

Expression of Glut-1 and HK-II in Pancreatic Cancer and Their Impact on Prognosis and FDG Accumulation¹



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Abstract

OBJECTIVE: The purpose of this article is to analyze the expression of Glut-1 and HK-II, the association between their expression and ¹⁸F-FDG accumulation in pancreatic cancer. **METHODS:** Fifty patients with histologically proven pancreatic cancer were included in this preliminary study, all of whom received ¹⁸F-FDG PET/CT performance before surgery. Immunohistochemical staining of tumor tissue and adjacent normal tissue was performed for Glut-1 and HK-II. By combining proportions and intensity of immunochemical staining, we obtained the modified immunohistological scores for Glut-1 and HK-II respectively. The relationship between expression of Glut-1, HK-II and series of parameters was analyzed, i.e. clinicopathological characteristics, prognosis of patients and SUV_{max} of PET-CT. **RESULTS:** Compared with normal tissue, the Glut-1 and HK-II expression in pancreatic cancer tissue was significantly increased ($P < .001$). There was no correlation between expression of Glut-1, HK-II and age, gender, tumor size, tumor location, tumor histological type, tumor differentiation, the nerve infiltration, vascular invasion, local infiltration, lymph node metastasis or tumor staging in pancreatic cancer ($P > .05$). During the follow-up period, the survival curves of low Glut-1 group and high Glut-1 group were statistically different ($P = .049$). Multivariate analysis (Cox regression) revealed that Glut-1 expression was not associated with mortality ($P > .05$). No statistical difference was found in the survival curves of negative HK-II group and positive HK-II group ($P = .545$). There was no correlation between ¹⁸F-FDG uptake and expression of Glut-1 and HK-II ($P > .05$). **CONCLUSION:** The Glut-1 and HK-II expression in pancreatic cancer tissue was significantly increased. There was no correlation between expression of Glut-1, HK-II and clinicopathological characteristics, prognosis and ¹⁸F-FDG uptake.

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Introduction

Pancreatic cancer is considered to be one of the most aggressive human cancers, and the prognosis is poor. The 5-year survival rate is less than 5% [1,2]. The main reasons of high mortality lay in difficult early-diagnosis and lack of special treatment. Therefore, understanding the molecular biology of pancreatic cancer is important for early diagnosis and treatment, and may eventually provide new therapeutic targets for pancreatic cancer.

The cancer cells typically depend more on aerobic glycolysis (a persistently high rate of glucose conversion into lactate even under normoxic condition). This increased glycolysis, which accompanied by accelerated glucose uptake, is known as the Warburg effect, after German biochemist Otto Warburg [3], who first described the phenomenon in 1920s. The Warburg effect is a universal property of cancers [4,5] and a distinctive metabolic characteristic of malignancies that distinguishes them from normal cells. It is reported that glucose uptakes increase in 60–90% of malignant tumors (including

pancreatic cancer). This particular way of energy supply makes it possible to kill tumor cells specially. Therapeutic strategies based on glucose metabolism become the hotspots in tumor studies.

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The exact mechanism for the accelerated glucose use seen in tumors is not clear. It is mentioned that the increased glucose uptakes mainly lay in two factors: transmembrane transport mediated by specific glucose transporters (Gluts) [6,7] and increased concentrations of hexokinases (HKs) [8,9]. As major subtypes of each family, glucose transporter protein, type 1 (Glut-1) and glucose phosphorylation enzyme type II (Hexokinase II, or HK-II) have become the focus of tumor research, and may provide new targets for tumor therapy.

The use of ^{18}F -FDG is based on the increase glucose metabolism of malignant cells, in which ^{18}F -FDG, an analogue of glucose, is absorbed, phosphorylated, and trapped in the cytosol of the cells. ^{18}F -FDG PET/CT has been widely used not only for detecting and staging pancreatic tumors but also for monitoring therapy response [6,10]. The exact mechanism of ^{18}F -FDG accumulation in pancreatic cancer has not been fully elucidated. Recent years, More and more studies have evaluated the relationship between temporal changes in ^{18}F -FDG uptake and expression of Glut-1 or HK-II [11,12]. It is indicated that the high expression of Glut-1 is an important factor for ^{18}F -FDG in malignant tumors [13,14]. Whereas, in different malignant tumors, the correlations between ^{18}F -FDG uptake and expression of Glut-1 or HK-II were different.

