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journal or publication title	博士論文本文Full
学位授与番号	13301甲第4202号
学位名	博士（保健学）
学位授与年月日	2015-03-23
URL	<a href="http://hdl.handle.net/2297/44680">http://hdl.handle.net/2297/44680</a>

doi: 10.1016/j.nucmedbio.2014.12.017





# A strategy for improving FDG accumulation for early detection of metastasis from primary pancreatic cancer: Stimulation of the Warburg effect in AsPC-1 cells



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## ARTICLE INFO

### Article history:

Received 12 December 2014

Received in revised form 25 December 2014

Accepted 29 December 2014

### Keywords:

FDG

Ascites tumor cells

Glycolysis

6-Phosphofructo-1-kinase

Hexose

## ABSTRACT

**Introduction:** Early detection and/or prediction of metastasis provide more prognostic relevance than local recurrence. Direct spread into the peritoneum is frequently found in pancreatic cancer patients, but positron emission tomography (PET) with 2-deoxy-2-fluoro-D-glucose (FDG) is not useful for identifying such metastasis. We investigated a method to enhance FDG accumulation using AsPC-1 human ascites tumor cells.

**Methods:** <sup>14</sup>C-FDG accumulation was assessed under the following conditions: 1) characteristics of <sup>14</sup>C-FDG transport were examined using phloridzin, a Na<sup>+</sup>-free buffer, and various hexoses, and 2) accumulation of <sup>14</sup>C-FDG was measured in cells that were pretreated with hexose for various time periods, and activity of 6-phosphofructo-1-kinase (PFK-1) was assayed.

**Results:** <sup>14</sup>C-FDG transport into AsPC-1 cells was mediated primarily by a Na<sup>+</sup>-independent transport mechanism. Aldohexoses such as D-glucose, D-mannose, and D-galactose inhibited <sup>14</sup>C-FDG transport. Cells pretreated with D-glucose, D-mannose, or D-fructose exhibited augmented <sup>14</sup>C-FDG accumulation. Pretreatment with higher concentrations of D-glucose or D-fructose tended to increase PFK-1 activity.

**Conclusions:** Very little information has been published about the association between PFK-1 and FDG accumulation, and we confirmed the impacts of various hexoses on the activity of PFK-1 and FDG accumulation in AsPC-1 cells. Clarifying the relevance of PFK-1 in FDG accumulation will contribute to developing new features of FDG-PET, because PFK-1 is the main regulator of glycolysis.

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## 1. Introduction

Pancreatic cancer (PC) is highly aggressive, and its mortality rates have been approximately stable in many European countries, the USA, Japan, and Australia over the last few years [1]. Retrospective analysis has demonstrated that a dramatic change in surgical resectability and patient survival occurs as the size of the PC tumor increases from 20 mm to 30 mm [2,3]. Moreover, PC patient survival is dependent on distant metastasis rather than local recurrence, and therefore, early detection and/or prediction of metastasis provide more prognostic relevance than local recurrence [4].

Direct spread into the peritoneum is not uncommon in PC patients, but positron emission tomography (PET) with 2-deoxy-2-fluoro-D-glucose (FDG) is not useful for identifying such metastasis, especially microscopic peritoneal dissemination (<10 mm) and intraperitoneal seeding, because of the low concentration of cancer cells [5–7].

In acute hyperglycemia, FDG transport may decrease because of competition between FDG and D-glucose [8]. Numerous reports examining the relationship between FDG accumulation and D-glucose have been published [9–13]. In contrast, whether other hexoses are involved in FDG accumulation has yet to be determined, either *in vivo* or *in vitro*.

A future objective of ours is to be able to assess peritoneal metastasis with FDG-PET. Thus, in the present study, we investigated a method to enhance FDG accumulation using AsPC-1 human ascites tumor cells from a PC patient.

## 2. Materials and methods

### 2.1. Materials

Reagent grade D-glucose, L-glucose, and D-allose were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and D-altrose, D-gulose, D-sorbose, and D-tagatose were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). D-mannose, D-galactose, D-fructose, L-fructose, D-fructose 6-phosphate (D-Fru-6-P), aldolase, α-glycerophosphate

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dehydrogenase-triosephosphate isomerase, and the other chemicals were purchased from Sigma-Aldrich Japan (Tokyo, Japan). The radiolabeled compounds  $^{14}\text{C}$ -FDG,  $^{14}\text{C}$ -maltose, and  $^{14}\text{C}$ -inulin were purchased from American Radiolabeled Chemicals Co. (St. Louis, MO, USA).

## 2.2. Cell culture

AsPC-1 human ascites tumor cells were obtained from the American Type Culture Collection (Rockville, MD, USA). Cells were incubated in Dulbecco's Modified Eagle's Medium (Sigma-Aldrich Japan) supplemented with L-glutamine (2 mM final concentration) and fetal bovine serum (10% final concentration; Nichirei Biosciences Inc., Tokyo, Japan) at 37 °C in a 10% CO<sub>2</sub> atmosphere. Cells were maintained in 25-cm<sup>2</sup> cell culture flasks (Nalge Nunc International, Roskilde, Denmark) and were passaged every 4 days using 0.1% trypsin and 0.04% EDTA. AsPC-1 cells ( $5 \times 10^5$  cells per dish) were seeded in a plastic culture dish (60-mm diameter; Nalge Nunc International) and were used in the experiments described below 4 days after seeding. At the time experiments were initiated, the cell density was approximately  $2.5 \times 10^6$  cells/dish. Before experiments, the culture medium was removed from the dish, and the cells were rinsed once with Dulbecco's phosphate-buffered saline (D-PBS; Sigma-Aldrich Japan).

