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Retrospective Study

Low glucose metabolism in hepatocellular carcinoma with GPC3 expression

You-Cai Li, Chuan-Sheng Yang, Wen-Lan Zhou, Hong-Sheng Li, Yan-Jiang Han, Quan-Shi Wang, Hu-Bing Wu

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Abstract

AIM

To investigate the relationship between glucose metabolism and glypican-3 (GPC3) expression in hepatocellular carcinoma (HCC).

METHODS

Immunohistochemical staining of pathological samples for GPC3 and glucose transporter 1 (GLUT1), and whole-body ^{18}F -FDG PET/CT for measuring tumour glucose uptake were performed in 55 newly diagnosed HCC patients. The maximum standard uptake value (SUV_{max}) and tumour-to-non-tumourous liver uptake (T/NT) ratio were used to quantify ^{18}F -FDG uptake. *In vitro* ^{18}F -FDG uptake assay of GPC3-expressing HepG2 and non-GPC3-expressing RH7777 cells was used to examine the effect of GPC3 in cellular glucose metabolism. The relationships between GPC3 expression and ^{18}F -FDG uptake, GLUT1 expression, tumour differentiation, and other clinical indicators were analysed using Spearman rank correlation, univariate

and multiple logistic regression analyses.

RESULTS

Positive GPC3 expression was observed in 67.3% of HCC patients, including 75.0% of those with well or moderately differentiated HCC and 36.4% of those with poorly differentiated HCC. There was an inverse relationship between GPC3 expression and SUV_{max} (Spearman correlation coefficient = -0.281, $P = 0.038$) and a positive relationship between GLUT1 expression and SUV_{max} (Spearman correlation coefficient = 0.681, $P < 0.001$) in patients with HCC. Univariate analysis showed that two glucose metabolic parameters (SUV_{max} and T/NT ratio), tumour differentiation, lymph node metastasis, and TNM stage were all significantly associated with GPC3 expression ($P < 0.05$), whereas GLUT1 expression, sex, age, tumour size, intrahepatic lesion number, and distant metastasis showed no statistical association ($P > 0.05$). Further multivariate analysis revealed that only the T/N ratio was significantly correlated with GPC3 expression in patients with HCC ($P < 0.05$). *In vitro* assay revealed that the uptake of ^{18}F -FDG in GPC3-expressing HepG2 cells was significantly lower than that of non-GPC3-expressing RH7777 cells ($t = -20.352$, $P < 0.001$).

CONCLUSION

The present study demonstrated that GPC3 expression is inversely associated with glucose metabolism, suggesting that GPC3 may play a role in regulating glucose metabolism in HCC.

Key words: Hepatocellular carcinoma; Glypican-3; ^{18}F -FDG; Maximum standard uptake value; T/NT ratio; Glucose metabolism; Glucose transporter 1

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Core tip: The present study demonstrated that glypican-3 (GPC3) was positively expressed in 67.3% of hepatocellular carcinoma (HCC) patients. GPC3 expression is found to be inversely associated with the glucose metabolism of HCC tumours in the patient study. Multivariate analysis revealed that only the glucose metabolism was significantly correlated with GPC3 expression ($P < 0.05$), but not GLUT1 expression, tumour differentiation, or other clinical indicators ($P < 0.05$). Low glucose metabolism was also observed in positive GPC3-expressing HepG2 cells in cellular uptake assay. Therefore, we suggested that GPC3 may play a role in regulating glucose metabolism in HCC.

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INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common and fatal malignancies worldwide and is especially prevalent in China. The outcome of patients with HCC is poor, with a low five-year survival rate of 25%-39%^[1]. Surgical resection remains the standard treatment for early stage HCC^[2,3]. Unfortunately, most patients present with advanced stage HCC at diagnosis, and there are few treatment options for them since current systemic therapy often cannot effectively control advanced stage HCC^[1,2]. Therefore, it is of utmost importance to develop a technique that can accurately diagnose early stage HCC as well as an effective treatment that can control advanced stage HCC.

Glypican-3 (GPC3) is a member of the glypican family of heparin sulfate proteoglycans (HSPGs). This protein has been reported to be highly expressed in HCC, but not in normal liver tissue, cirrhosis tissue, or paracancerous tissue^[4-6]. GPC3 plays an important role in regulating malignant transformation and promoting the growth of HCC by stimulating the canonical Wnt signalling pathway^[7]. Therefore, GPC3 is suggested to be an important target for diagnosis and therapy. Recently, positron emission tomography (PET) imaging using a ^{89}Zr -conjugated monoclonal antibody or a F(ab')₂ fragment directed against GPC3 was shown to successfully enable the non-invasive quantification and visualization of tumour GPC3 expression *in vivo*^[8-10], which has potential to be a specific probe for HCC detection. In addition, GPC3-targeted therapies are emerging as novel molecular treatments for HCC patients^[11-15].