But as far as immunohistochemical study of Glut-1 and HK-II in human pancreatic cancer is concerned, only a few cases have been reported. In this study, we examined the immunohistochemical expression of Glut-1 and HK-II in the resected pancreatic tumor. The relationships between expression of Glut-1 or HK-II and clinicopathological characteristics, prognosis of pancreatic cancer patients were analyzed to explore new methods for diagnose and treatment. The correlations between ^{18}F -FDG accumulations and expression of Glut-1 or HK-II were analyzed in order to elucidate the mechanism of ^{18}F -FDG accumulation in pancreatic cancer.

Materials and Methods

Materials

This study consisted of 50 patients who had an pancreatic operation between June 2011 and December 2013. All patients underwent ^{18}F -FDG PET/CT imaging before operation. Final diagnoses were confirmed histopathologically in resected specimens obtained by operation of all patients. None of them received any previous chemotherapeutic or radiotherapeutic treatments. All patients have full clinical data. There were 33 men and 17 women with ages ranging from 43 years to 80 years (median 64.4 years). Tumor staging was based on the TNM system proposed by AJCC (American Joint Committee on Cancer Staging): 8 cases in Stage I, 36 cases in Stage II, 5 cases in Stage III and 1 case in Stage IV. The study was performed with institutional review board approval. An informed consent was obtained from all the patients who participated in this study.

The survival datas of patients were obtained by telephone contact or direct home visit. Survival was determined from the date of surgery until death of patients. Death from a cause other than cancer relapse or survival until the end of observation period (December 1, 2013) was considered a censoring event or patient death. The follow-up time was 6 to 35 months (the median follow-up time was 17 months). We lost track of 5 patients during the observation period, and the follow-up rate was 90%. Patient information and tumor characteristics are summarized in Table 1.

Table 1. Patient Characteristics

Patient Characteristics	Number
Age	
≥60	36
<60	14
Gender	
Male	34
Female	16
Tumor size	
≤4 cm	33
>4 cm	17
Tumor location	
Head of pancreas	37
Body and tail of pancreas	12
Both	1
Tumor histological type	
Other types	12
Ductal adenocarcinoma	38
Tumor differentiation	
II, III, III	25
I, II	21
Nerve infiltration	
Present	24
Absent	26
Vascular invasion	
Present	19
Absent	31
Local infiltration	
Present	37
Absent	13
Lymph node metastasis	
Present	24
Absent	26
Tumor staging	
I-IIA	24
IIB-IV	26

Methods

PET/CT study. The patients fasted for at least six hours before the ^{18}F -FDG injection. Serum levels of glucose were monitored immediately before the ^{18}F -FDG injection. ^{18}F -FDG (approximately 7.4 MBq/kg) was administered intravenously. Simultaneous emission-transmission PET/CT scans were performed one hour after ^{18}F -FDG injection with a dedicated scanner (Siemens Biograph HD 64).

PET images were compared with the corresponding CT images. ^{18}F -FDG accumulation was analyzed semiquantitatively by calculating the standardized uptake value (SUV) in the regions of interest (ROI) placed over the suspected lesions on one-hour images after injection of ^{18}F -FDG. The ROI placed over the tumor was 10 × 10 mm (independent of tumor size) and was placed in tumor areas that showed the highest ^{18}F -FDG activity. The transaxial slice with the highest radioactivity concentration within the tumor was identified, and the decay-corrected maximal count in the tumor area was divided by the injected dose of ^{18}F -FDG normalized for body weight to calculate the maximum standardized uptake value (SUV_{max}).

$$\text{SUV} = \frac{C \left(\frac{\text{kBq}}{\text{ml}} \right)}{\frac{\text{ID}(\text{kBq})}{\text{body}} \text{weight}(\text{kg})}$$

Immunohistochemical staining. The expression of Glut-1 and HK-II was detected by immunohistochemistry (enhance labeled polymer system, ELPS) in 50 cases of pancreatic cancer tissue and 50 cases of corresponding adjacent tissue. The resected specimens were fixed in 10% formalin. The immunoperoxidase procedure (avidin-biotin-complex

method) was performed on the paraffin embedded sections to allow the detection of Glut-1 and HK-II expression. Antigen retrieval was performed by heating the deparaffinized and rehydrated sections in 10 mmol/l citrate buffer for five minutes. The polyclonal rabbit anti-human Glut-1 antibody (DAKO Cytomation A/S, Copenhagen, Denmark) and monoclonal mouse anti-human HK-II antibody (Abcam ab104836, Cambridge, MA) were used as the primary antibodies at a dilution of 1:100 and 1:200 respectively.