## 2.3. Characteristics of $^{14}\text{C}$ -FDG transport into AsPC-1 cells

$^{14}\text{C}$ -FDG accumulation experiments were performed according to previously reported methods, with modifications [14,15]. For time-course experiments, AsPC-1 cells were rinsed with D-PBS and then incubated with 2 ml D-PBS containing  $^{14}\text{C}$ -FDG ( $^{14}\text{C}$ -FDG D-PBS; 9.25 kBq/dish) at 37 °C for a specific period (15 to 180 min). The  $^{14}\text{C}$ -FDG D-PBS was then removed by aspiration, and the cells were immediately rinsed twice with 5 ml ice-cold D-PBS. Next, the cells were lysed overnight in 2 ml 0.2 M NaOH, and the radioactivity in the NaOH extract was assayed using a Tri-carb 2910TR liquid scintillation analyzer (PerkinElmer Japan Co., Ltd., Kanagawa, Japan). Similar experiments using radiolabeled  $^{14}\text{C}$ -maltose D-PBS (9.25 kBq/dish) or  $^{14}\text{C}$ -inuline D-PBS (9.25 kBq/dish) were performed as described above.

To examine the characteristics of  $^{14}\text{C}$ -FDG transport into AsPC-1 cells, we carried out experiments using  $^{14}\text{C}$ -FDG D-PBS in 1 mM phloridzin or Na<sup>+</sup>-free buffer. Phloridzin is a specific inhibitor of the Na<sup>+</sup>-dependent glucose transporter family [16]. In experiments with the Na<sup>+</sup>-free buffer, NaCl and Na<sub>2</sub>HPO<sub>4</sub> in the D-PBS were replaced with the same concentrations of choline chloride and K<sub>2</sub>HPO<sub>4</sub>, respectively. Cells were incubated in phloridzin-containing buffer or Na<sup>+</sup>-free buffer for 60 min, and then the radioactivity present in the cell extract was measured as described above. To suppress  $^{14}\text{C}$ -FDG entry via transporters, cells were kept on ice during all incubations.

## 2.4. Competitive inhibition of $^{14}\text{C}$ -FDG transport

To confirm the competitive effects of hexoses on  $^{14}\text{C}$ -FDG transport into AsPC-1 cells, the following modifications were incorporated into competitive inhibition experiments: 1)  $^{14}\text{C}$ -FDG D-PBS contained one of the following 11 hexoses (D-/L-glucose, D-mannose, D-allose, D-galactose, D-altrose, D-gulose, D-/L-fructose, D-sorbose, or D-tagatose) at a concentration of 1 mM, and 2)  $^{14}\text{C}$ -FDG D-PBS contained an indicated concentration (0.01 to 100 mM) of one select hexose (D-/L-glucose, D-mannose, D-galactose, or D-fructose). After incubation in  $^{14}\text{C}$ -FDG D-PBS for 60 min, the radioactivity present in the cell extract was measured as described above.

## 2.5. $^{14}\text{C}$ -FDG accumulation in pretreated AsPC-1 cells

To examine the effect of pretreating AsPC-1 cells with hexoses on  $^{14}\text{C}$ -FDG accumulation, the following modifications were incorporated into the pretreatment experiments: 1) D-PBS contained one of the

following 11 hexoses (D-/L-glucose, D-mannose, D-allose, D-galactose, D-altrose, D-gulose, D-/L-fructose, D-sorbose, or D-tagatose) at a concentration of 1 mM, and 2) D-PBS contained an indicated concentration (0.01 to 100 mM) of one select hexose (D-/L-glucose, D-mannose, D-galactose, or D-fructose). Cells were incubated in one of the above D-PBS solutions for 60 min, and then immediately rinsed twice with D-PBS and incubated with  $^{14}\text{C}$ -FDG D-PBS for 60 min. Following cell lysis, the radioactivity present in the cell extract was measured as described above. A pretreatment time of 60 min was chosen because incubations less than 60 min produced no augmentation of FDG accumulation in our preliminary experiments.

## 2.6. 6-Phosphofructo-1-kinase activity in pretreated AsPC-1 cells

For the time-course experiments, AsPC-1 cells were exposed to 1 or 100 mM D-glucose or D-fructose for a specific period (15 to 60 min), after which the cells were immediately rinsed twice with D-PBS and removed from the culture dish with a rubber policeman.

To measure the 6-phosphofructo-1-kinase (PFK-1) activity in cells pretreated with various hexoses, cells were incubated at 37 °C for 60 min in D-PBS containing D-/L-glucose, D-mannose, D-galactose, or D-/L-fructose at concentrations ranging from 1 to 100 mM, and then removed from the culture dish with a rubber policeman.

The collected cells were homogenized on ice in extraction buffer comprised of 50 mM Tris-phosphate (pH 8.0), 0.2 mM ethylenediamine tetraacetic acid (EDTA), 90 mM potassium fluoride, 10 mM dithiothreitol (DTT), and 1 mM phenylmethylsulfonyl fluoride [17]. The homogenates were centrifuged at 29,000 × g for 30 min at 4 °C using a himac CS100FX ultracentrifuge (Hitachi Koki Co. Ltd., Tokyo, Japan).

PFK-1 activity in the supernatants was measured as previously described [14]. The reaction mixture contained 50 mM Tris-HCl (pH 8.0), 1 mM EDTA, 2 mM MgCl<sub>2</sub>, 2 mM DTT, 5 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 1 mM D-Fru-6-P, 1.2 mM adenosine 5'-triphosphate, 0.2 mM reduced nicotinamide adenine dinucleotide (NADH), 0.8 U/ml aldolase, 0.8 U/ml α-glycerophosphate dehydrogenase, and 8.7 U/ml triosephosphate isomerase in a final volume of 1 ml. The reaction was initiated by addition of the extract (30 μl), and the absorbance at 340 nm was measured at 37 °C using a UV-2200 spectrophotometer (Shimadzu Corp., Kyoto, Japan). PFK-1 activity was calculated as follows: 1 mol D-fructose 1,6-bisphosphate (D-Fru-1,6-P<sub>2</sub>) = 2 mol NADH consumed. The NADH content in the homogenates was calculated using the absorption coefficient of NADH (6220 M<sup>-1</sup>·cm<sup>-1</sup>) [18].