Malignant cells require accelerated glycolysis to generate ATP, in order to meet their high energy demands for cell proliferation and survival. Accelerated glycolysis has been widely confirmed to be a common biological phenomenon in malignant tumours by positron emission tomography combined with computed tomography (PET/CT) using 2-[fluorine-18]-fluoro-2-deoxy-D-glucose (^{18}F -FDG), a glucose analogue^[16-18]. However, glucose metabolism varies greatly in HCC. Low glucose metabolism was often observed in well- and moderately differentiated HCC^[19,20]. Previous studies have revealed that low ^{18}F -FDG uptake was correlated with high FDG-6-phosphatase activity, low expression of GLUT1 or GLUT2, and high expression of P-glycoprotein^[21,22]. However, Cho *et al.*^[23] found that GPC3 may also be an important regulator for glucose metabolism in HCC. They reported that GPC3 could bind to GLUT1 and decrease glucose uptake by HCC cells. Nevertheless, this phenomenon has not yet been confirmed in patients. Therefore, we performed the present study to elucidate their relationship in HCC patients. This work may contribute to a better understanding of the biological role of GPC3 in HCC and could be useful to predict the potential utility of GPC3

targeted imaging in the clinic.

MATERIALS AND METHODS

Patients

This study included 55 patients (46 males, 9 females; mean age: 52.9 years [range: 18–78 years]) with newly diagnosed HCC who underwent ^{18}F -FDG PET/CT for staging before local hepatectomy or biopsy at Nanfang Hospital from August 2013 to October 2017. The inclusion criteria were as follows: (1) final diagnosis of HCC established by pathologic examination; (2) no adjuvant therapy administered before the PET/CT scans; and (3) available GPC3 and GLUT1 immunohistochemical staining. A total of 55 patients met the criteria and were enrolled in this study.

^{18}F -FDG PET/CT scans

^{18}F -FDG PET/CT scans were performed using a Biograph mCTx scanner (Siemens, Germany). The patients were instructed to fast for at least 6 h, and their blood glucose levels were monitored with a glucometer prior to ^{18}F -FDG injection. All the patients had blood glucose levels below 7.0 mmol/L. ^{18}F -FDG was manufactured using a tracer synthesis system (TRACERlab FX_{FDG}; GE Healthcare, United States) and had a > 95% radiochemical purity. Approximately 60 min after the intravenous injection of 318–524 MBq (8.6–14.2 mCi, 150 $\mu\text{Ci/kg}$) ^{18}F -FDG, whole-body PET/CT was performed at our centre according to established protocols^[24].

Image interpretation

The acquired PET and CT images were registered and analysed using the syngo MI workplace (Siemens, Germany). All the PET/CT images were independently read by two nuclear medicine physicians with over five years of experience. Both physicians were blinded to the findings of other imaging modalities and the GPC3 expression data. For visual analysis, tumours with higher ^{18}F -FDG uptake than that of non-tumour liver tissue were considered PET positive. For the semi-quantitative analysis, a region of interest (ROI) was drawn along the margin of the HCC lesion to measure the SUV_{max}, which was used to quantify glucose metabolism. We also calculated the tumour-to-non-tumourous liver uptake (T/NT) ratio by dividing the tumour SUV_{max} by the SUV_{mean} of the non-tumourous liver tissue, which was measured by automated computation of the average SUV_{mean} of three 1-cm ROIs in non-tumourous liver tissue, two in the right lobe and one in the left lobe, using the syngo MI workplace (Siemens, Germany)^[25]. For lesions without obvious ^{18}F -FDG uptake, the ROI was drawn on CT images and copied to the corresponding region on the PET images in order to measure the SUV_{max} and T/NT ratio. Non-contrast-enhanced CT images obtained from PET/CT

were reviewed by two experienced radiologists.

Immunohistochemical analysis

HCC tissue samples were acquired *via* biopsy or surgical resection. Paraffin-embedded tissue sections were deparaffinized with xylene and rehydrated in a graded series of ethanol solutions. Antigen retrieval was performed by heating the slides twice in 0.01 mol/L sodium citrate buffer, pH 6.0, in a microwave oven (13 min, 850 W). Endogenous peroxidase was then blocked with 0.3% H₂O₂ in methanol for 15 min at room temperature. Immunohistochemical staining was performed by incubating the slides with a mouse anti-GPC3 antibody (sc-65443 1G12; Santa Cruz Inc., Santa Cruz, CA, United States) or rabbit anti-GLUT1 antibody (ZA-0471; ZSGB-BIO, China) at a dilution of 1:100 at 4 °C overnight. Serial sections were stained with a horseradish peroxidase enzyme-labelled polymer conjugated to anti-mouse/rabbit immunoglobulins, according to the instructions of the Chemmate EnVision/Mo&Rb Detection Kit (GK500705, Gene Tech Company Limited, Shanghai, China).

The total GPC3 immunostaining score was calculated based on the percent positivity of stained tumour cells and the staining intensity. The percent positivity was scored as 0 (< 5%), 1 (5%–10%), 2 (11%–50%), or 3 (> 50%). The staining intensity was scored as 0 (no staining), 1 (weak staining), 2 (moderate staining), or 3 (strong staining). The percent positivity and staining intensity were determined in a double-blinded manner. The GPC3 expression score based on membrane and cytoplasmic immunostaining was calculated as percent positivity score \times staining intensity score and ranged from 0 to 9. The GPC3 expression level was defined as -, 0; 1⁺, 1–2; 2⁺, 3–5; or 3⁺, 6–9^[26].

Glucose transporter-1 expression in tumour cells was evaluated using a semiquantitative scoring method: score 0 = absence of immunostaining; score 1 = 1%–10% of cells stained; score 2 = 10%–50% of cells stained; and score 3 = > 50% of cells stained. No account was taken for the intensity of staining and necrotic areas were excluded from the evaluation^[27]. An immunoreactive score above 2 was defined as high GLUT1 expression, while a score of 0 or 1 was defined as low expression.

