GPC 3 is more reliable and could allow the earlier diagnosis of HCC as compared to serum AFP [79,90] The diagnostic sensitivity could be further enhanced if GPC 3 is combined with AFP for the diagnosis of HCC [91]. Besides, another study also found that it is more advantageous to combine other markers than GPC 3 single stain alone to diagnose HCC [92] GPC 3 also represents a promising therapeutic target for HCC [6,11,93]. Targeted immune therapy using dendritic cells against GPC 3 as one of the target antigens is found to be clinically beneficial to the patients even with advanced HCC. This adjuvant therapy is well tolerated in phase I/IIa clinical trial study andhas been found to be safe to use [94]. A separate study targeting the Heparan Sulfate chains of GPC 3 using a targeted antibody which affects Hepatocyte Growth Factor (HGF) signalling pathway, has also yielded promising therapeutic implications [93]. In view of the important role of GPC 3 in the progression of malignant tumours especially for HCC, a recent study has confirmed GPC 3 as a valuable tumour marker for the early diagnosis of HCC [95]. Investigation of GPC 3 as a diagnostic and therapeutic target may enhance progress in preventing and treating liver cancer [96].

GPC 3 in Lung Cancer

GPC 3 expression was reported to be lower in smokers' healthy lung tissue than in non-smokers, and lower in adenocarcinoma of the lung than in healthy lung tissue [50,51]. These findings suggest that GPC 3 may be a lung tumour suppressor gene that is downregulated by tobacco exposure [97,98]. However, GPC 3 expression has been described in several lung squamous cell carcinomas (LSCC) [21,26,27]. A study by Kim et al. investigated GPC 3 expression and its role in lung cancer [97]. They profiled gene expression of lung adenocarcinoma and compared normal tissue from smokers and non-smokers using cDNA microarray analysis. They observed that GPC 3 expression was diminished in lung adenocarcinoma. Within the normal lung, expression of GPC 3 was reduced in smokers as compared to non-smokers, suggesting that expression may be decreased by cigarette smoking. The altered GPC 3 expression phenotype suggests that GPC 3 may suppress tumour growth and inhibit cell proliferation. Overall, GPC 3 expression was suppressed or lost in tumour tissue and in smokers, suggesting that GPC 3 is a tumour suppressor gene for lung carcinogenesis whose expression may decrease upon exposure to cigarette smoke [97]. Aviel-Ronen et al. conducted a study of GPC 3 protein and mRNA expression in non-small-cell lung carcinoma patients. GPC 3 was found to be overexpressed in lung SCC compared with normal lung tissue. Adenocarcinoma and LSCC expressed GPC 3 differentially, with elevated expression in LSCC patients who were smokers. GPC 3 was not expressed in normal lung tissue, but was expressed in 23% of lung carcinomas. There was higher GPC 3 expression in LSCC (55%) as compared to adenocarcinoma (8%). The authors noted that expression of GPC 3 renders it a promising biomarker for early detection of LSCC of the lung [21]. Yu et al. found that GPC 3 protein expression was significantly stronger in lung SCC than in lung adenocarcinoma, suggesting that GPC 3 may be a diagnostic marker for lung SCC [95]. A meta-analysis of gene expression databases by Parmigiani et al. also found that GPC 3 expression was associated with longer survival times in lung adenocarcinoma [99].

GPC 3-targeted immunotherapy is also being explored for lung SCC. Li et al. explored the potential therapeutic effect of engineered T lymphocytes against GPC 3 positive LSCC, where glypican 3 (GPC 3)-redirected Chimeric Antigen Receptor (CAR)-engineered T lymphocytes (CARGPC 3 T cells), were found to inhibit the *in vivo* growth of the cells in two established LSCC xenograft models [26]. Similarly, another study conducted by Iwama et al. which used liposome-coupled GPC 3-derived CTL epitope peptide (pGPC 3-lipsome) to inhibit GPC 3 positive tumour growth, also yielded promising antitumour potential [34].