Immunohistochemical analysis for anti-Glut-1 antibody and anti-HK-II antibody was independently performed by three well-experienced physicians who were unaware of patients' datas. A random selection of ten photographic cuts was examined in each paraffin tissue. According to the criteria of Higashi et al [15], the staining intensity was classified as 0, 1, 2 or 3 points for no staining, weak, moderate and strong intensity, respectively. Moreover, The percentage of positive cells was rated as: 1 point, 0%–10% positive cells; 2 points, 11–50% positive cells; 3 points, 51%–75% positive cells; 4 points, >75% positive cells. The expression levels of Glut-1 and HK-II were assessed semiquantitatively using the product of these scores (intensity \times % positive): 0–3 points, negative(-), 4–5 points, weakly positive(+), 6–8 points, positive(++), 9–12 points, strongly positive(+++) [16]. For statistical reasons, tumors were classified into two groups: (-), negative; (+), (++) and (+++), positive. In survival analysis, tumors were classified into two groups according to the Glut-1 expression: (-), (+) and (++) , low reactivity group, (+++)high reactivity group.

Statistical Analysis

Statistical analysis was performed using the SPSS 19.0 statistical software package. The quantitative data was expressed as mean \pm

standard deviation. The difference between groups of continuous data was identified by Student's *t*-test. Categorical variables were assessed by χ^2 or Fisher's exact test. Spearman's rank correlation coefficient test was carried out for testing the association between ordinal variables. Survival curves were calculated using the Kaplan–Meier method and compared by the log-rank test. The Cox proportional hazards regression model was used for multivariate analyses after univariate analysis defined relevant prognostic variables. Significance was presumed at $P < .05$.

Results

Immunohistochemical Findings

Glut-1 expression in pancreatic tumors occurred mainly in the cytoplasm and in the plasma membrane of tumor cells (Figure 1). In the examined patients, pancreatic cancer cells showed different expression of Glut-1: negative in 9 cases, weakly positive in 3 cases, positive in 11 cases and strongly positive in 25 cases. In adjacent normal tissue, negative, weakly positive, positive and strongly positive expression was detected in 48, 2, 0 and 0 cases respectively. The protein expression of Glut-1 in tumor tissue was 81.3%, being significantly higher than that in adjacent normal tissue (4%, $P < .001$) (Figure 2).

HK-II was mainly expressed in the cytoplasm of tumor cells (Figure 1). As well as Glut-1 staining, pancreatic cancer cells showed different expression of HK-II ranged from negative in 18 cases, weakly positive in 3 cases, positive in 18 cases, to strongly positive in 9 cases. The expression of HK-II was 62.5% in tumor cells. There were 50, 0,