In vitro assay of cellular glucose metabolism

In vitro assay was performed to evaluate the effect of GPC3 expression on the cellular glucose metabolism. GPC3-expressing HepG2 and non-GPC3-expressing RH7777 cells^[8,10] were seeded into 12-well plates at a density of 5×10^5 cells per well for overnight incubation. Cells were rinsed three times with phosphate buffered saline (PBS), followed by the addition of ^{18}F -FDG (111 kBq/well) to the cultured wells in quadruplicate. After incubation at 37 °C for 60 min, cells were rinsed three times with PBS and lysed with NaOH sodium

Table 1 Univariate analysis of the variables related to GPC3 expression in hepatocellular carcinoma *n* (%)

Variable	GPC3 expression		χ^2 or <i>t</i>	<i>P</i> value
	Negative	Positive		
Gender			1.458	0.227
Male	13 (28.3)	33 (71.7)		
Female	5 (55.6)	4 (44.4)		
Age (yr)			0.014	0.907
< 50	7 (31.8)	15 (68.2)		
≥ 50	11 (33.3)	22 (66.7)		
Tumour differentiation			4.341	0.037
Well or moderate	11 (25.0)	33 (75.0)		
Poor	7 (63.6)	4 (36.4)		
Tumour size (cm)			2.542	0.111
< 5	3 (17.6)	14 (82.4)		
≥ 5	15 (39.5)	23 (60.5)		
¹⁸ F-FDG			3.135	0.077
Positive	15 (40.5)	22 (59.5)		
Negative	3 (16.7)	15 (83.3)		
Intrahepatic lesion number			0.461	0.497
Solitary	11 (29.7)	26 (70.3)		
Multiple	7 (38.9)	11 (61.1)		
Lymph node metastasis			4.341	0.037
Positive	7 (63.7)	4 (36.4)		
Negative	11 (25.0)	33 (75.0)		
Distant metastasis			0.836	0.361
Positive	5 (50.0)	5 (50.0)		
Negative	13 (28.9)	32 (71.1)		
TNM stage			4.969	0.026
I - II	7 (21.2)	26 (78.8)		
III-IV	11 (50.0)	11 (50.0)		
Serum AFP (μg/L)			2.645	0.104
< 20	11 (44.0)	14 (56.0)		
≥ 20	7 (23.3)	23 (76.7)		
HBV infection			0.836	0.361
Positive	13 (28.9)	32 (71.1)		
Negative	5 (50.0)	5 (50.0)		
Liver cirrhosis			0.445	0.505
Positive	10 (29.4)	24 (70.6)		
Negative	8 (38.1)	13 (61.9)		
GLUT1			1.863	0.172
High	6 (54.5)	5 (45.5)		
Low	12 (27.3)	32 (72.7)		
SUV _{max}	9.56 ± 5.95	6.01 ± 3.55	2.341	0.028
T/N ratio	4.52 ± 2.92	2.62 ± 1.55	2.597	0.017

GPC3: Glypican-3; GLUT1: Glucose transporter 1; SUV: Standard uptake value.

dodecyl sulfate (SDS) (0.2 mol/L NaOH, 1% SDS). The cell lysate was collected and the cell-associated radioactivity was then measured using a gamma counter (GC-1200, USTC Chuangxin Co. Ltd. Zonkia Branch, China). The cell uptake was normalized with inputted radioactivity. Experiments were conducted in quadruplicate^[28].

Statistical analysis

All statistical analyses were performed using SPSS version 20.0. Differences in glucose metabolic parameters (SUV_{max}, T/NT ratio) between groups were compared using the *t*-test (unpaired). GPC3 positive rates were compared using the crosstabs χ^2 test. Spearman rank correlation was used to determine the association between GPC3 expression, GLUT1

expression, and glucose metabolism. Univariate and multiple logistic regression analyses were used to analyse the association between GPC3 expression and ¹⁸F-FDG uptake, GLUT1, histopathological diagnosis, and other clinical parameters. A *P*-value < 0.05 indicated statistical significance.

RESULTS

Patient characteristics

Of the 55 included patients, 44 (80.0%) were diagnosed with well or moderately differentiated HCC, and 11 (20.0%) were diagnosed with poorly differentiated HCC. Immunohistochemical analysis showed that the expression of GPC3 was positive in 67.3% (37/55) of patients, including 75.0% (33/44) of those with well or moderately differentiated HCC and 36.4% (4/11) of those with poorly differentiated HCC patients. The GPC3 expression score was 3⁺ in 34.5% (19/55) of the patients, 2⁺ in 14.5% (8/55), 1⁺ in 18.2% (10/55), and 0 in 32.7% (18/55). Twenty percent (11/55) of tumours had high GLUT1 expression and 80% (44/55) tumours had low GLUT1 expression. Multiple intrahepatic lesions were found in 18 (32.7%) patients, and solitary lesions were observed in 37 (67.3%) patients. Most intrahepatic lesions (69.1%) were larger than 5 cm in diameter. The disease was categorized into TNM stage I in 29 patients, TNM stage II in 4, TNM stage III in 6, and TNM stage IV in 16. Other related clinical information is shown in Table 1.