GPC 3 in Colon Cancer

Studies on the role of GPC 3 in colon cancer have given inconsistent and contradictory findings. Initial studies demonstrated GPC 3 mRNA to be overexpressed in colon cancer compared to the corresponding normal tissues [100]. The upregulation of GPC 3 in cancer suggested that GPC 3 acts as an oncofetal protein in the colon [9]. Filmus et al. found that while normal colon is negative for GPC 3 expression, it is expressed in a significant proportion of colorectal tumours. [2] The authors suggested that re-expression of GPC 3 appears to play a role in the progression of colorectal tumours. However, in a recent study by Joo et al. comparing the expression of proteoglycans between patient-matched normal and cancerous tissue, GPC 3 was downregulated in cancer tissue as compared to normal tissue. [101] Foda et al. studied the expression of GPC 3 and E-cadherin in colorectal Mucinous Adenocarcinoma (MA) and Non-Mucinous Adenocarcinoma (NMA) using manual TMA technology. They found that although GPC 3 and E-cadherin expression were not independent prognostic factors in colorectal carcinoma,they were significantly interrelated in NMA as compared to MA [102,103]. Decreased expression of GPC 3 in

colorectal cancer progression could be due to hypermethylation of the GPC 3 gene promoter [14]. Further studies will be required to clarify the role of GPC 3 in colon cancer.

GPC 3 in Testicular Germ Cell Tumours

Expression of GPC 3 was reported to be upregulated in testicular yolk sac tumours by gene expression microarray study [104]. Subsequently, some studies have been published regarding the value of GPC 3 immunohistochemistry in differentiating testicular germ cell tumours [68,105,106]. In these researches, all yolk sac tumours express GPC 3, even more so than α-fetoprotein (AFP). On the other hand, all intratubular germ cell neoplasia, seminomas and benign testicular tissue were observed to have no GPC 3 expression [13]. The reported percentage of GPC 3 positivity in embryonic carcinoma was 0-8%, and 0-40% in teratomas in the immature elements [58,59]. GPC 3 may therefore be useful in differentiating germ cell tumours since it is expressed in virtually all yolk sac tumours [11].

GPC 3 in Embryonal Tumours

Embryonal tumours are a group of paediatric malignant neoplasms. They include medulloblastoma, neuroblastoma and Wilms' tumour. They contain undifferentiated malignant cells that resemble their immature counterparts in their organs of origin [11]. As it has been shown that GPC 3 mutations result in the Simpson-Golabi-Behmel syndrome [107], researchers have studied GPC 3 expression in some of the embryonal tumours associated with this syndrome [11]. Saikali and Sinnett found GPC 3 mRNA expression in some neuroblastomas and all Wilms' tumours, but not in medulloblastoma [108]. No correlation was found between GPC 3 expression and N-myc, an indicator of neuroblastoma prognosis. The same authors also found that all GPC 3-expressing samples expressed insulin-like growth factor-II (IGF2), indicating that GPC 3 may be involved in the development of embryonic tumours through a signalling pathway involving this growth factor [108]. Toretsky et al. reported GPC 3 upregulation in Wilms' tumour and hepatoblastoma by microarray analysis [109]. GPC 3 expression was low or undetectable in the surrounding normal tissue, supporting the GPC 3 role in the tumourigenesis of these embryonal tumours [108,109]. In addition, GPC 3 point mutations were detected in 5% of Wilms' tumours [110].

Kinoshita et al. assessed GPC 3 expression in paediatric malignant solid tumours. They found that most cases of hepatoblastoma and yolk sac tumour and some cases of neuroblastoma, Wilms tumour and rhabdomyosarcoma expressed GPC 3. Because GPC 3 is normally expressed during the fetal and neonatal periods in subjects below 1 year old, patients above 1 year of age who were positive for GPC 3 were considered suitable candidates to obtain immunotherapy using GPC 3 peptide [111]. Xiong et al. conducted a systematic analysis which found that GPC 3 expression is highly associated with pediatric hepatoblastoma [112]. Tretiakova et al. showed that GPC 3 was overexpressed in most primary and metastatic Wilms tumours, but not in other renal neoplasms, suggesting that GPC 3 can be used as a biomarker for Wilms tumours. GPC 3 protein was found in fetal kidneys but not in adult kidneys, indicating its possible role as an oncofetal protein [113].

