

Review Article

GP73 expression and its significance in the diagnosis of hepatocellular carcinoma: a review

Ming-Chen Ba¹, Hui Long², Yun-Qiang Tang¹, Shu-Zhong Cui¹

¹Department of Hepatobiliary Tumor Surgery, Cancer Hospital of Guangzhou Medical College, Guangzhou 510095, PR China; ²Department of Pharmacy, Guangzhou Dermatology Institute, Guangzhou 510095, PR China

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Abstract: Hepatocellular carcinoma (HCC) is one of the most common malignant tumors, and its incidence has been increasing worldwide. Serum alpha-fetoprotein (AFP) levels and abdominal ultrasound have been widely used for diagnosis as well as surveillance of HCC. However, the sensitivity and specificity of both AFP levels and ultrasound for HCC surveillance have some shortcomings, particularly in the early stages of the disease. Golgi protein-73 (GP73) is a type II Golgi-localized integral membrane protein that is normally expressed in epithelial cells of many human tissues. It is essential for human survival, and might have multiple roles for GP73 in epithelial cell function such as in the kidney and liver. However, details of its biochemical function and regulation of GP73 expression are unknown at present. GP73 expression is upregulated in serum samples from patients with liver disease, with expression being highest in HCC. Therefore, it may be useful as a new serum marker for detection of HCC in at high-risk population. But, this hypothesis needs to be proven in large cohorts.

Keywords: Hepatocellular carcinoma, liver disease, alpha-fetoprotein, golgi protein73, tumor marker

Introduction

Hepatocellular carcinoma (HCC) is a major health problem, being the sixth most common cancer with 626,000 new cases in 2002. Its incidence continues to increase all over the world [1]. Unfortunately, patient survival with HCC has only been marginally improved over the last 20 years. Between 1981 and 1998, the 5-year survival rate only rose from 2% to 5%. The poor survival rate is mainly related to late diagnosis of HCC, when effective therapies are lacking. Surveillance of patients at highest risk for developing HCC (i.e., patients with cirrhosis) is an important strategy that can potentially decrease the HCC related mortality rate [2]. Although HCC meets the criteria of a tumor that would benefit from a surveillance program, the poor sensitivity and specificity of currently available tools has prevented widespread implementation of HCC surveillance.

Alpha-fetoprotein (AFP) is a serum marker that is most widely used for diagnosis as well as surveillance of HCC. However, AFP levels may be

normal in up to 40% of patients with HCC, particularly during the early stages of HCC. Furthermore, elevated AFP levels may be seen in patients with cirrhosis or exacerbations of chronic hepatitis. Prospective studies evaluating the performance characteristics of AFP for HCC surveillance reported sensitivities of 39% to 64%, specificities of 76% to 91%, and positive predictive values of 9% to 32% [3-5]. Abdominal ultrasound is the most common imaging modality used for surveillance of HCC, and it can lead to increased survival of patients with cirrhosis who develop HCC by allowing earlier implementation of disease management strategies. The sensitivity, specificity, and positive predictive value of ultrasound for HCC have been reported to be 71% to 78%, 90% to 93%, and 14% to 73%, respectively [6]. However, the accuracy of ultrasound can be limited by the ability of the operator to differentiate HCC from non-neoplastic lesions such as regenerative nodules [7]. Development of more sensitive and specific serum biomarkers for the early detection of HCC in the at-risk population may lead to improved survival of patients. Given

improvements in the overall survival of high-risk population, and the continued rise in the incidence of HCC in the world, strategies for the detection of early HCC are urgently needed.

Golgi Protein-73 (GP73) is a novel type II Golgi-localized integral membrane protein that is normally expressed in epithelial cells of many human tissues, it can also be detected in the sera of patients with liver disease, and its serum levels appear to be predictive of HCC [8-13]. In the present article, we reviewed the literature regarding GP73 structure, biological functions, expression distribution, regulation in humans, and especially its potential value as a diagnostic biomarker for HCC.