Association of ¹⁸F-FDG uptake with GPC3 expression, GLUT1 expression, and tumour differentiation

HCC lesions were noted to be positive for ¹⁸F-FDG PET/CT in 37 (67.3%) patients and negative in 18 (32.7%) patients by the visual analysis. The SUV_{max} for primary tumours ranged from 2.07 to 18.60 (7.17 ± 4.73) and the T/NT ratio ranged from 0.86 to 10.0 (3.24 ± 2.26). In the lesions with negative ¹⁸F-FDG uptake, GPC3 expression was positive in 15/18 (83.3%) patients. Combining ¹⁸F-FDG uptake with GPC3 expression, the total positivity reached 94.5% (52/55).

There was an inverse relationship between GPC3 expression and ¹⁸F-FDG uptake (SUV_{max}: Spearman correlation coefficient = -0.281, *P* = 0.038; T/NT ratio: Spearman correlation coefficient = -0.303, *P* = 0.025), and ¹⁸F-FDG uptake in HCC lesions with GPC3 positivity was significantly lower than that of lesions with GPC3 negativity (SUV_{max}: 6.01 ± 3.55 vs 9.56 ± 5.95, *t* = -2.341, *P* = 0.028; T/NT ratio: 2.62 ± 1.55 vs 4.52 ± 2.92, *t* = -2.597, *P* = 0.017) (Figure 1A and B, Figure 2). On the contrary, a positive association was found between GLUT1 expression and ¹⁸F-FDG uptake (SUV_{max}: Spearman correlation coefficient = 0.681, *P* < 0.001; T/NT ratio: Spearman correlation coefficient = 0.616, *P* < 0.001). ¹⁸F-FDG uptake in the high GLUT1 expression group was significantly higher than that in the low GLUT1 expression group (SUV_{max}: 13.58 ± 3.44 vs