GPC 3 in melanoma

The function of GPC 3 in melanoma remains unclear [11]. Expression of serum GPC 3 was reported in 40% of primary melanomas (PMs) [5].Nakatsura et al. conducted a study to investigate GPC 3 expression in human melanoma [114]. They used a polyclonal rabbit anti- GPC 3 antibody to investigate the GPC 3 expression in Japanese melanoma patients. The same authors reported that >80% of cases with melanoma and melanocytic nevi exhibited GPC 3 immunoreactivity. Soluble GPC 3 protein could be detected by Enzyme-Linked Immunosorbent Assay (ELISA) in cell culture supernatants of 5 of 6 melanoma cell lines. They also detected GPC 3 protein in the sera of 40% of melanoma patients irrespective of clinical stage (including stage 0 in situ melanoma), but not in healthy donor sera. In addition, GPC 3 could not be detected in the sera of 6 patients after surgical excision of the skin tumour, suggesting that this protein may be useful as a diagnostic marker for melanoma, particularly in its early stages [114]. In contrast, hiring a monoclonal antihuman GPC 3, a cytologic study performed on 60 cases of Fine Needle Aspirates (FNA)-diagnosed metastatic melanoma (MM) showed negative GPC 3 immunoreactivity in all individuals. This argued against metastatic melanoma as being GPC 3-positive [11]. These observations were supported by the data obtained from another study. Kandil et al. investigated expression of GPC 3 protein in MM FNAs, and in corresponding primary tumour histologic sections [5]. In this study, all MM cases and the corresponding primary tumour tissue sections were observed to be GPC 3-negative, which . are inconsistent with those of Nakatsura and co-workers [114]. The authors noted that Nakatsura et al. had used a different antibody (H-162 cloneobtained from Santa Cruz Biotechnology) from the one used in their study (1G12 clone from BioMosaics Inc). They raised the possibility that the 2 antibodies may react to different GPC 3 epitopes. The strongly negative data obtained from this study also argued against the reliability that melanoma is truly a GPC 3-positive tumour [5]. Further investigations will be necessary to clarify the role of GPC 3 in melanoma.

Clinical implications of GPC 3 in human cancers

As earlier discussed, GPC 3 may serve as tumour markers for diagnostic purposes such as early detection of cancer. Tumour markers may also be used to assess the severity and prognosis of cancer, and to determine the treatment regime [20]. Because levels of tumour marker may vary over time, they provide an indication

of the molecular changes during cancer progression [19]. Although not discussed in detail in this review, GPC 3 may represent a viable target for cancer therapy. The functional attributes of therapeutic targets may include specific GPC 3 core protein domains and/or heparan sulfate chains. In addition, the proteoglycan modifying enzymes, especially heparanase and endosulfatases, represent potential therapeutic targets. Reports that heparan sulfate in the nucleus can regulate gene transcription has led to speculation that GPC 3 may be directly involved in the transcription regulation of genes that control tumour behavior [20]. This possibility remains unexplored for now. Eventually, it is anticipated that GPC 3 may prove to be a potential effective therapeutic target for certain types of cancer [19].

Conclusion

We have reviewed the expression, mutations and changes in expression of GPC 3 in various human cancers, as summarised in Table 1. In some instances, these changes correlate with a poorer patient outcome. GPC 3 mediates an array of tumour functions and has the ability to either enhance or suppress the initiation and progression of cancer, depending on the tissue and tumour type. The inherent diversity of GPC 3 function suggests the possibility of multiple layers of tumour regulation [20]. When considering the role of GPC 3 in cancer, there are still many areas meriting further studies. For example, little is known of the possible alterations in heparan sulfate structure that may relate to changes in GPC 3 expression during cancer progression. This may be important, considering the ability of GPC 3 to interact with many potent growth factors [19]. The structural or biological relationships between heparan sulfate fine structures and the biological effects of these molecules on cancers are highly specific [115]. Clearly, there are many facets that need to be addressed before any effective GPC 3-based anti-cancer therapies can be developed. It is also not clear if the elevated presence of GPC 3 in tumours is a contributor to cancer progression or an effect of increased cell growth and invasive tumour behaviour. Further study of the GPC 3 mutations in cancer may help to clarify this. It is possible that elevated levels of GPC 3 contribute to tumour pathogenesis since their interaction with mitogenic proteins and ability to act as co-receptors with high-affinity tyrosine kinase receptors are crucial in cell communication [19]. The role of GPC 3 in various cancers, including those highlighted in this review, are important and relevant for further study. Current research provides the prospect that GPC 3 may be a basis for the future treatment for cancer. One key factor in its favour is that it is located on the cell surface and is thus an accessible target. Further research in animal and human models will be necessary to clarify the key issues regarding GPC 3 in cancer.