GP73 discovery and biological characteristics

GP73 was first identified in a genetic screen for proteins with differential expression in adult giant-cell hepatitis (GCH). Kladney et al [8] isolated and characterized GP73, a 73 kDa human Golgi protein. *In vitro* transcription-translation studies indicated that GP73 is an integral membrane protein, and immunolocalization experiments using epitope-tagged GP73 demonstrated that the protein is localized to the Golgi apparatus. Northern blot analysis of RNA from multiple human tissues revealed a single GP73 mRNA transcript with a size of approximately 3.0 kb.

GP73 was originally described as a resident Golgi type II transmembrane protein, with a single, N-terminal transmembrane domain and an extensive, C-terminal coiled-coil domain located on the luminal surface of the Golgi apparatus [11]. In recent years, a number of Golgi membrane proteins with N- or C-terminal membrane anchors similar to GP73 have been described. They include the recently described Golgin-84, p63, and GPP130 [11]. A common feature of these proteins is the presence of coiled-coil domains in the N- or C-termini of the molecule. The biochemical functions of these proteins are unknown, and there are few studies addressing the regulation of their expression. In a study by Kladney et al [11], the porcine homologue of p63 was found to be up-regulated in hepatocytes during cardiogenic shock, a finding that raises the possibility that the protein might be involved in a cellular stress response. It has been suggested that their coiled-coil domains function as homotypic or

heterotypic protein interaction sites that are essential for the binding, docking, and trafficking of transport vesicles to the cisternal membranes. All resident Golgi proteins are peripheral or integral membrane proteins, and the majority of them are involved in the intracellular modification of secretory proteins. GP73 has no homologies to the known glycosyltransferases, and is unlikely to have catalytic functions in this regard. In addition, GP73 has no significant sequence homologies or structural similarities to any of the known nucleotide, sugar, or ATP transporters of the Golgi apparatus [11-15].

GP73 protein expression distribution

GP73 is preferentially expressed by epithelial cells in many human tissues. It is consistently present in biliary epithelial cells in normal livers, and hepatocytes show little or no signal. However, GP73 expression is upregulated in hepatocytes, and in serum samples from patients with liver disease, with expression present at low levels in hepatitis, and highest in HCC [8-13]. Kladney and colleagues [9] showed that the GP73 signal in normal livers was predominantly derived from biliary epithelial cells, hepatocytes contributed little to the overall GP73 expression. In contrast, high-level expression of GP73 was present in diseased hepatocytes, regardless of the cause of liver disease. GP73 was not expressed in inflammatory cells or in cells within the cirrhotic septa, and its expression levels in biliary epithelial cells did not appear to be elevated. Based on the decreased percentage of hepatocytes compared to non-parenchymal cells in cirrhotic liver disease, the degree of up-regulation of GP73 expression in hepatocytes likely exceeds the 36- to 72-fold increase measured at the whole tissue level. This striking increase suggests that GP73 may participate in an important cellular pathway of liver disease [9-12].

In order to investigate the localization of GP73, Kladney et al [12] established sinusoidal lining cells as potential sources of GP73, especially in diseased livers. Their study results demonstrated that the co-localization of GP73 with α -SMA in a subpopulation of sinusoidal lining cells, and its absence in CD68-positive cells in the same patients, strongly suggested that activated hepatic stellate cells are a potential source

of GP73. It is tempting to speculate that GP73 expression in myofibroblasts represents a feature of disease-related cellular activation that parallels the increased expression in hepatocytes.

Because it is an integral membrane protein localized to the *cis* Golgi, it is not known how GP73 can appear in serum as a biomarker [8, 9, 12, 14, 15]. More recently, Bachert et al [16] showed that a soluble form of GP73 was released from cultured cells. Compared with the Golgi-localized full length protein, the molecular weight of the soluble form was slightly lower, suggesting that proteolytic cleavage releases the GP73 ectodomain. Sequence analysis revealed a proprotein convertase (PC) consensus site, and, indeed, the ubiquitous PC furin was found to be capable of cleaving purified GP73. Furthermore, alanine substitutions in the PC site blocked both the *in vitro* and the *in vivo* cleavage of GP73. Using a cleavage-specific antibody, cleaved GP73 was found in the *trans* Golgi network and endosomes, suggesting that GP73 cleavage occurs as GP73 cycles distally to the early Golgi. Bachert and his colleagues [17] reported that the endosomal trafficking of GP73 allowed for PC-mediated cleavage, resulting in GP73 secretion, and provided a molecular mechanism for its presence as a serum biomarker for HCC. These studies provided a plausible model to explain the higher serum GP73 concentration observed in patients with liver disease and HCC.