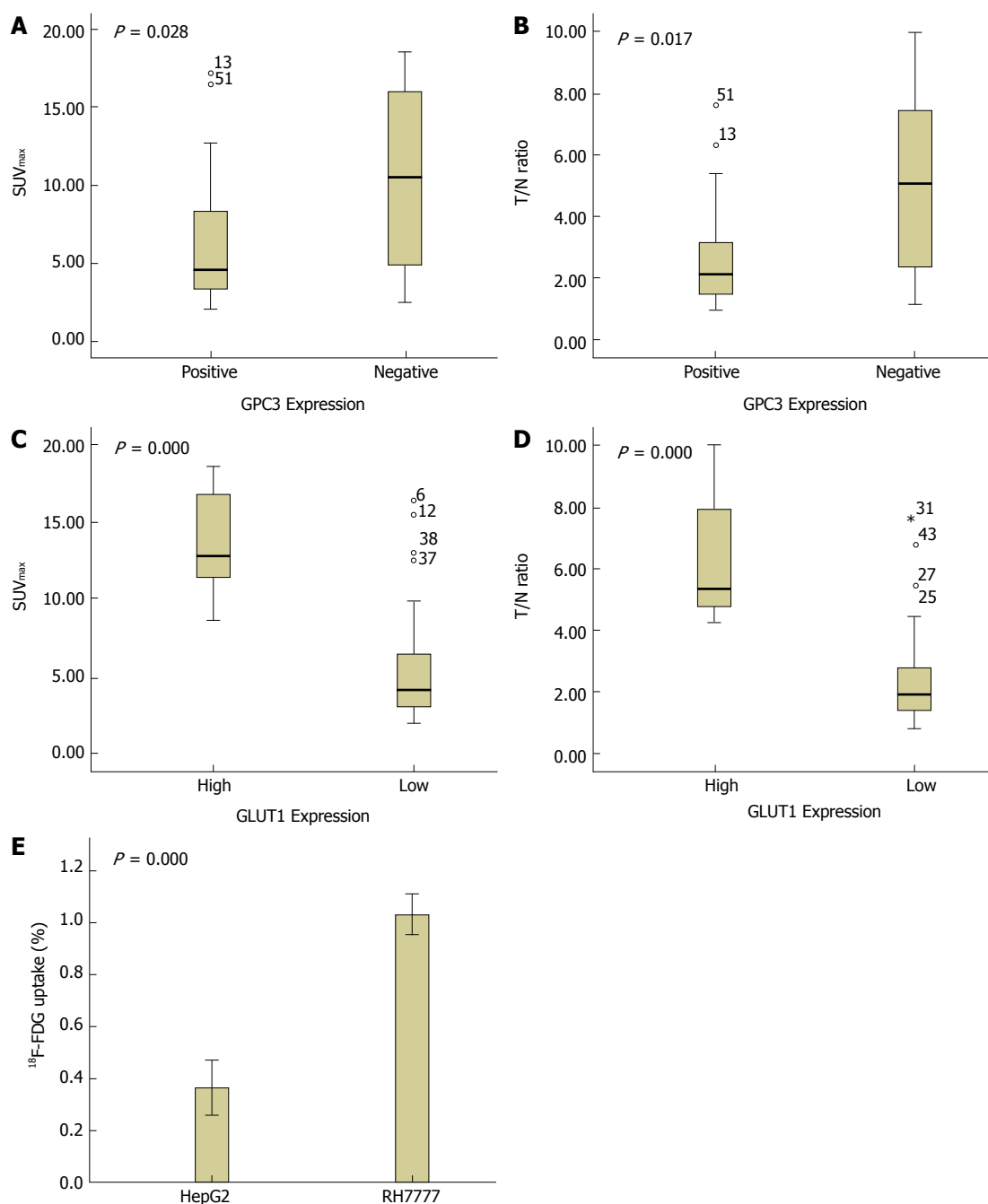


Figure 1 The relationship of ¹⁸F-FDG uptake with GPC3 and GLUT1 expression, and the cellular ¹⁸F-FDG uptake assay. A and B: ¹⁸F-FDG uptake in hepatocellular carcinoma (HCC) lesions with positive and negative GPC3 expression. (A) SUV_{max}: 6.01 ± 3.55 vs 9.56 ± 5.95, $t = -2.341$, $P = 0.028$; (B) T/NT ratio: 2.62 ± 1.55 vs 4.52 ± 2.92, $t = -2.597$, $P = 0.017$. C and D: ¹⁸F-FDG uptake in HCC lesions with high and low expression of GLUT1. (C) SUV_{max}: 13.58 ± 3.44 vs 5.57 ± 3.49, $t = 6.898$, $P < 0.001$; (D) T/NT ratio: 6.38 ± 1.91 vs 2.46 ± 1.55, $t = 6.307$, $P < 0.001$. E: ¹⁸F-FDG uptake in GPC3-expressing HepG2 cells and non-GPC3-expressing RH7777 cells (0.37% ± 0.05% vs 1.03% ± 0.04% of inputted radioactivity, $t = -20.352$, $P < 0.001$).

5.57 ± 3.49, $t = 6.898$, $P < 0.001$; T/NT ratio: 6.38 ± 1.91 vs 2.46 ± 1.55, $t = 6.307$, $P < 0.001$) (Figure 1C and D). We then investigated the relationship between GPC3 and GLUT1 expression. Low GLUT1 expression was found in 86.5% of GPC3-positive tumours and in 66.7% of GPC3-negative tumours, respectively. Although there was an inverse trend of relationship between GPC3 and GLUT1 expression, it did not reach statistical significance (Spearman correlation coefficient = -0.232, $P = 0.088$).

There were significant differences in SUV_{max} and T/NT

ratio between different degrees of tumour differentiation. Poorly differentiated HCC had a significantly higher SUV_{max} and T/NT ratio than well- or moderately differentiated HCC (SUV_{max}: 10.96 ± 6.08 vs 6.22 ± 3.86, $t = 2.465$, $P = 0.030$; T/NT ratio: 5.16 ± 3.06 vs 2.76 ± 1.74, $t = 2.499$, $P = 0.028$, respectively).

Univariate and multivariate analyses of the relationship of ¹⁸F-FDG uptake, tumour differentiation, and other factors with GPC3 expression