Cancer	Expression levels and functional roles	References	Expression	Protein Localisation
Breast	GPC 3 expression inhibits breast cancer invasion and metastasis in a syngeneic murine model. Ectopic expression of GPC 3 increased apoptosis in the LM3 mammary tumour cell line.	[17]	mRNA, Protein	NA
	Enhanced GPC 3 expression induced apoptosis in MCF-7 breast cancer cells.	[36]	Protein	NA
	Promoter hyper methylation silenced GPC 3 gene in human breast cancer. Ectopic expression of GPC 3 inhibited growth in MCF-7, T47D, ZR75, BT20, BT549, Hs578T, MDAMB157, MDAMB468 breast cancer cell lines.	[38]	mRNA, Protein	NA
	Mechanism of GPC 3 silencing in human breast cancer due, in part, to hypermethylation of the GPC 3 promoter, especially in hormone receptor–negative tumours. CpG island of GPC 3 was found extensively hypermethylated in ZR75, Hs578T, MCF7, MDAMB-231, and MDAMB468 breast cancer cell lines.	[39]	NA	NA
	Active location of GPC 3 gene on X chromosome (Xq26.1-q27.1 region) was found to be frequently deleted in breast cancers.	[40]	NA	NA
Ovary	GPC 3 expression found to be associated with histotype of clear cell carcinoma but not associated with tumour stage. GPC 3 expression has no major role in development of platinum-based chemo-responsiveness in ovarian cancer.	[16]	Protein	Memb, Cyto
	Meta-analysis confirmed that GPC 3 is of high diagnostic efficacy to improve the early diagnosis for HCC.	[32,70]	Protein	NS
	GPC 3 expression is downregulated and undetectable in ES-2, OV1063, A222, and A224 ovarian cancer cell lines. Where GPC 3 expression inhibited, promoter hypermethylated was found in GPC 3 gene. Restoration of ectopic GPC 3 expression inhibited colony-forming activity and cell growth in ES-2, and A224 ovarian cancer cell lines.	[41]	mRNA	NA
	GPC 3 absent or undetectable in majority of clear cell, serous, endometrioid, and mucinous tumours.	[28]	Protein	Memb, Cyto
	GPC 3 expressed more in stage I clear cell carcinoma and was associated with a shorter survival. GPC 3 expression may be associated with taxane-based chemotherapy resistance.	[42]	Protein	Memb
	GPC 3 overexpressed in ovarian clear cell adenocarcinomas but was rarely found in mucinous, endometrioid, and serous subtypes. GPC 3 expressed more in stage III/IV clear cell adenocarcinoma and was associated with a shorter overall survival.	[43]	Protein	Memb, Cyto
	Downregulation of GPC 3 enhanced migration, invasion, and tumorigenicity in human ovarian cancer cells.	[44]	mRNA, Protein	NS
	Decreased expression of GPC 3 enhanced development of human ovarian cancer cells in nude mice by upregulating matrix metalloproteinase (MMP)-2 and MMP-9 and downregulating tissue inhibitor of metalloproteinase-1.	[44]	mRNA	NA