GP73 biological function

GP73 function is presently unknown. Multiple search algorithms for protein motifs have not revealed any obvious catalytic or enzymatic properties [16]. Recent gene knock-down studies in HepG2.2.15 cells demonstrated that GP73 inhibition is associated with a reduction in the surface area of the Golgi complex. Therefore, some researchers think that GP73 expression might be involved in maintaining the structural integrity of the Golgi complex during cellular stress [17, 18].

In order to investigate the physiological role of GP73, some researchers [19] used a gene trap approach to generate mice with a severe truncation of the GP73 C-terminus (GP73tr/tr). Wright et al [20] showed that GP73tr/tr mice were born at the expected rate, and were fer-

tile, but cumulative survival was significantly decreased compared to wild-type controls, particularly in females, and GP73tr/tr mice developed varying degrees of renal disease, most notably focal segmental glomerulosclerosis and hyaline thrombi. In addition to renal abnormalities, GP73tr/tr mice developed marked microvesicular hepatic steatosis, hepatocyte nuclear membrane irregularities and intranuclear inclusions. GP73tr/tr expression in morphologically normal kidneys and livers was constitutively low, but was strikingly upregulated in the diseased kidney cortex, and in livers in animals of advanced age. Despite the substantial morphological changes in the kidneys and liver, routine screening serum assays provided no evidence of renal or hepatic dysfunction. The cause of the increased mortality of GP73tr/tr animals is still unclear at present. These results indicate that GP73 is essential for normal survival, and suggests multiple roles for GP73 in epithelial cell function in the kidney and liver [19].

GP73 mRNA and protein are expressed in highly differentiated HepG2 hepatoma cells after infection with adenovirus *in vitro*. GP73 may be expressed in response to infection with hepatotropic viruses. Iftikhar et al [17] observed that GP73 was highly expressed in hepatocytes of patients with decompensated liver cirrhosis, so they thought that GP73 may function in the processing of viral proteins in the Golgi apparatus or in the formation of infective virions, and might be beneficial to the invading virus. Alternatively, GP73 might be part of the host cell's antiviral response, and might limit viral toxicity or replication [17, 18].

Regulation of GP73 expression

Based on studies *in vitro* in an adenovirus infection model and on preliminary immunohistochemical studies in biopsies of patients with GCH, some researchers have shown that livers of patients with GCH display strong GP73 immunoreactivity in multinucleated hepatocytes [8, 17]. Kladney et al [12] reported that acute liver disease might result in a similar upregulation of GP73 expression. Its expression was found to be, upregulated in hepatocytes from patients with liver disease, viral and non-viral. Kladney et al [11] demonstrated that acute hepatitis results in a rapid and profound upregulation of

hepatocyte GP73 expression. This effect is potentially reversible upon the resolution of the acute disease. Chronic hepatitis is associated with a gradual, stage-dependent increase over a longer time period. Maximal GP73 levels were achieved in fully established cirrhosis, with a magnitude similar to that observed in acute hepatitis [12]. Subsequently, Kladney et al [9] observed that adenovirus infection induced the expression of GP73 in HepG2 and Hep3B hepatoma cells. GP73 expression in this model was rapid in onset, and dependent on the presence of the adenoviral E1A protein, suggesting regulation at the level of transcription. HepG2 2.2.15 cells were originally developed by stable transfection of the entire HBV genome into HepG2 cells. They expressed HBV mRNA, and supported the formation infectious HBV particles *in vitro*. Kladney et al [9] demonstrated that HepG2 2.2.15 cells had robust expression of GP73, whereas HepG2 cells and HepG2T14 cells, an HBV-transfected cell line incapable of supporting productive HBV infection, did not express GP73.