SUV_{max}, T/NT ratio, tumour differentiation, and other

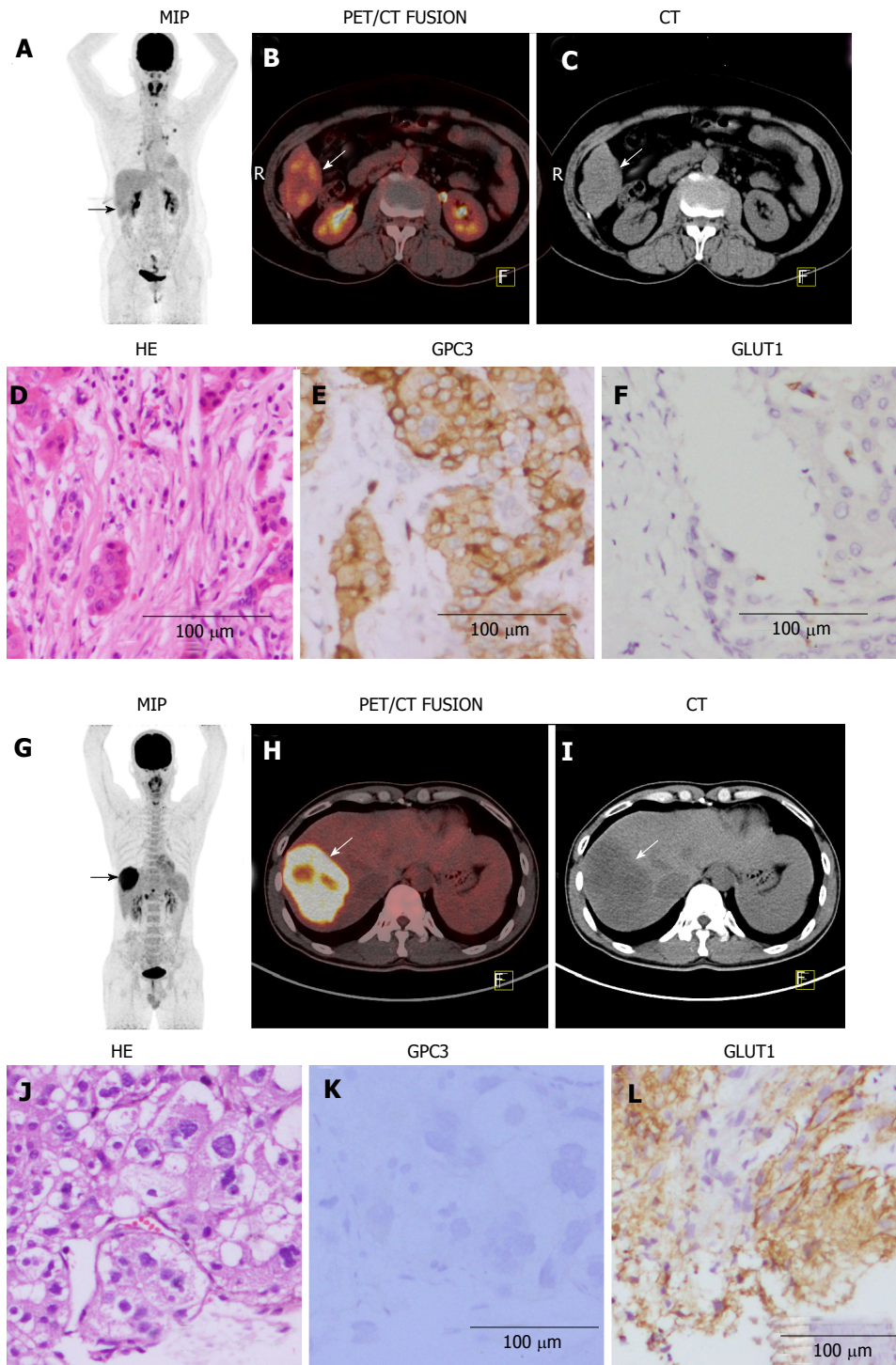


Figure 2 A 60-year-old woman with moderately differentiated hepatocellular carcinoma positive for GPC3 expression (A-E) and a 38-year-old man with poorly differentiated HCC negative for GPC3 (G-J). A-C: ^{18}F -FDG PET/CT showed slight ^{18}F -FDG uptake ($\text{SUV}_{\text{max}} = 3.4$, $\text{T/NT} = 1.54$) in the tumour (black arrow in A, white arrows in B and C). D: Moderately differentiated hepatocellular carcinoma (HCC) was diagnosed by pathological examination using HE staining. E: Immunohistochemical analysis revealed positive expression of GPC3. F: Immunohistochemical analysis revealed low expression of GLUT1 in tumour tissue. G-I: ^{18}F -FDG PET/CT scans showed intense accumulation of ^{18}F -FDG ($\text{SUV}_{\text{max}} = 16.5$, $\text{T/NT} = 7.03$) in the tumour (black arrow in G, white arrows in H and I). J: Poorly differentiated HCC was confirmed by pathological examination using HE staining. K: Immunohistochemical analysis revealed that the tumour was negative for GPC3 expression. L: Immunohistochemical analysis revealed high GLUT1 expression in tumour tissue.

clinical factors were analysed for their relationship with GPC3 expression. Univariate analysis showed that two glucose metabolic parameters (SUV_{max} and T/NT ratio), tumour differentiation, lymph node metastasis, and TNM stage were all significantly associated with

GPC3 positivity in HCC patients ($P < 0.05$) (Table 1). Low ^{18}F -FDG uptake was observed in GPC3-positive HCCs. Well- or moderately differentiated HCCs also showed a significantly higher GPC3 positive rate than poorly differentiated HCC tumours (75.0% vs

36.4%, $\chi^2 = 4.341$, $P = 0.037$). Similar trends were observed for lymph node metastasis and TNM stage. Higher GPC3 positivity rates were found in patients with no lymph node metastasis and those with TNM stage I-II disease, than in patients with lymph node metastasis and TNM stage III-IV disease, respectively (75.0% vs 36.4%, $\chi^2 = 4.341$, $P = 0.037$ for lymph node status; 78.8% vs 50.0%, $\chi^2 = 4.969$, $P = 0.026$ for TNM stage). Other clinical factors, such as GLUT1 expression, sex, age, tumour size, ^{18}F -FDG positivity, intrahepatic lesion number, distant metastasis, HBV infection, and liver cirrhosis, were not significantly related to GPC3 expression ($P > 0.05$) (Table 1).

The five factors (two glucose metabolic parameters, tumour differentiation, lymph node metastasis, and TNM stage) that showed a significant relationship with GPC3 expression on univariate analysis were further analysed using multivariate analysis. The multivariate analysis demonstrated that only T/N ratio was significantly correlated with GPC3 expression in patients with HCC ($P = 0.007$, OR = 1.479, 95.0%CI: 1.113-1.964), while SUV_{max}, tumour differentiation, lymph node metastasis, and TNM stage had no significant association ($P > 0.05$).

Effect of GPC3 expression on cellular uptake of ^{18}F -FDG

To evaluate the effect of GPC3 expression on the glucose metabolism, GPC3-expressing HepG2 cells and non-GPC3-expressing RH7777 cells were incubated with ^{18}F -FDG for 60 min and the cellular uptake was measured. The results revealed that HepG2 cells had a significantly lower ^{18}F -FDG uptake than that of RH7777 cells ($0.37\% \pm 0.05\%$ vs $1.03\% \pm 0.04\%$ of inputted radioactivity, $t = -20.352$, $P < 0.001$) (Figure 1E), which is consistent with the findings in the patient study.