## 2.7. Statistical analysis

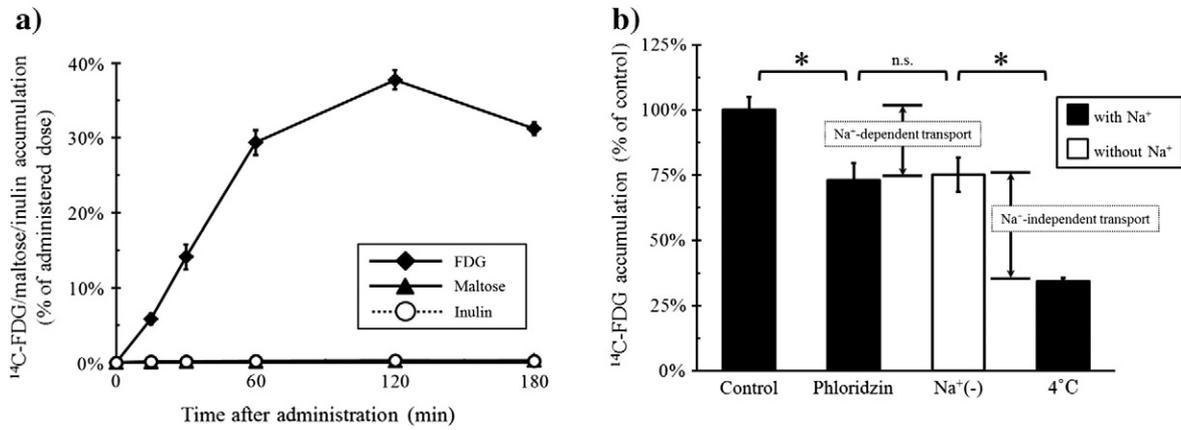
Values obtained in each experiment are presented as the mean ± SD. Groups were compared using ANOVA followed by Dunnett's *post-hoc* analysis. Differences were considered statistically significant at  $P < 0.05$ .

## 3. Results

### 3.1. Characterization of $^{14}\text{C}$ -FDG accumulation in non-pretreated AsPC-1 cells

$^{14}\text{C}$ -FDG transport into AsPC-1 cells was time dependent during the initial 60 min, and was saturated at approximately 35% of the administered dose by 120 min (Fig. 1a). No  $^{14}\text{C}$ -inulin was detected in the cells, indicating that no error was present in the methodology that would have resulted in accumulation of radioactivity not associated with  $^{14}\text{C}$ -FDG. In addition, radiolabeled  $^{14}\text{C}$ -maltose did not accumulate in cells. Maltose must be enzymatically metabolized (e.g., maltase in the small intestine) before entry into cells.

The accumulation of  $^{14}\text{C}$ -FDG after 60 min in various conditions is shown in Fig. 1b. Low temperature suppressed total  $^{14}\text{C}$ -FDG accumulation to 35% of the control, suggesting that FDG transport is

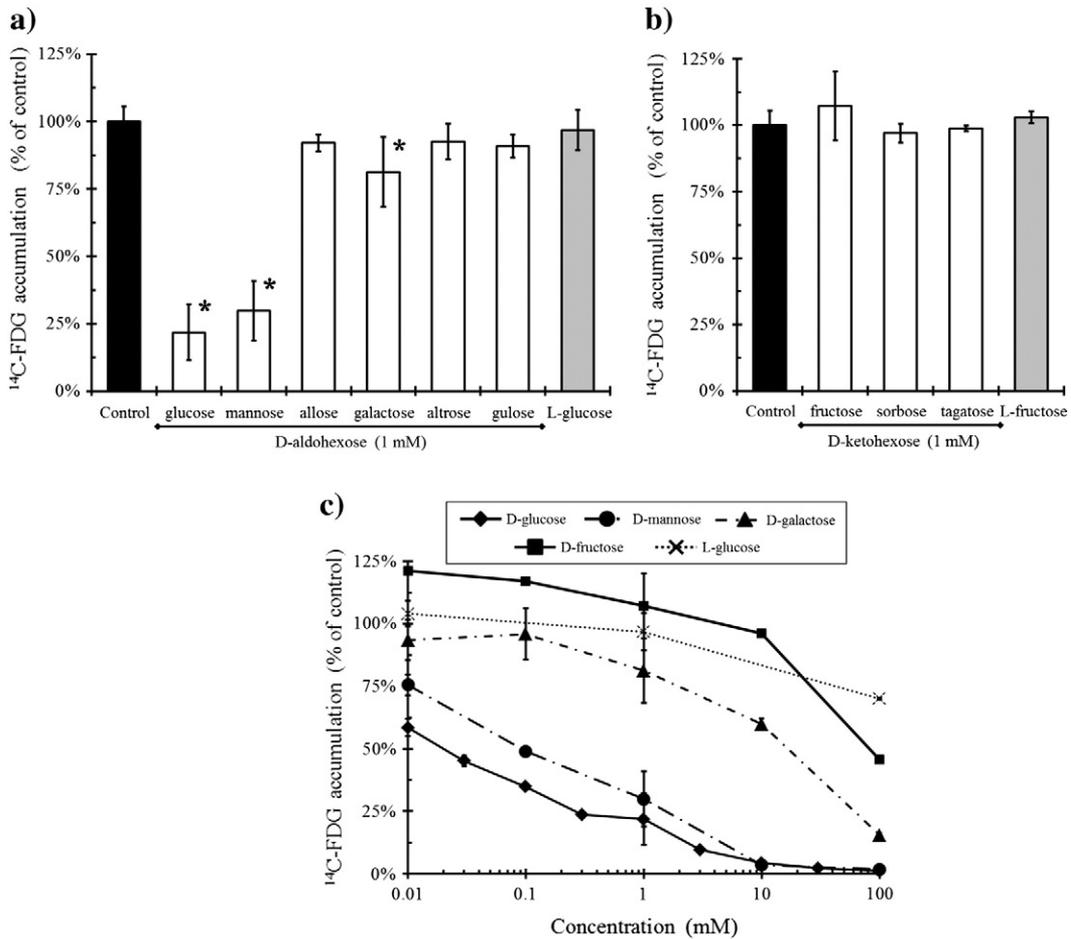


**Fig. 1.** Characteristics of  $^{14}\text{C}$ -FDG accumulation in AsPC-1 cells. (a) Time course of  $^{14}\text{C}$ -FDG (◆),  $^{14}\text{C}$ -maltose (▲), and  $^{14}\text{C}$ -inulin (○) accumulation. Accumulation (% of administered dose) is plotted against time after administration. (b)  $^{14}\text{C}$ -FDG transport in AsPC-1 cells under various conditions. Black columns and white columns represent  $^{14}\text{C}$ -FDG accumulation in cells incubated in D-PBS and in  $\text{Na}^+$ -free buffer, respectively. Values represent the mean  $\pm$  SD.  $n = 4\text{--}8$ . \* $P < 0.05$ ; n.s., not significant.