Studies in hepatoma cell lines demonstrated that interferon- γ (*IFN*- γ) increased GP73 expression in SK-Hep1 hepatoma cells. Increased levels of circulating and intrahepatic *IFN*- γ have been demonstrated in patients with acute and chronic hepatitis, suggesting that this cytokine may play a role in the upregulation of GP73 expression *in vivo*. *TNF*- α has immunomodulatory, proinflammatory, and pro-apoptotic effects in acute and chronic liver disease. Inhibitory pathway may involve *TNF*- α , which strongly antagonized GP73 expression in SK-Hep1 cells, and *TNF*- α may be involved in the down regulation of GP73 during the recovery phase of acute hepatitis or during the early stages of chronic hepatitis [12].

Interestingly, *IFN*- γ and *TNF*- α had no consistent effects on GP73 expression in HepG2 and Hep3B cells, even though both cell lines expressed GP73 in response to adenovirus infection, and HepG2-derived HepG2 2.2.15 cells expressed large amounts of the protein. HepG2, Hep3B, and SK-Hep-1 cells are members of a cluster of hepatocyte-derived tumor cell lines that are genetically related, but distinct. These data suggest that the cytokine response of GP73 varies in response to the cellular genetic background [12]. Iftikhar et al [17]

proposed that there are two mechanisms to regulate GP73, the first of which is triggered during acute hepatocellular injury, and the second during progression of chronic liver disease.

GP73 expression and its relationship with hepatic disease, but no-HCC

Many studies have demonstrated that significant increases of serum GP73 levels are found in liver disease due to viral causes (HBV, HCV) or non-viral causes (alcohol-induced liver disease, autoimmune hepatitis). Therefore, some researchers consider that an increase in GP73 expression is a common feature of hepatocyte response to a variety of disease etiologies [8, 9, 12, 17, 21].

Iftikhar et al [17] found that immunohistochemical scores and quantitative GP73 measurements were highly correlated in culture cell lines expressed GP73. At low expression levels, increased GP73 tissue levels were associated with comparable increases in immunohistochemical scores, suggesting that the increase in GP73 tissue levels was mainly due to an increase in the number of GP73-expressing cells. The immunohistochemical scores reached saturation at high tissue levels, indicating that increased GP73 expression occurred at the level of individual cells. Iftikhar et al [17] studied cases according fibrosis stage, and demonstrated marked increases in GP73 expression in stage 3 and 4 (due to HCV) and stage 2, 3, and 4 (due to alcohol) disease. Unlike the stage-dependent increase in GP73 expression in acute HBV hepatitis, no significant differences in GP73 expression were found with respect to the same disease stages of liver disease regardless of etiology (HCV- vs. alcohol). This suggests that the increase in GP73 expression is a common feature of the hepatocyte response to various causes of inflammation.

Gu and colleagues [21] have shown that the median serum GP73 levels in patients with liver disease were significantly higher ($p < 0.001$) than in healthy individuals, and in patients with other diseases. When GP73 was used to detect liver disease, it had a sensitivity of 82% and a specificity of 80% at the optimal cut-off value of 85.5 mg/L. The area under the receiver-operating characteristic curve (AUROC) was 0.9. Gu et

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Table 1. Comparison of the efficacy of serum GP73 and AFP levels in the diagnosis of HCC

References	AFP-negative (%)	GP73			AFP		
		AUROC	Sensitivity	specificity	AUROC	Sensitivity	specificity
Marrero et al 2005	62% (32/52)	0.79	69%	86%	0.61	30%	96%
Mao et al 2008	35.1%(12/37)	—	76.9%	92.9%	—	48.6%	75%
Wang et al 2009	—	0.94	95 %	70 %	0.83	95%	28%
Hu et al 2010	49.1% (61/124)	0.89	77.4%	83.9%	0.77	48.4%	96.8%

GP73, Golgi protein-73; AFP, Alpha-fetoprotein; AUROC, area under the receiver operating curve.

al [21] concluded that GP73 concentrations in patients with liver disease were three-fold higher than in healthy individuals. However, GP73 concentrations did not differ significantly between patients in each liver disease group. Furthermore, GP73 was not significantly elevated in patients with diseases of organs other than the liver, compared with healthy individuals. These results suggest that GP73 may be used as a serum marker for the diagnosis of liver disease.