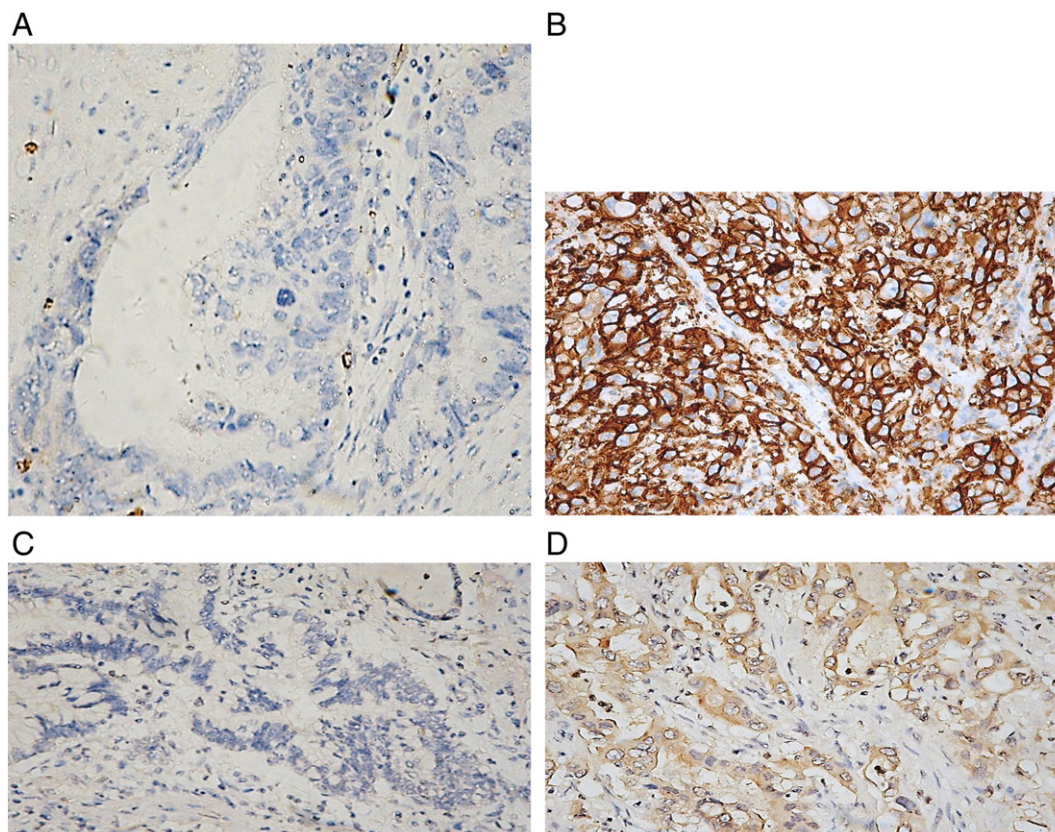


Figure 1. A: Negative expression of Glut-1 in adjacent normal tissue. B: Positive expression of Glut-1 in pancreatic cancer. C: Negative expression of HK-II in adjacent normal tissue. D: Positive expression of HK-II in pancreatic cancer.

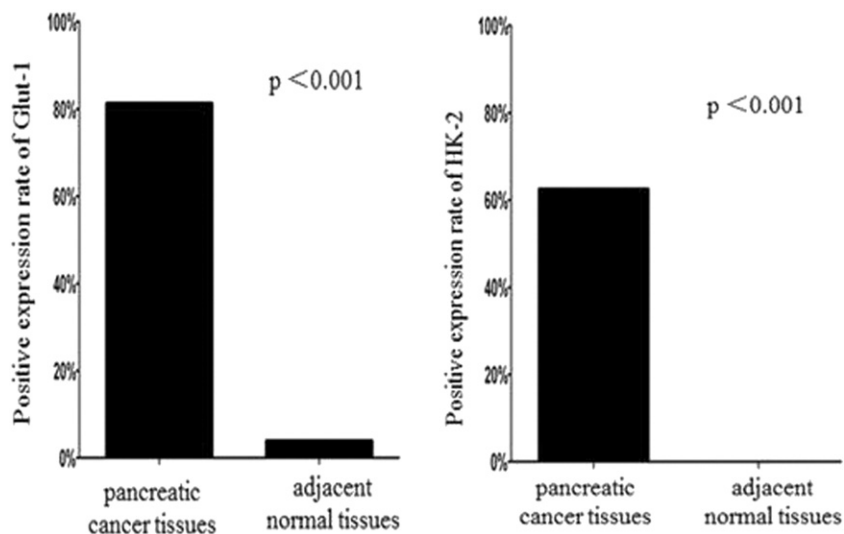


Figure 2. The positive expression rate of Glut-1 and HK-II in pancreatic cancer tissue and adjacent normal tissue.

0 and 0 cases of adjacent normal tissue showing negative, weakly positive, positive and strongly positive HK-II expression. That is to say, there was no HK-II expression in adjacent normal tissue. The HK-II expression between two groups was statistically different (62.5% vs 0%, $P < 0.001$) (Figure 2).

Figure 3 showed the comparative analysis of immunohistochemical staining results using anti-Glut-1 and anti-HK-II antibody. The Glut-1 expression had no correlation with the HK-2 expression ($P > .05$).

3.2. Correlations Between Glut-1 or HK-II Expression and Clinicopathological Parameters and Patient Survival

There was no correlation between Glut-1 expression and age, gender, tumor size, tumor location, tumor histological type, tumor differentiation, the nerve infiltration, vascular invasion, local infiltration, lymph node metastasis or tumor staging ($P > .05$) (Table 2). Meanwhile, the HK-II expression had no correlation with age, gender, tumor size, tumor location, tumor histological type, tumor differentiation, the nerve infiltration, vascular invasion, local infiltration, lymph node metastasis or tumor staging in pancreatic cancer ($P > .05$) (Table 3).

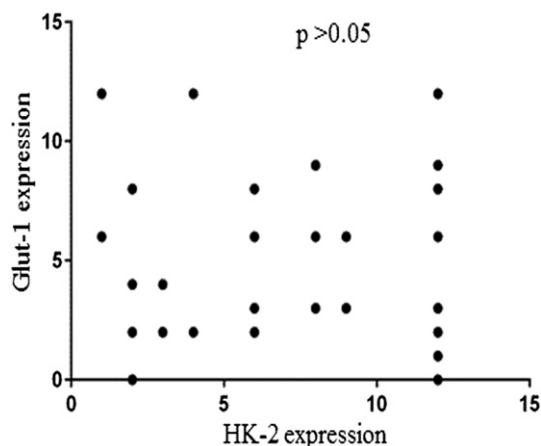


Figure 3. The correlation between Glut-1 expression and HK-2 expression.