carrier mediated. Transport of  $^{14}\text{C}$ -FDG via  $\text{Na}^+$ -independent and  $\text{Na}^+$ -dependent mechanisms was approximately 40% and 25%, respectively. In the presence of phloridzin, a  $\text{Na}^+$ -dependent glucose transporter inhibitor,  $^{14}\text{C}$ -FDG accumulation was reduced by approximately 25% relative to the control.  $^{14}\text{C}$ -FDG transport into AsPC-1 cells is thus mediated primarily by a  $\text{Na}^+$ -independent transport mechanism.

### 3.2. Competitive inhibition of $^{14}\text{C}$ -FDG transport

The results of competitive inhibition experiments of  $^{14}\text{C}$ -FDG transport by D-/L-hexoses (1 mM) are shown in Fig. 2a. Three aldohexoses including D-glucose, D-mannose, and D-galactose inhibited  $^{14}\text{C}$ -FDG accumulation. As L-glucose is not transported via glucose transporter proteins and does not inhibit 2-deoxyglucose transport [19,20],



**Fig. 2.** Competitive inhibition of  $^{14}\text{C}$ -FDG transport by various hexoses. The effect of 1 mM aldohexose (a) or ketohexose (b).  $^{14}\text{C}$ -FDG accumulation was normalized to that of the control. (c) Effect of 0.01 to 100 mM D-glucose (◆), D-mannose (●), D-galactose (▲), D-fructose (■), or L-glucose (×) in  $^{14}\text{C}$ -FDG D-PBS. Values represent the mean  $\pm$  SD.  $n = 4\text{--}8$ . \* $P < 0.05$  vs. corresponding control.

$^{14}\text{C}$ -FDG transport was not inhibited by L-glucose in AsPC-1 cells. None of the D-/L-ketohexoses we examined had any significant inhibitory effect (Fig. 2b). As shown in Fig. 2c, D-glucose and D-mannose strongly inhibited  $^{14}\text{C}$ -FDG transport at all concentrations tested, and D-galactose had an inhibitory effect at concentrations above 1 mM. Lower concentrations (0.01 and 0.1 mM) of D-fructose augmented  $^{14}\text{C}$ -FDG accumulation ( $121.4 \pm 1.1\%$  and  $117.1 \pm 0.5\%$  of control, respectively), and 100 mM D-fructose had an inhibitory effect ( $45.7 \pm 1.4\%$  of control).

### 3.3. $^{14}\text{C}$ -FDG accumulation in AsPC-1 cells pretreated with hexoses

As shown in Fig. 3a and b,  $^{14}\text{C}$ -FDG accumulation was augmented in AsPC-1 cells pretreated with 1 mM D-glucose, D-mannose, or D-fructose ( $147.8 \pm 18.6\%$ ,  $129.0 \pm 4.3\%$ , or  $125.0 \pm 15.6\%$  of control, respectively). In contrast, pretreating cells with 1 mM D-galactose, D-allose, D-gulose, or L-glucose had no significant effect on  $^{14}\text{C}$ -FDG accumulation. Pretreatment with D-allose resulted in a slight decrease in  $^{14}\text{C}$ -FDG accumulation. Ketohexoses other than D-fructose (D-sorbose, D-tagatose, and L-fructose) did not have any effect on  $^{14}\text{C}$ -FDG accumulation. The effect of pretreating cells with D-glucose, D-mannose, D-galactose, or D-fructose at concentrations ranging from 0.01 to 100 mM is shown in Fig. 3c. We chose to also include D-galactose because Miller demonstrated that D-galactose increased the velocity of hexose transport [21]. Significant effects were observed in cells pretreated with D-glucose (0.01 to 100 mM), D-mannose (1 to 100 mM), D-galactose (10 and

100 mM), and D-fructose (1 to 100 mM). Pretreating cells with 100 mM D-mannose led to a decrease in  $^{14}\text{C}$ -FDG accumulation. Pretreatment with L-glucose had no effect on  $^{14}\text{C}$ -FDG accumulation at any concentration tested.