Liver	GPC 3 strongly expressed in HCC but not in healthy liver and benign hepatic disorders such as focal nodular hyperplasia, or liver cirrhosis.	[7]	mRNA	NA
	GPC 3 overexpressed in HCC and in hepatoma cell lines (e.g. HuH7, Hep G2, HuH6, HT17, Hep3B and PLC/PRF/5). Over-expression of GPC 3 supressed the cell growth by inhibiting FGF2 and BMP-7 signalling pathways.	[9]	mRNA, Protein	Memb
	GPC 3 mRNA expressions significantly increased in most HCCs compared with healthy liver and benign liver lesions. GPC 3 more commonly upregulated in HCC than α-fetoprotein (AFP).	[3]	mRNA	NA
	Upregulation of GPC 3 enhances HCC development-related mRNA expression and was associated with dysplasia in liver cirrhosis, where GPC 3 may serve as a premalignant molecular marker.	[44]	mRNA, Protein	Memb, Cyto
	HCCs overexpress GPC 3, whereas GPC 3 not detected in healthy liver cells (hepatocytes), cirrhotic liver or benign liver lesions such as focal nodular hyperplasia. While GPC 3was detectable in sera of most HCC patients, it could not be found in normal sera.	[55]	mRNA, Protein	Memb, Cyto
	GPC 3 showed granular or dot-like cytoplasmic or diffuse cytoplasmic staining pattern, in the presence or absence of membranous enhancement; GPC 3 showed high sensitivity in poorly differentiated HCC and GPC 3 is associated with high aggressiveness.	[58,61,62]	Protein	Memb, Cyto
	GPC 3 showed intense granular staining at intracytoplasmic pigment in 89% of the pigmented hepatocellular adenomas cases, without any diffuse cytoplasmic staining.	[63]	Protein	Cyto
	Soluble GPC 3 protein could be found in culture supernatants from most HCC cell lines (Hep G2, Hep 3B, PLC/PRF/5 and HUH-7) and in sera from many patients with HCC, showing that GPC 3 may be secreted.	[116]	mRNA, Protein	Memb, Cyto
	Immunohistochemical staining for GPC 3 is highly sensitive and specific and can differentiate HCC from metastatic liver adenocarcinoma in liver fine-needle aspiration biopsies.	[65]	Protein	Cyto
	Immunohistochemical quantification of GPC 3, β-catenin and claudin-1 protein expression was useful in differentiating hepatoblastoma subtypes and distinguishing paediatric hepatoblastoma from HCC.	[66]	Protein	Cyto
	GPC 3 is abundantly expressed in HCCs. GPC 3 can be used as a differential diagnostic biomarker in primary tumours of the liver.	[69]	mRNA, Protein	Memb, Cyto
	GPC 3 overexpressed in HCC but downregulated in cholangiocarcinoma.	[71]	mRNA	NA
	90% of HCC cases in hepatic fine-needle aspiration (FNA) revealed strong cytoplasmic expression for GPC 3, with membranous expression in 70%. All benign liver lesions and other non-HCC metastatic liver carcinomas were found nonreactive.	[72]	Protein	Memb, Cyto
	GPC 3 enhances the *in vivo* and *in vitro* growth of HCC cells through canonical Wnt growth factor signalling pathway.	[75,78]	mRNA, Protein	NS
	Measurement of serum N-terminal subunit containing-GPC 3 is useful for identifying GPC 3 expression in HCC and high preoperative levels are associated with poor prognosis in HCC patients after curative hepatectomy.	[83]	Protein	NA
	GPC 3 promotes epithelial-mesenchymal transition and thus development and migration of HCC via activation of extracellular signal-regulated kinase signalling. GPC 3 silencing using RNA interference led to a reduction in HCC cell proliferation and a rise in apoptosis. GPC 3 regulates HCC migration and metastasis through epithelial-mesenchymal transition.	[90]	mRNA, Protein	NA
	GPC 3-targeted human heavy chain ntibody may be therapeutically used as a drug carrier for HCC. A heavy-chain antibody, HN3, was more effective than YP7, a conventional IgG, as a targeting agent against HCC.	[87]	NA	NA
	Diagnostic sensitivity for HCC is enhanced when combined with other markers, not just GPC 3 alone	[91,92]	Protein	NS
Lung	Unlike lung adenocarcinoma, SCC of the lung had overexpression of GPC 3.	[21]	mRNA, Protein	Memb, Cyto
	GPC 3 protein expression was significantly more in lung SCC than in lung adenocarcinoma, suggesting that GPC 3 may be a diagnostic marker for lung SCC.	[95]	mRNA, Protein	NA
	GPC 3 expression decreased in lung adenocarcinoma compared with healthy tissue and also in smokers compared with non-smokers. GPC 3 may be a tumour suppressor gene for lung carcinogenesis.	[97]	mRNA, Protein	Memb
Colon	GPC 3 mRNA is highly expressed in colon cancer compared to the corresponding normal tissues.	[100]	mRNA	NA
	Compared to normal tissues, GPC 3 was downregulated in colon cancer tissues.	[101]	Protein	NS
	GPC 3 and E-cadherin expression are not independent prognostic factors in colorectal carcinoma. However, expression of both is significantly interrelated in non-mucinous adenocarcinoma as compared to mucinous adenocarcinoma.	[103]	Protein	Cyto
	Downregulation of GPC 3 was observed and affected modulation of the Wnt signalling pathway in colorectal cancer; the decreased gene expression was due to hypermethylation	[14]	mRNA, Protein	NS
Testicular germ cell tumours	GPC 3 was found to be overexpressed in testicular yolk sac tumours using gene microarray study. However, RT-PCR analysis showed no significant changes.	[104]	mRNA	NA
	All yolk sac tumours, several choriocarcinoma, some mature teratomas and few embryonal carcinomas were immunoreactive for GPC 3. In yolk sac tumours, the positive area expressing GPC 3 was vaster than that of α-fetoprotein (AFP). In contrast, GPC 3 was undetectable in all intratubular germ cell neoplasia, seminomas, and benign testicular tissue.	[4,53,68,73]	mRNA, Protein	Memb, Cyto