GP73 significance in the diagnosis of HCC

GP73 was found to be elevated in the serum in the woodchuck model of HCC compared to non-neoplastic liver. Therefore, many researchers think that the significant increase in serum GP73 levels in patients with HCC potentially provides a marker for early detection of HCC [10, 22-26]. Several reports have stated that GP73 is a better marker than AFP for diagnosing HCC.

Marrero et al [23] showed that serum GP73 levels were significantly higher in patients with HCC compared to those with cirrhosis. Serum GP73 levels had an AUROC of 0.79 (95%CI: 0.72–0.82) and a sensitivity of 69%, specificity of 75%, while, AFP with an AUROC 0.61 (95%CI: 0.59–0.71) and a sensitivity of 30%, specificity of 96%. Serum GP73 levels above the optimal cutoff were found in 62 and 71% of HCC patients who had AFP levels below 20 and 100 ng/ml, respectively, demonstrating the utility of GP73 in the diagnosis of HCC in patients with normal or mildly elevated AFP, and the performance of GP73 as determined by AUROC was found to be better than AFP. The findings suggest an advantage of GP73 over AFP as a serum marker for early detection of HCC. Marrero et al. [23] confirmed that GP73 is upregulated in hepatocytes from patients with HCC, and suggested that GP73 may be a serum marker of HCC.

Mao and colleagues [24] showed that the serum GP73 levels in HCC patients that were HBV positive were significantly higher than those of the HBV carriers, patients with non-liver diseases, and healthy controls. There was no difference in GP73 levels between healthy controls and patients with non-liver diseases (P=0.2925). The sensitivity of GP73 for the diagnosis of HCC was 76.9%, significantly higher than that of AFP 48.6%. The specificity for the diagnosis of HCC of GP73 was 92.9%, significantly higher than that of AFP 75%. In the Mao study, nearly 35.1% of the patients with HCC were AFP-negative (<25 ng/l). Mao et al [24] reported that serum GP73 had a higher sensitivity and specificity in diagnosis of hepatitis B-related HCC than AFP, and that it could be a new effective HCC tumor marker in Chinese Patients.

Wang et al [25] measured the levels of fucosylated kininogen (Fc-Kin) and fucosylated alpha-1-antitrypsin and analyzed them individually and in combination with AFP, and GP73 for the ability to distinguish between cirrhosis and HCC. The GP73 had the best individual performance characteristic, with an AUROC of 0.89, a specificity of 43% and a sensitivity of 95%. For comparison, AFP performance was found to be similar to GP73 with an AUROC of 0.83, a specificity of 28%, and a sensitivity of 95%. The best performance was achieved with a combination of Fc-Kin, AFP, and GP73, giving, an AUROC of 0.94, a sensitivity of 95%, and a specificity of 70%. Wang et al [25] reported that altered glycosylation of serum glycoproteins can act as potential biomarkers of primary HCC when used independently or in combination with other markers of HCC include GP73.

Hu et al [26] studied ROC curves comparing all the HCC patients in Chinese patients, and showed that the HBV-related HCC AUROC curve for GP73 was 0.89 (95%CI: 0.82–0.97), with a sensitivity of 77.4%, specificity of 83.9%,

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Table 2. Comparison of AUROC, positive predictive value, sensitivity, specificity of serum GP73, AFP level and abdominal ultrasound in the diagnosis of HCC

	PPV	AUROC	Sensitivity	Specificity
GP73		0.79-0.94	69%-95%	70%-93%
AFP	61%~83%		30%-95%	28%-96%
Abdominal ultrasound	14%~73%		71%-78%	90%-93%