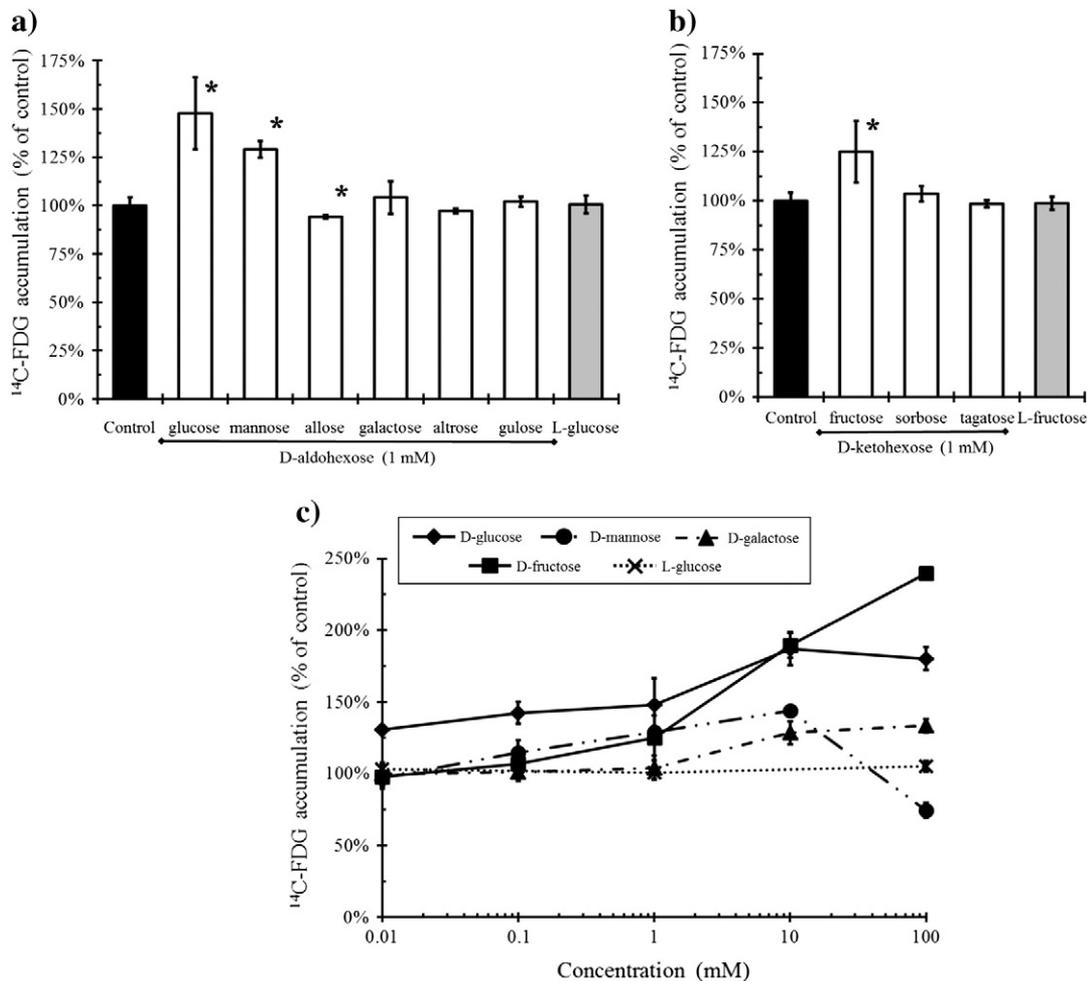
### 3.4. PFK-1 activity in AsPC-1 cells pretreated with hexoses

Longer pretreatments resulted in greater enhancement of PFK-1 activity in control cells or in cells pretreated with D-glucose (1 mM) or D-fructose (1, 100 mM) (Table 1). An increase in PFK-1 activity relative to the control in cells pretreated with 100 mM D-glucose or D-fructose was observed at all time points examined.

To examine the effect of pretreatment with various concentrations of hexoses, we chose D-glucose, D-mannose, D-galactose, and D-fructose, which augmented  $^{14}\text{C}$ -FDG accumulation. As shown in Fig. 4a and b, pretreatment with higher concentrations of D-glucose or D-fructose tended to increase PFK-1 activity. We found no difference in PFK-1 activity relative to the control in cells pretreated with L-glucose (1 mM), L-fructose (1 mM), D-mannose (1 to 100 mM), or D-galactose (1 to 100 mM).

## 4. Discussion

In this study,  $^{14}\text{C}$ -FDG accumulation was augmented significantly compared to controls in AsPC-1 cells pretreated with 1 mM D-glucose, D-mannose, or D-fructose for 60 min. We previously reported that L-



**Fig. 3.**  $^{14}\text{C}$ -FDG accumulation in AsPC-1 cells pretreated with one hexose. Pretreatment with 1 mM (a) aldohexose or (b) ketohexose.  $^{14}\text{C}$ -FDG accumulation was normalized to that of the control (black columns). \* $P < 0.05$  vs. corresponding control. (c) Pretreatment with 0.01 to 100 mM D-glucose (◆), D-mannose (●), D-galactose (▲), D-fructose (■), or L-glucose (×). Values represent the mean  $\pm$  SD.  $n = 4$ –8. Significant effects were observed with all of the hexoses we examined.

**Table 1**  
PFK-1 activity in AsPC-1 cells pretreated with 1 or 100 mM D-glucose or D-fructose.

Pretreatment	Concentration (mM)	Pretreatment span (min)	PFK-1 activity (nmol NADH/min/mg protein)	% of PFK-1 activity (Pretreatment/each Control)
Control	0	15	20.88 ± 0.16	–
D-glucose	1	15	22.78 ± 0.34	109 *
	100	15	24.39 ± 0.20	117 *
D-fructose	1	15	23.71 ± 0.34	114 *
	100	15	23.85 ± 0.48	114 *
Control	0	30	24.90 ± 0.87	–
D-glucose	1	30	26.21 ± 0.17	105
	100	30	27.63 ± 0.51	111 *
D-fructose	1	30	25.86 ± 0.40	104
	100	30	27.70 ± 0.04	111 *
Control	0	60	25.66 ± 3.92	–
D-glucose	1	60	23.99 ± 0.35	94 *
	100	60	32.02 ± 0.16	125 *
D-fructose	1	60	28.32 ± 0.27	110
	100	60	37.11 ± 0.94	145 *

The activity of PFK-1 was expressed as the amount of NADH consumed (nmol/min/mg protein). Values represent the mean ± SD. n = 3–6.

\* P < 0.05 vs. corresponding control.