Embryonal tumours	GPC 3 expressed strong and difuse in more than 90% of hepatoblastoma cases.	[68]	Protein	Cyto
	GPC 3 mRNA was detectable in Wilms' tumours and several neuroblastomas, but undetectable in medulloblastoma. All GPC 3 expressing samples expressed IGF2.	[108]	mRNA	NA
	GPC 3 was found highly expressed in Wilms' tumour and hepatoblastoma. The expression of GPC 3 of the normal tissues around the tumour was found low or undetectable.	[109]	mRNA, Protein	NA
	Most hepatoblastoma and yolk sac tumours and some neuroblastoma, Wilms tumour and rhabdomyosarcoma expressed GPC 3.	[111]	Protein	NA
	Systematic analysis found that GPC 3 expression is highly associated with pediatric hepatoblastoma.	[112]	Protein	NA
	GPC 3 overexpressed in most primary and metastatic Wilms tumours, but not in other renal neoplasms. GPC 3 protein was found in fetal kidneys but not in adult kidneys, indicating its possible role as an oncofetal protein.	[113]	mRNA, Protein	Memb, Cyto
Skin	All metastatic melanoma (MM) tissues and their corresponding primary cutaneous melanomas were reported non-reactive or weakly reactive for GPC 3.	[5]	Protein	Memb, Cyto
	GPC 3 immunoreactivity present in most patients with melanoma and melanocytic nevi. Soluble GPC 3 protein was found in culture supernatants of most melanoma cell lines. GPC 3 protein detected in sera of many melanoma patients irrespective of clinical stage but not in sera from normal individuals. GPC 3 was not detectable in sera of individuals whose melanomas were surgically removed.	[114]	mRNA, Protein	Cyto
Stomach	GPC 3 immunoreactivity was positive in hepatoid component of all 10 cases in series of α-fetoprotein (AFP)-producing gastric carcinomas. GPC 3 was reported concurrently with AFP, but its expression was stronger compared to that of AFP.	[117]	Protein	Memb, Cyto
Urinary tract	GPC 3 expressed in a significant percentage of urothelial carcinomas, primarily the high-grade tumours. GPC 3 expression can be used to differentiate low and high-grade urothelial carcinomas as well as distinguish between neoplastic and non-neoplastic urothelium.	[118]	Protein	NA
Kidney	GPC 3 is downregulated in renal clear cell carcinoma. GPC 3 reduces cell proliferation in renal cell carcinoma through G1 phase cell cycle arrest.	[119]	mRNA, Protein	NA

Memb: membranous expression; Cyto: cytoplasmic expression; NS: not specified; NA: not applicable.

Table 1: Characteristics of GPC 3 in human cancer.

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