DISCUSSION

^{18}F -FDG PET/CT has often been used to non-invasively evaluate tumour glycolysis *in vivo* by measuring the uptake of ^{18}F -FDG, a glucose analogue^[29-33]. This radiotracer is transported into cells *via* glucose transporters (GLUTs) and is then phosphorylated to ^{18}F -FDG-6-phosphate by the rate-limiting glycolytic enzyme hexokinase type 2. ^{18}F -FDG-6-phosphate then becomes trapped within cells^[29-33]. High ^{18}F -FDG uptake is indicative of accelerated glycolysis. Although ^{18}F -FDG is consistently taken up intensively by a variety of cancers, ^{18}F -FDG accumulation in HCCs appears to be variable. It is well established that ^{18}F -FDG uptake by well and moderately differentiated HCCs is low, whereas ^{18}F -FDG uptake by poorly differentiated HCC is high^[34-36]. On the contrary, ^{11}C -acetate and ^{11}C -choline, which are probes for lipid metabolism, have been reported to be intensively taken up by well- and moderately differentiated HCCs^[37,38], indicating that low glucose metabolism and high lipid metabolism are

the specific energy metabolism patterns of low grade HCC. In the present study, we found that SUV_{max} was actually lower in well- or moderately differentiated HCC than in poorly differentiated HCC, which consolidated the above views^[34-36]. Low ^{18}F -FDG uptake has also been found to correlate with low expression of GLUT1 or GLUT2 and high expression of P-glycoprotein^[21,22]. Our study confirmed the above findings that low GLUT1 expressing tumours actually had a significantly low ^{18}F -FDG uptake than that of high GLUT1 expressing tumours ($P < 0.001$).

In the present study, for the first time, we found the phenomenon that low glucose metabolism also occurred in the HCCs with positive GPC3 expression, not only in the patient study, but also in the *in vitro* cellular uptake assay. In the patient study, an inverse association was noted between GPC3 expression and ^{18}F -FDG uptake ($P < 0.05$). ^{18}F -FDG uptake in HCC lesions with GPC3 positivity was significantly lower than that of lesions with GPC3 negativity (SUV_{max}: 6.01 ± 3.55 vs 9.56 ± 5.95 , $t = -2.341$, $P = 0.028$; T/NT ratio: 2.62 ± 1.55 vs 4.52 ± 2.92 , $t = -2.597$, $P = 0.017$). Furthermore, multivariate analysis revealed that only the glucose metabolism was significantly correlated with GPC3 expression ($P < 0.05$), but not other clinical factors. *In vitro* cellular uptake assay also revealed that GPC3-expressing HepG2 cells had a low ^{18}F -FDG uptake than non-GPC3-expressing RH7777 cells ($0.37 \pm 0.05\%$ vs $1.03 \pm 0.04\%$ of inputted radioactivity, $t = -20.352$, $P < 0.001$). Consistent with these findings, we observed that GPC3 expression was highly expressed in well- or moderately differentiated HCCs, which always have low ^{18}F -FDG uptake^[34-36]. Therefore, our study implied that GPC3 may be another underlying factor that contributes to the complex ^{18}F -FDG uptake characteristics in HCCs. Cho *et al.*^[23] reported that GPC3 could bind to GLUT1 with an equilibrium dissociation constant (K_d) of 1.61 nmol/L and decrease glucose uptake by HCC cells, which might be helpful to explain this phenomenon. However, in the present study, low GLUT1 expression was found in most (86.5%) of GPC3-positive tumours. In addition, although an inverse trend of relationship was observed between GPC3 and GLUT1 expression, their association did not reach statistical significance (Spearman correlation coefficient = -0.232, $P = 0.088$). Therefore, the present study had no enough evidence to identify that GPC3 inversely regulates the glucose *via* GLUT1 and further basic research is warranted to uncover the mechanism.

Both SUV_{max} and T/NT ratio can be used to quantify ^{18}F -FDG uptake in tumours, however, in the present study, multivariate analysis revealed that only the T/NT ratio was significantly correlated with GPC3 expression ($P < 0.05$), but not the SUV_{max} ($P > 0.05$). A rational explanation for this result is that T/NT ratio can be more accurate to define ^{18}F -FDG uptake in HCC since it is not influenced by serum glucose level, the uptake period, or measurement variation, which often make the

measurement of SUV_{max} inaccurate^[25].

GPC3 is currently under consideration as a potential molecular therapeutic target for HCC^[11-15]. GPC3-targeted treatments that utilize siRNA or anti-GPC3 antibodies have shown potential in altering cell migration, metastasis, and invasion, and in inhibiting xenograft tumour growth^[13,15,39,40]. A GPC3-derived peptide vaccine has also been tested in a phase II study as an adjuvant therapy for HCC^[41]. GPC3-targeted PET imaging might be useful for the non-invasive analysis of GPC3 expression in HCC patients and for selecting those suitable for GPC3-targeted therapy. The present study also indicated that GPC3-targeted PET imaging might be helpful for detection of early stage HCC, which often presents with a low uptake of ^{18}F -FDG and appears as well- and moderately differentiated HCC in pathology tests. In the present study, we demonstrated that GPC3 expression was positive in most (75.0%) of the well- or moderately differentiated HCC tumours. More importantly, in the lesions with negative ^{18}F -FDG uptake, GPC3 expression was positive in 15/18 (83.3%) patients. Combining ^{18}F -FDG uptake with GPC3 expression, the total positivity reached 94.5% (52/55). Therefore, we propose that GPC3-targeted PET imaging may improve diagnostic sensitivity for early stage HCC and can serve as an effective complement to ^{18}F -FDG imaging for diagnosing HCC.