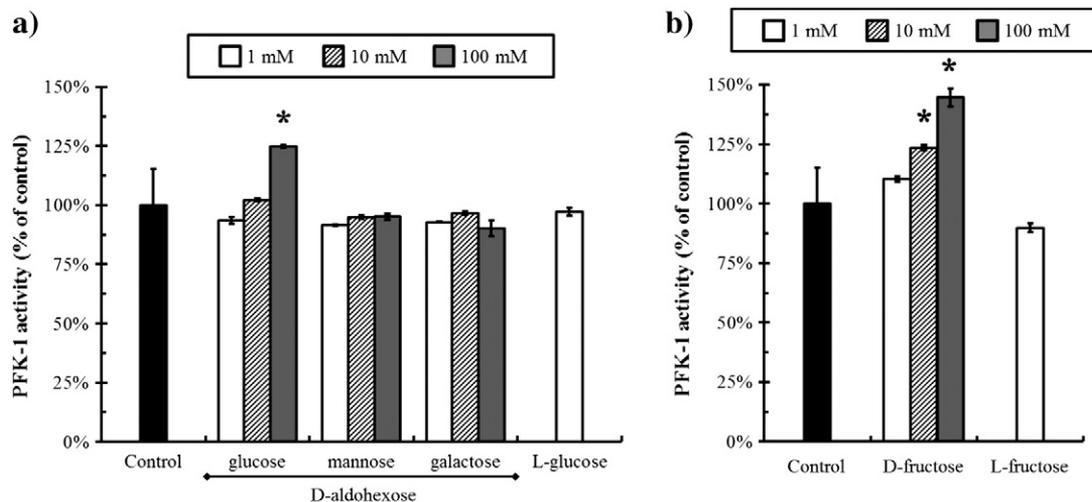
tyrosine esters effectively enhance transfer of a radiolabeled L-tyrosine analog, which prompted us to examine effects on FDG accumulation in cells pretreated with hexoses [15]. Similar induction of sugar transport was reported by Miller, who observed accelerated sugar out-flow [21]. Such an acceleration of substrate transport is an obligatory exchange mechanism in amino acid transport [15,22]. Our preliminary experiments, however, did not show acceleration of the out-flow of <sup>14</sup>C-FDG when hexose was present outside the cell, and we hypothesized that augmentation of FDG accumulation is due to elevation in the levels of intracellular glycolytic enzymes, not acceleration in the obligatory exchange.

Control of glycolysis is usually explained in terms of the allosteric properties of three enzymes: hexokinase, PFK-1, and pyruvate kinase. Many (or all) of the glycolytic enzymes in cancer cells have been suggested to be overexpressed [23,24]. Although numerous studies investigating the association between FDG accumulation and glucose transporters or hexokinase have been published, the effects of the main regulator of glycolysis, PFK-1, on FDG accumulation have not been thoroughly explored [25–28].

We focused our investigation on PFK-1, because its phosphorylation step is considered the major rate-limiting reaction and surmounts the regulatory roles of hexokinase and pyruvate kinase [29]. Consistent with the results obtained from Malaisse et al. [30] and Colomer et al. [31], our results demonstrated elevation of PFK-1 activity in AsPC-1 cells pretreated with D-glucose or D-fructose. The roles of these hexoses in the metabolism of cancer cells have been thoroughly investigated. In the early 20th century, D-fructose was shown to be converted into lactic acid more rapidly than D-glucose, and cancer cells were shown to rely on aerobic glycolysis, a phenomenon known as “the Warburg effect” [32,33]. PFK-1 mediates the phosphorylation of D-Fru-6-P to D-Fru-1,6-P<sub>2</sub> using ATP, and is regulated allosterically by the level of D-fructose 2,6-bisphosphate (D-Fru-2,6-P<sub>2</sub>) [34]. The level of the allosteric regulator D-Fru-2,6-P<sub>2</sub> is controlled by a bifunctional enzyme, 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase (PFK-2/FBPase). PFK-1 and PFK-2/FBPase share the same substrate, and PFK-2/FBPase is capable of phosphorylating D-Fru-6-P to D-Fru-2,6-P<sub>2</sub> or dephosphorylating D-Fru-2,6-P<sub>2</sub> to D-Fru-6-P [35].

Human PFK-1 is expressed as three isozymes, type-M, -L, and -P, which undergo random tetramerization to produce various homo- and heterotetrameric isozymes, each of which shows different kinetic properties [36]. Active investigations continue to elucidate alterations in gene expression and enzymatic activity of these isozymes regarding high energy production in cancer [29,35,37]. Colomer et al. have reported that PFK-1 from cancer cell extracts is more sensitive to an activation effect by D-Fru-2,6-P<sub>2</sub> than normal cells, although the intracellular levels of D-Fru-2,6-P<sub>2</sub> are equal in both normal and cancer cells [31]. Sanchez-Martinez et al. demonstrated that ascites tumor cells mainly express PFK-1 type-P, whereas type-L is the more abundant isozyme in normal mammary glands [38]. Wang et al. recently reported that enhancement of glycolytic activity depends on the conversion of PFK-1 isozyme from type-L to type-P during the development of cancer [39]. Consequently, PFK-1 type-P may contribute to maintaining a high glycolytic status in cancer cells [40]. Another report, however, showed that PFK-1 activity is negligible for glycolytic flux control in hepatoma cells [41]. The characteristics of PFK-1 should be further clarified in future studies.

Because of the inhibitory effects of PFK-2/FBPase, which regulates the D-Fru-2,6-P<sub>2</sub> level and leads to a reduction in PFK-1 activity, antimetabolic agents have been investigated [29,42,43]. Expression of the PFK-2/FBPase gene is markedly elevated in cancer cells, resulting in stimulation of glycolysis [44]. Clem et al. concluded that their novel class of antimetabolic agents selectively suppresses glucose uptake by



**Fig. 4.** PFK-1 activity in homogenates of AsPC-1 cells pretreated with one hexose. Pretreatment with 1 mM (white columns), 10 mM (hatched columns), or 100 mM (gray columns) aldohexose (a) or ketohexose (b). PFK-1 activities were normalized to the activity of the control (black columns). Values represent the mean ± SD. n = 3–6. \*P < 0.05 vs. corresponding control.

cancer *in vivo* and that glucose uptake measurements by FDG scans can be used as pharmacodynamic endpoints in clinical trials [42]. We expect that FDG-PET will play a role as a predictive modality for the therapeutic effects of PFK-1 inhibitors.

Because the only curative treatment for PC patients is surgical resection, detection and/or assessment of dissemination or seeding in the peritoneum by FDG-PET are important for selecting patients who will benefit from PC resection and for avoiding unnecessary surgery (surgical resection benefits only patients with localized disease) [45]. In this study, we demonstrated that pretreating cells with hexoses augments FDG accumulation, and D-fructose was the most effective at stimulating an increase in FDG accumulation among the hexoses we examined. Nutrient treatment before injection of metabolic imaging agents may effectively enhance tracer accumulation by cancer cells [22]. Oral administration of D-fructose leads to transient elevation of the level of D-fructose in the blood [46].