During the follow-up period, the survival curve of low Glut-1 group and high Glut-1 group was statistically different ($P = .049$) (Figure 4). No statistical difference was found in the survival curves of negative HK-II group and positive HK-II group ($P = .545$) (Figure 5). Univariate analysis revealed that higher expression of Glut-1 was correlated with poor prognosis. In addition, we evaluated the impact of clinicopathological features on overall survival. As a result, large tumor size, present of lymph node metastasis, advanced tumor staging significantly predicted patients'

Table 2. Associations Between Glut-1 Levels and Clinicopathological Variables in Pancreatic Cancer Patients

Patient Characteristics	Negative (-)	Positive (+ - +++)	P-Value
Age			>.05
≤60	6	28	
>60	3	11	
Gender			>.05
Male	6	26	
Female	2	13	
Tumor size			.460
≤4 cm	7	24	
>4 cm	2	15	
Tumor location			.178
Head of pancreas	4	30	
Body and tail of pancreas	4	8	
Tumor histological type			>.05
Other types	2	9	
Ductal adenocarcinoma	7	30	
Tumor differentiation			>.05
II, III, III	4	20	
I,II	4	16	
Nerve infiltration			.466
Present	3	20	
Absent	6	19	
Vascular invasion			.451
Present	5	14	
Absent	4	25	
Local infiltration			.199
Present	5	31	
Absent	4	8	
Lymph node metastasis			.279
Present	6	17	
Absent	3	22	
Tumor staging			.235
I-IIA	2	20	
IIB-IV	6	19	

Table 3. Associations Between HK-II Levels and Clinicopathological Variables in Pancreatic Cancer Patients

Patient Characteristics	Negative (-)	Positive (+ - +++)	Pvalue
Age			.870
≥60	13	21	
<60	5	9	
Gender			.252
Male	13	18	
Female	4	12	
Tumor size			.527
≤4 cm	11	21	
>4 cm	7	9	
Tumor location			.737
Head of pancreas	12	22	
Body and tail of pancreas	5	7	
Tumor histological type			>.05
Other types	4	7	
Ductal adenocarcinoma	14	23	
Tumor differentiation			>.05
II, III, III	6	17	
I, II	3	18	
Nerve infiltration			.881
Present	7	14	
Absent	10	16	
Vascular invasion			.441
Present	8	10	
Absent	10	20	
Local infiltration			0.513
Present	12	23	
Absent	6	7	
Lymph node metastasis			.551
Present	8	16	
Absent	10	14	
Tumor staging			.805
I-IIA	9	13	
IIB-IV	9	17	

survival, whereas age, gender, tumor location, tumor histological type, tumor differentiation, nerve infiltration, vascular invasion and local infiltration had no predictive value. All factors that were significant for predicting overall survival by univariate analysis were included in the multivariate Cox regression analysis, which revealed that Glut-1 was not associated with mortality ($P > .05$) (Table 4).

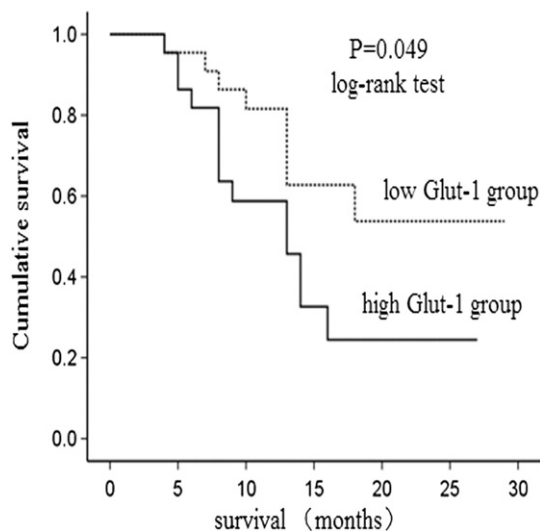


Figure 4. The survival analysis in Glut-1 low group and Glut-1 high group.