There are some limitations to the present study. First, the sample size of patients was small, especially the number of poorly differentiated HCC patients, which may cause the results of this study to fail to reflect the real correlation between GPC3 and glucose metabolism. Second, this was a retrospective study, and thus, there may have been a certain degree of bias.

ARTICLE HIGHLIGHTS

Research background

Glypican-3 (GPC3) is a cell surface proteoglycan overexpressed in most hepatocellular carcinomas (HCCs), but not in normal liver tissue, cirrhosis tissue, or paracancerous tissue. Therefore, GPC3 is suggested to be an important target for diagnosis and therapy. Elucidating the relationship between GPC3 expression and glucose metabolism may contribute to a better understanding of the biological role of GPC3 in regulating glucose metabolism. In addition, the research also could be useful to predict the potential utility of GPC3-targeted imaging in the clinic.

Research motivation

In this study, we investigated the relationship between GPC3 expression and glucose metabolism in HCC with an aim to uncover how GPC3 regulates the glucose metabolism in HCCs and predict the potential utility of GPC3-targeted imaging in the clinic.

Research objectives

This study aimed to investigate the relationship between glucose metabolism and GPC3 expression in HCC.

Research methods

A retrospective analysis was performed on 55 HCC patients who had undergone ^{18}F -FDG PET/CT before therapy. Tumour SUV_{max} and T/N ratio were used to quantify ^{18}F -FDG uptake. The relationship between ^{18}F -FDG uptake and expression of GPC3 and glucose transporter 1 (GLUT1) was analyzed

by immunohistochemical analysis. *In vitro* cellular ^{18}F -FDG uptake was also measured in GPC3-expressing HepG2 and non-GPC3-expressing RH7777 cells to determine the effect of GPC3 on glucose metabolism. The relationships between GPC3 expression and ^{18}F -FDG uptake, GLUT1 expression, tumour differentiation, and other clinical indicators were analysed using spearman rank correlation, and univariate and multiple logistic regression analyses.

Research results

In the present study, we found a phenomenon that the glucose metabolism in the GPC3-expressing HCC tumours is low in the patient study. ^{18}F -FDG uptake in HCC lesions with GPC3 positivity was significantly lower than that of lesions with GPC3 negativity (SUV_{max} : 6.01 ± 3.55 vs 9.56 ± 5.95 , $t = -2.341$, $P = 0.028$; T/N ratio: 2.62 ± 1.55 vs 4.52 ± 2.92 , $t = -2.597$, $P = 0.017$). Furthermore, multivariate analysis revealed that only the glucose metabolism was significantly correlated with GPC3 expression ($P < 0.05$), but not other clinical factors. In *in vitro* cellular uptake experiments, GPC3-expressing HepG2 cells were also found to have low ^{18}F -FDG uptake than that of non-GPC3-expressing RH7777 cells ($0.37\% \pm 0.05\%$ vs $1.03\% \pm 0.04\%$ of inputted radioactivity, $t = -20.352$, $P < 0.001$). Although an inverse trend of relationship was observed between GPC3 and GLUT1 expression, their association did not reach statistical significance (Spearman correlation coefficient = -0.232 , $P = 0.088$).

Research conclusions

GPC3 was reported to play an important role in regulating malignant transformation and promoting the growth of HCC by stimulating the canonical Wnt signalling pathway. Besides, glucose is very important for malignant cell survival and proliferation. Both of them are very important for tumour growth. Therefore, we suggested that there might be a correlation between GPC3 and tumour glucose metabolism. We used ^{18}F -FDG PET/CT for non-invasively measuring tumour glucose uptake *in vivo* in HCC patients and ^{18}F -FDG uptake assay to measure the cellular glucose metabolism. In conclusion, the expression of GPC3 was observed to be positive in 67.3% (37/55) of HCC patients. The patient study and *in vitro* cellular uptake assay demonstrated that the glucose metabolism is inversely correlated with the expression of GPC3 in HCC. These results implied that GPC3 may be another underlying factor that contributes to the complex ^{18}F -FDG uptake characteristics in HCCs. We believe that it is helpful for clarifying the mechanism of anti-GPC3 treatment by uncovering how GPC3 regulates the glucose metabolism in HCC. In addition, we found that GPC3 expression was positive in 15/18 (83.3%) of the lesions with negative ^{18}F -FDG uptake. Combining ^{18}F -FDG uptake with GPC3 expression, the total positivity reached 94.5% (52/55). Therefore, we propose that GPC3-targeted PET imaging may improve diagnostic sensitivity for early stage HCC and can serve as an effective complement to ^{18}F -FDG imaging for diagnosing HCC.

Research perspectives

For the future research, we want to investigate the mechanism concerning how GPC3 regulates the glucose and lipid metabolism in HCC. In the previous study, we found ^{11}C -choline, as a probe of lipid metabolism, could be highly taken up by well- and moderately differentiated HCC. So, we deduce that GPC3 may have a potential to promote the lipid metabolism in HCC, which may conversely reduce the glucose metabolism. We want to do further basic research confirm this hypothesis.

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