Very little has been published in the literature about the association between PFK-1 and FDG accumulation, and we confirmed the impact of various hexoses on the activity of PFK-1 and FDG accumulation in AsPC-1 cells. Although PFK-1 in cancer cells has been thoroughly investigated, further studies are needed to clarify the PFK-1 characteristics that are relevant to FDG accumulation. Such investigations will identify new features of FDG-PET; e.g., the detection of microscopic ascites tumor cells and prediction of the effect of novel metabolic agents. In future work, we will use *in vivo* studies to investigate how each treatment with each hexose impacts FDG accumulation in various cancer cells.

## Acknowledgements

We wish to thank Akihiro Imura, Takahiro Nadamura, Tomohiro Kurosawa, Misa Kozaka, Tomoki Miyazaki, and Kazuaki Arai of Ibaraki Prefectural University for their valuable technical assistance. This work was supported by Grants-in-Aid for Scientific Research (#10770451, #14770498, #13557075, #15659283, #16659322, #17390336, #22591372, and #26461801) from the Ministry of Education, Science, Sports and Culture of Japan and the Japan Society for the Promotion of Science. Financial support was also provided by Ibaraki Prefectural University Grants-in-Aid for the Encouragement of Young Scientists in 2001, 2002, 2004, 2005, 2006, 2008, 2009, 2010, 2012 and 2014 and by a Japan Atherosclerosis Research Foundation Grant in 2014.

## References

- [1] Bosetti C, Bertuccio P, Negri E, La Vecchia C, Zeegers MP, Boffetta P. Pancreatic cancer: overview of descriptive epidemiology. *Mol Carcinog* 2012;51:3–13.
- [2] Agarwal B, Correa AM, Ho L. Survival in pancreatic carcinoma based on tumor size. *Pancreas* 2008;36:e15–20.
- [3] Tummala P, Junaidi O, Agarwal B. Imaging of pancreatic cancer: an overview. *J Gastrointest Oncol* 2011;2:168–74.
- [4] Kang MJ, Han SS, Jang JY, Park JW, Kwon W, Chang YR, et al. Cancer cells with p53 deletion detected by fluorescent *in situ* hybridization in peritoneal drainage fluid is correlated with early peritoneal seeding in resectable pancreatic cancer. *J Korean Surg Soc* 2013;84:209–15.
- [5] Lim JS, Kim MJ, Yun MJ, Oh YT, Kim JH, Hwang HS, et al. Comparison of CT and <sup>18</sup>F-FDG pet for detecting peritoneal metastasis on the preoperative evaluation for gastric carcinoma. *Korean J Radiol* 2006;7:249–56.
- [6] Shimada H, Okazumi S, Koyama M, Murakami K. Japanese Gastric Cancer Association Task Force for Research Promotion: clinical utility of <sup>18</sup>F-fluoro-2-deoxyglucose positron emission tomography in gastric cancer. A systematic review of the literature. *Gastric Cancer* 2011;14:13–21.
- [7] Smyth EC, Shah MA. Role of <sup>18</sup>F 2-fluoro-2-deoxyglucose positron emission tomography in upper gastrointestinal malignancies. *World J Gastroenterol* 2011;17: 5056–74.
- [8] Gorenberg M, Hallett WA, O'Doherty MJ. Does diabetes affect [(18)F]FDG standardised uptake values in lung cancer? *Eur J Nucl Med Mol Imaging* 2002;29: 1324–7.
- [9] Wahl RL, Henry CA, Ethier SP. Serum glucose: effects on tumor and normal tissue accumulation of 2-[F-18]-fluoro-2-deoxy-D-glucose in rodents with mammary carcinoma. *Radiology* 1992;183:643–7.
- [10] Torizuka T, Clavo AC, Wahl RL. Effect of hyperglycemia on *in vitro* tumor uptake of tritiated FDG, thymidine, L-methionine and L-leucine. *J Nucl Med* 1997;38:382–6.
- [11] Diederichs CG, Staib L, Glattig G, Beger HG, Reske SN. FDG PET: elevated plasma glucose reduces both uptake and detection rate of pancreatic malignancies. *J Nucl Med* 1998;39:1030–3.
- [12] Hara T, Higashi T, Nakamoto Y, Suga T, Saga T, Ishimori T, et al. Significance of chronic marked hyperglycemia on FDG-PET: is it really problematic for clinical oncologic imaging? *Ann Nucl Med* 2009;23:657–69.
- [13] Lindholm H, Brodin F, Jonsson C, Jacobsson H. The relation between the blood glucose level and the FDG uptake of tissues at normal PET examinations. *EJNMMI Res* 2013; 3:50.
- [14] Xie P, Liu ML, Gu YP, Lu J, Xu X, Zeng WM, et al. Oestrogen improves glucose metabolism and insulin signal transduction in HepG2 cells. *Clin Exp Pharmacol Physiol* 2003;30:643–8.
- [15] Shikano N, Ogura M, Sagara J, Nakajima S, Kobayashi M, Baba T, et al. Stimulation of 125I-3-iodo-alpha-methyl-L-tyrosine uptake in Chinese hamster ovary (CHO-K1) cells by tyrosine esters. *Nucl Med Biol* 2010;37:189–96.
- [16] Wright EM. Renal Na(+)-glucose cotransporters. *Am J Physiol Renal Physiol* 2001; 280:F10–8.
- [17] Narabayashi H, Lawson JW, Uyeda K. Regulation of phosphofructokinase in perfused rat heart. Requirement for fructose 2,6-bisphosphate and a covalent modification. *J Biol Chem* 1985;260:9750–8.
- [18] Di Luccio E, Elling RA, Wilson DK. Identification of a novel NADH-specific aldolase reductase using sequence and structural homologies. *Biochem J* 2006; 400:105–14.
- [19] Burant CK, Bell GI. Mammalian facilitative glucose transporters: evidence for similar substrate recognition sites in functionally monomeric proteins. *Biochemistry* 1992; 31:10414–20.
- [20] Colville CA, Seatter MJ, Gould GW. Analysis of the structural requirements of sugar binding to the liver, brain and insulin-responsive glucose transporters expressed in oocytes. *Biochem J* 1993;294:753–60.
- [21] Miller DM. The kinetics of selective biological transport. 3. Erythrocyte-monosaccharide transport data. *Biophys J* 1968;8:1329–38.
- [22] Lahoutte T, Cavelliers V, Franken PR, Bossuyt A, Mertens J, Everaert H. Increased tumor uptake of 3-(123)I-Iodo-L-alpha-methyltyrosine after preloading with amino acids: an *in vivo* animal imaging study. *J Nucl Med* 2002;43:1201–6.
- [23] Szutowicz A, Kwiatkowski J, Angielski S. Lipogenic and glycolytic enzyme activities in carcinoma and nonmalignant diseases of the human breast. *Br J Cancer* 1979;39: 681–7.
- [24] Nakashima RA, Paggi MG, Scott LJ, Pedersen PL. Purification and characterization of a bindable form of mitochondrial bound hexokinase from the highly glycolytic AS-30D rat hepatoma cell line. *Cancer Res* 1988;48:913–9.
- [25] Som P, Atkins HL, Bandyopadhyay D, Fowler JS, MacGregor RR, Matsui K, et al. A fluorinated glucose analog, 2-fluoro-2-deoxy-D-glucose (F-18): nontoxic tracer for rapid tumor detection. *J Nucl Med* 1980;21:670–5.
- [26] Reske SN, Grillenberger KG, Glattig G, Port M, Hildebrandt M, Gansauge F, et al. Overexpression of glucose transporter 1 and increased FDG uptake in pancreatic carcinoma. *J Nucl Med* 1997;38:1344–8.
- [27] Smith TA. The rate-limiting step for tumor [18 F]fluoro-2-deoxy-D-glucose (FDG) incorporation. *Nucl Med Biol* 2001;28:1–4.
- [28] Mochizuki T, Tsukamoto E, Kuge Y, Kanegae K, Zhao S, Hikosaka K, et al. FDG uptake and glucose transporter subtype expressions in experimental tumor and inflammation models. *J Nucl Med* 2001;42:1551–5.
- [29] Yalcin A, Telang S, Clem B, Chesney J. Regulation of glucose metabolism by 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatases in cancer. *Exp Mol Pathol* 2009;86:174–9.
- [30] Malaisse WJ, Malaisse-Lagae F, Sener A. The glycolytic cascade in pancreatic islets. *Diabetologia* 1982;23:1–5.
- [31] Colomer D, Vivels-Colomer JL, Pujades A, Bartrons R. Control of phosphofructokinase by fructose-2,6-bisphosphate in B-lymphocytes and B-chronic lymphocytic leukemia cells. *Cancer Res* 1987;47:1859–62.
- [32] Dickens F, Greville GD. The metabolism of normal and tumour tissue: the conversion of fructose and glucose to lactic acid by embryonic tissues. *Biochem J* 1932;26: 1251–69.
- [33] Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science* 2009;324: 1029–33.
- [34] Kole HK, Resnick RJ, Van Doren M, Racker E. Regulation of 6-phosphofructo-1-kinase activity in ras-transformed rat-1 fibroblasts. *Arch Biochem Biophys* 1991;286: 586–90.
- [35] Hasawi NA, Alkandari MF, Luqmani YA. Phosphofructokinase: a mediator of glycolytic flux in cancer progression. *Crit Rev Oncol Hematol* 2014;92:312–21.
- [36] Vora S, Halper JP, Knowles DM. Alterations in the activity and isozymic profile of human phosphofructokinase during malignant transformation *in vivo* and *in vitro*: transformation- and progression-linked discriminants of malignancy. *Cancer Res* 1985;45:2993–3001.
- [37] Pedersen SN. The glycolytic enzyme activity of the human cervix uteri. *Cancer* 1975; 35:469–74.
- [38] Sánchez-Martínez C, Aragón JJ. Analysis of phosphofructokinase subunits and isozymes in ascites tumor cells and its original tissue, murine mammary gland. *FEBS Lett* 1997;409:86–90.
- [39] Wang G, Xu Z, Wang C, Yao F, Li J, Chen C, et al. Differential phosphofructokinase-1 isoenzyme patterns associated with glycolytic efficiency in human breast cancer and paracancer tissues. *Oncol Lett* 2013;6:1701–6.
- [40] Moreno-Sánchez R, Rodríguez-Enríquez S, Marín-Hernández A, Saavedra E. Energy metabolism in tumor cells. *FEBS J* 2007;274:1393–418.
- [41] Marín-Hernández A, Rodríguez-Enríquez S, Vital-González PA, Flores-Rodríguez FL, Macías-Silva M, Sosa-Garrocho M, et al. Determining and understanding the control of glycolysis in fast-growth tumor cells. Flux control by an over-expressed but strongly product-inhibited hexokinase. *FEBS J* 2006;273:1975–88.