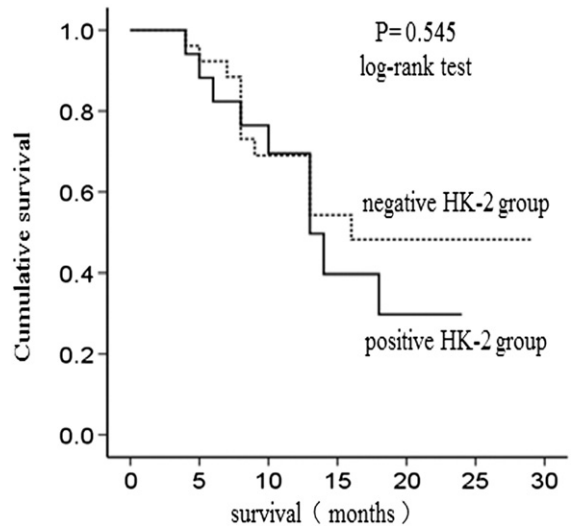


Figure 5. The survival analysis in HK-2 negative group and HK-2 positive group.

Relationships Between ¹⁸F-FDG Accumulation and Expression Levels of Glut-1 and HK-II

In the first place, difference analysis was carried out on our data. The mean value of SUV_{max} in Glut-1 negative group was 5.0 ± 2.8 , and the mean value of SUV_{max} in Glut-1 positive group was 5.5 ± 3.0 , the SUV_{max} increased as the grade of expression increased from negative to positive, but there was no significant difference between them in this study ($P = .661$). The mean value of SUV_{max} in HK-II negative and positive group was 5.7 ± 2.6 , 5.4 ± 3.1 respectively. No statistical difference was found between them ($P = .664$) (Table 5).

In the second place, correlation analysis was done on our data. Picture 8 showed the comparative analysis between immunohistochemical findings (Glut-1 expression and HK-II expression) and SUV_{max} . ¹⁸F-FDG accumulation had no correlation with the expression levels of Glut-1 or HK-II ($P > .05$) (Figure 6).

Discussion

The tumor tissue have accelerated glycolysis under both anaerobic and aerobic conditions [17]. The increased expression and activity of Gluts and HKs are considered to be the priority for sufficient energy supply in tumor cells [18].

Table 4. Univariate and Multivariate Analysis of Overall Survival of Patients with Pancreatic Cancer

Variables	Univariate Analysis P value	Multivariate Analysis P value
Age	>0.05	
Gender	>0.05	
Tumor size	0.041*	>0.05
Tumor location	>0.05	
Tumor histological type	>0.05	
Tumor differentiation	>0.05	
Nerve infiltration	>0.05	
Local infiltration	>0.05	
Vascular invasion	0.053	
Lymph node metastasis	0.003*	>0.05
Tumor staging	0.006*	>0.05
Glut-1	0.049*	>0.05
HK-II	0.545	

Table 5. Relationship Between SUV_{max} and the Expression of Glut-1 or HK-II in Patients of Pancreatic Cancer. There was no Significant Difference in SUV_{max} Between Tumors That Exhibited Negative and Positive Glut-1 Expression ($P > .05$). The Same was True for Tumors That Exhibited Negative and Positive HK-II Expression. ($P > .05$)

Patients of Pancreatic Cancer	SUV_{max}	P Value (t-Test)
Glut-1 negative group	5.0 ± 2.8	0.661
Glut-1 positive group	5.5 ± 3.0	
HK-2 negative group	5.7 ± 2.6	0.664
HK-2 positive group	5.4 ± 3.1	

Fourteen subtypes of human facilitative glucose transporters have been described. Glut-1 is the most ubiquitously distributed subtype. It has been shown to overexpress in many tumor tissue, such as: colorectal cancer [19], lung cancer [20], thyroid carcinoma [21], ovarian cancer [22], breast cancer [23], prostate cancer [24], gastric cancer [25] and musculoskeletal tumors. In tumor tissue, The glucose uptake through plasma membrane is mentioned to be the rate-limiting step of glucose metabolism [26].

It is reported that Glut-1 is overexpressed in pancreatic cancer [27] and in pancreatic cancer cell lines [28]. Higashi T et al [15] got the same conclusion in one immunohistochemical study about Glut-1. Such overexpression may reflect enhanced glucose consumption. The Glut-1 expression could be regulated by many factors. It is rapidly induced experimentally by glucose, hypoxia ischemia [29], cAMP or VEGF. These same factors have been demonstrated to be alkaline fibroblast growth factor (FGF) [30], tumor necrosis factor alpha (TNF- α) and so on. Our study indicated that the Glut-1 expression in pancreatic tumor was significantly higher than that in normal tissue ($P < .001$).