GP73, Golgi protein-73; AFP, Alpha-fetoprotein; AUROC, area under the receiver operating curve; PPV, positive predictive value.

whereas the AUROC for AFP was 0.77 (95%CI: 0.65–0.89), with a sensitivity of 48.4%, specificity of 96.8%. The findings suggested that GP73 may be more useful as a serum marker for the detection of HCC compared to AFP. Hu et al [26] showed that nearly 49.1% of the patients with HCC were AFP-negative (<25 ng/l). After comparing GP73 in patients with AFP positive HCC, no significant difference was found. Hu et al [26] showed that GP73 may be a serum marker of HCC in the Chinese population.

A comparison of serum GP73 and AFP level in the diagnosis of HCC from recently documents is shown in **Table 1**. Serum GP73 levels were with an AUROC of 0.79 to 0.94, sensitivity of 69% to 95% and specificity of 70% to 93% in diagnosis of HCC, significantly higher than that of AFP with an AUROC 0.61 to 0.83, sensitivity of 30% to 95% and specificity of 28% to 96%. Moreover, serum GP73 levels in patients with AFP-positive HCC were not significantly different compared to those with AFP-negative HC. A comparison of AUROC, sensitivity, specificity of serum GP73, AFP levels and abdominal ultrasound in the diagnosis of HCC obtained from recent publications is shown in **Table 2**. Serum GP73 levels had an AUROC of 0.79-0.94, sensitivity of 69-95% and specificity of 70-93% in diagnosis of HCC, which were significantly higher than that of AFP with a positive predictive value 61-83%, sensitivity of 30-95% and specificity of 28-96%. In contrast, abdominal ultrasound had a positive predictive value 14-73%, sensitivity 71-78%, and sensitivity 90-93%, respectively.

Serum AFP levels have been shown to correlate with tumor size. But, currently there are no data on the relationship between tumor size of HCC and serum GP73 levels. Marrero et al [23] showed that serum GP73 levels were signifi-

cantly higher in patients with early stage HCC (T1/T2) compared to those with cirrhosis (regardless of etiology). Wang et al [26] showed that GP73 had the best individual performance characteristic in differentiating cirrhosis from stage I or II HCC. Further study is required to determine the details of the relationship between serum GP73 levels and HCC size. Mao et al [24]

showed that in a few HCC patients, the GP73 levels were not markedly lower a week after surgical resection, but became lower 1.5-2 years after surgery. However, AFP levels usually decrease substantially within a week post-resection. These results demonstrate that serum GP73 levels change slower than serum AFP levels.

Serum AFP levels have been found to be high in patients with HCC, but at low levels in cholangiocellular hepatic carcinoma. Mao et al [24] showed that the GP73 levels in 4 of 6 intra-hepatic cholangiocarcinoma patients were intermediate between those of the HCC patients and HBV carriers. Aside from this report, there are no data on whether GP73 expression is significantly increased in metastatic tumors or non-hepatocellular hepatic carcinoma.

In summary, GP73 is a type II Golgi-localized integral membrane protein that is normally expressed in epithelial cells of many human tissues. Details of its biochemical function and regulation of expression are unknown at present. GP73 expression increased in serum samples from patients with liver disease, with expression being highest in HCC. Therefore, it may be a better serum marker of HCC than AFP. However, these data need to be confirmed in larger cohorts of patients to determine if GP73 is a reliable serum marker of early HCC, to compare its accuracy with AFP in large cohorts to determine its role in HCC surveillance.

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Address correspondence to: Dr. Ming-chen Ba, Department of Hepatobiliary Tumor Surgery, Cancer Hospital of Guangzhou Medical College, Guangzhou 510095, P.R. China. Tel: +86-13825017156; Fax: +86-20-83509106; E-mail: bamingchen@126.com or Dr. Hui Long, Department of Pharmacy, Guangzhou Dermatology Institute, Guangzhou 510095, PR China. Tel: +86-13535448232; Fax: +86-20-83489984; E-mail: 798587414@qq.com

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