- [42] Clem BF, O'Neal J, Tapolsky G, Clem AL, Imbert-Fernandez Y, Kerr II DA, et al. Targeting 6-phosphofructo-2-kinase (PFKFB3) as a therapeutic strategy against cancer. *Mol Cancer Ther* 2013;12:1461–70.
- [43] Brooke DG, van Dam EM, Watts CK, Khoury A, Dziadek MA, Brooks H, et al. Targeting the Warburg effect in cancer; relationships for 2-arylpyridazinones as inhibitors of the key glycolytic enzyme 6-phosphofructo-2-kinase/2,6-bisphosphatase 3 (PFKFB3). *Bioorg Med Chem* 2014;22:1029–39.
- [44] Atsumi T, Chesney J, Metz C, Leng L, Donnelly S, Makita Z, et al. High expression of inducible 6-phosphofructo-2-kinase/fructose-2,6-bisphosphate (iPFK-2; PFKFB3) in human cancers. *Cancer Res* 2002;62:5881–7.
- [45] Wang Z, Chen JQ, Liu JL, Qin XG, Huang Y. FDG-PET in diagnosis, staging and prognosis of pancreatic carcinoma: a meta-analysis. *World J Gastroenterol* 2013;19:4808–17.
- [46] Hui H, Huang D, McArthur D, Nissen N, Boros LG, Heaney AP. Direct spectrophotometric determination of serum fructose in pancreatic cancer patients. *Pancreas* 2009;38:706–12.