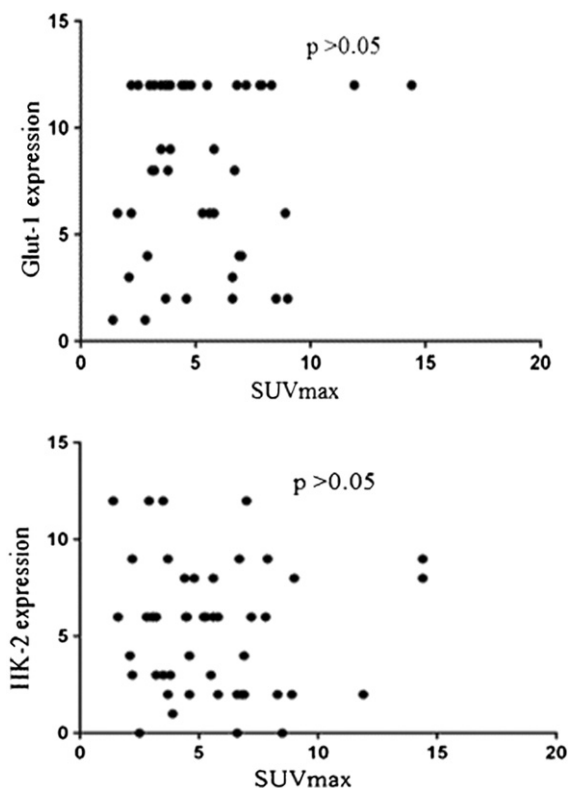


Figure 6. The correlation between immunohistochemical findings (Glut-1 expression and HK-II expression) and SUV_{max} .

Among the four HK isoenzymes (type 1–4), HK -2 is suggested to be the main subtype in regulating glucose metabolism in cancer cells [31–33]. Warburg discovered that the liver cancer cells had accelerated glycolysis, accompanied by enhanced HK activity [3]. The subsequent studies demonstrated that HK-II proteins were overexpressed in cancer cells of primary breast cancer, liver cancer, gastric cancer [34], colon cancer [35], lung cancer, cervical cancer [36] and musculoskeletal tumors [37]. Up to now, few researchers have focused on the HK-II expression in pancreatic cancer at home and abroad.

Our study firstly showed that the HK-II expression in pancreatic tumors was obviously higher than that in adjacent normal tissue (62.5% vs 0%, $P < .001$). There was no expression of HK-II in normal pancreatic tissue. The result can be explained as follows: first, the enhanced expression and activity of relevant enzymes, which act as decomposing HK-II, eventually lead in the rapid degradation of HK-II; second, the transcription and translation of the corresponding HK-II gene is weakened, causing the low expression of HK-II protein. Further studies are needed to elucidate the exact mechanism.

Tumor cells or transformed cells are known to have enhanced anaerobic glycolysis as a whole, which requires both Gluts and HKs overexpression, although the relative importance (as the rate-limiting step) of the two factors is still controversial. Among the family of Gluts and HKs, Glut-1 and HK-II were selected because each is known as a major subtype of each family for various cancer cells. Higashi et al [38] observed that there was a close correlation between Glut-1 and HK-II expression in one immunohistochemical study of twenty-one pancreatic cancer patients ($P = .0022$). The 2 immune molecules were known to promote the glycolysis of pancreatic tumors cooperatively. In the present study, we accelerated sample amounts, examined the expression of Glut-1 and HK-II by analyzing the staining intensity and percentage of positively stained cells, and obtained the opposite conclusion. In other words, the Glut-1 expression had no relationship with the HK-II expression ($P > .05$). More studies on large populations are necessary to elucidate their correlations.

The relationship between Glut-1 expression and clinical pathological characteristics has been controversial in tumors. In esophageal cancer, Glut-1 expression has been correlated to pathological grade, invasion and lymphatic metastasis of tumors [39]. In breast cancer, a positive correlation was found between Glut-1 expression and pathological grade [40]. In non-small cell lung cancer (NSCLC) [41] and colorectal cancer [42], Glut-1 expression has been associated with invasion and tumor stage. However, in nasopharyngeal cancer [43], Glut-1 expression had no association with ages, tumor size, tumor location, histological type or lymphatic metastasis. In order to determine whether up-regulated Glut-1 had influence on patients of pancreatic cancer or not, we further investigated its correlation with clinicopathological parameters as well as its impact on prognosis. It was demonstrated that there was no correlation among Glut-1 expression and tumor size, tumor location, tumor differentiation, lymph node metastasis, vascular invasion, the nerve infiltration, local infiltration, tumor histological type or tumor staging in pancreatic cancer. It is generally accepted that the Glut-1 expression may indicate a poor prognosis [19,44]. Nevertheless, one previous research has demonstrated no association of Glut-1 expression and survival in pancreatic cancer [27]. Lyshchik A et al [45] discovered the same conclusion. In our research, we did not find a correlation of Glut-1 expression to survival. Although the overexpressed Glut-1 may

enhance the transporting of glucose across plasma membrane, eventually, accelerating the rates of glycolysis, it can't provide sufficient energy for the proliferation, invasion and metastasis of pancreatic cancer cells. Whether other less commonly expressed Gluts contribute to the glycolysis in this model was not specifically addressed here.

More and more attention was drawn to the correlation between HK-II expression and clinical pathological characteristics of tumors. The HK-II expression appeared to be associated with carcinogenesis in an early research about cervical cancer [36]. Rho et al. concluded that the increased expression of HK-II in gastric carcinoma is closely related to tumor invasion [34]. But up to now, no study was carried out on the connection between HK-II expression and clinical pathological characteristics of pancreatic cancer. Our study firstly discovered that HK-II expression had no correlation with the clinical pathological features of patients. Furthermore, it is indicated that HK-II expression was not associated with patient survival, in contrary to the conclusion in Lyshchik A's study [45]. Therefore, it is suggested that HK-II may not be useful in predicting survival and further studies with larger number of patients are required to determine the clinical significance of HK-II in pancreatic cancer. The HK protein is the regulator of glycolysis and aerobic oxidation [46], maintaining a proper ratio between the two in the normal tissue. However, in tumors, the HK-II proteins were expressed in high levels, overcharging the balance. The HK-II expression may provide energy for the proliferation and development of cancer cells, but this protein was not the determinant of energy supply in pancreatic cancer.

So far, the exact mechanism of ^{18}F -FDG accumulation in pancreatic cancer is not elucidated. Three steps are required for FDG accumulation in cancer cells: (1) facilitated diffusion through Gluts; (2) subsequent phosphorylation by HK isoforms producing FDG-6-phosphate; (3) decreased dephosphorylation. It is believed that the dephosphorylation process is negligible and the ^{18}F -FDG-6-P is neither transported out of cells nor subjected to glycolytic breakdown; it is metabolically trapped inside cells. Thus, ^{18}F -FDG accumulation depends basically on the rate of transport through the cell membrane and the activity of hexokinases [47,48].

Recent years, more and more studies have focused on the expression of Glut-1 and HK-II to define the role of these proteins in the regulation of ^{18}F -FDG accumulation. The two immune molecules play different roles in different tumor tissue. In some tumor tissue, the FDG accumulation had correlation with both Glut-1 and HK-II expression. In other cancer lesions, the FDG accumulation had no correlation with Glut-1 expression or HK-II expression. However, it was also reported that the FDG accumulation had connection with each of the two immune molecules.

In one study consisted of 21 pancreatic cancer patients, Higashi et al [38] indicated that Glut-1 and HK-II expression was not strongly related to ^{18}F -FDG uptake ($P = .055$, $P = .1852$). The same conclusion was detected on our research although using different immunohistochemical analysis methods and with a large number of patients. Some early investigators indicated that other Gluts and HKs may mediate glucose uptake in certain tumors [38,49–51]. Glut-3 and Glut-4 were overexpressed and might serve in ^{18}F -FDG uptake in gastrointestinal cancer [52]. In addition, the Glut-3 expression had been demonstrated to have considerable impact on ^{18}F -FDG uptake in head and neck cancer [53], lymphoma [54], thyroid cancer [55] and malignant melanoma [11]. So it can be deduced that the Glut-1 and HK-II expression was not the main factor in ^{18}F -FDG accumulation of

pancreatic cancer, maybe there are other subtypes which contribute to a high rate of entry of the FDG into the tumor cells, such as: Glut-2, Glut-3, Glut-4, HK-1 and so on.

There were a few limitations in our study. A small number of patients were enrolled in this study. In addition, subtypes other than Glut-1 and HK-II were not included. Therefore, further studies, on large populations and with more subtypes of Gluts and HKs, are necessary to elucidate the role of these immune molecules and the molecular mechanism underlying the ^{18}F -FDG uptake.

Conclusions

Our data show that Glut-1 and HK-II proteins are over-expressed in pancreatic cancer, but they have no correlation with clinicopathological features, survival and ^{18}F -FDG uptake. We guess that maybe there are other subtypes which contribute to a high rate of entry of the FDG into pancreatic cancer cells.

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