Early monitoring of response to antimetabolic treatment of gemcitabine: A comparison of ^{18}F-FLT and ^{18}F-FDG uptake in Patu 8988 human pancreatic carcinoma cells

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Abstract It is essential to predict the treatment efficacy of pancreatic carcinoma early. The purpose of this study was to examine whether ^{18}F-FDG (2'-deoxy-2'-[^{18}F]fluoro-D-glucose) or ^{18}F-FLT (3'-deoxy-3'-^{18}F-fluorothymidine) PET can be used for chemosensitivity testing by investigating the binding characteristic of ^{18}F-FDG or ^{18}F-FLT with Patu 8988 human pancreatic carcinoma cell and the influence of gemcitabine in the uptake of ^{18}F-FDG or ^{18}F-FLT on Patu 8988. Under the conditions of 1×10^6 cells, 3.7 kBq ^{18}F-FDG or ^{18}F-FLT, and incubation at 37ºC for 100 min, the cell uptake of ^{18}F-FDG and ^{18}F-FLT was (60.60±3.05)% and (50.57±2.81)%, respectively. There was a significant decrease in TK1-LI (thymidine kinase 1 labeling index) 24 h after administration of gemcitabine. The uptakes of ^{18}F-FDG and ^{18}F-FLT were negatively correlated with the doses of gemcitabine (r= –0.928 for ^{18}F-FDG, r= –0.876 for ^{18}F-FLT，P<0.01). When same doses of gemcitabine were administered, the ^{18}F-FLT uptake inhibition rate was significantly higher than that of ^{18}F-FDG (P<0.01). These results indicate that the response to gemcitabine could be predicted as early as 24 h by ^{18}F-FDG or ^{18}F-FLT PET scans. ^{18}F-FLT is more sensitive than ^{18}F-FDG to predict the response to therapy.

Key words Pancreatic carcinoma, Uptake, Gemcitabine, ^{18}F-FDG, ^{18}F-FLT

1 Introduction

Pancreatic cancer is a fatal disease. With a 5-year survival rate of <5%, it has the poorest likelihood of survival among all major malignancies. And due to aggressive nature of the cancer, only 10% to 20% of them are resectable at the time of diagnosis. Therefore, a sensitive method is needed for its diagnosis and therapeutic alliance. Also, a sensitive, repeatable and noninvasive testing method is needed for evaluating efficacy of the chemotherapeutics. Gemcitabine is a first-line antimetabolitas to improve symptoms of patients. Currently, the RECIST and WHO criterion are main standards of the treatment efficacy of solid tumors[1].

For many malignancies, PET or PET/CT has emerged as a clinical cornerstone in early diagnosis of cancer, antidiastole, recurrence monitoring, prognostic evaluation, staging and restaging. Some authors suggested that ^{18}F-FDG PET might be able to discriminate sensitive from insensitive tumors if the imaging is performed immediately after a test dose of chemotherapy[2]. Other authors demonstrated the potential role of ^{18}F-FDG PET in the early monitoring of therapy for a variety of cancers, though most of them were lymphoma, lung cancer, etc[3,4]. Recently, a fluorine-labeled thymidine analog, 3'-deoxy-3'-^{18}F-fluorothymidine (^{18}F-FLT), was developed as a candidate for imaging cell proliferation[5]. Some studies *in vivo* demonstrated that the change in ^{18}F-FLT uptake was supposed to reflect proliferative activity after anticancer treatment[6]. Other studies reported that ^{18}F-FLT PET was useful for early evaluation of tumor response to anticancer drugs[7,8].

However, to the authors' knowledge, there is no report about the evaluation of treatment efficacy of

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gemcitabine on pancreatic carcinoma by ^{18}F-FDG or ^{18}F-FLT in cellular level. In this work, we used ^{18}F-FLT and ^{18}F-FDG for monitoring response of gemcitabine in pancreatic carcinoma in cellular level.

2 Materials and methods

2.1 Chemicals

Fetal bovine serum and RPMI 1640 culture medium were purchased from GIBCO Products International Ltd. (USA). ^{18}F-FDG and ^{18}F-FLT, in radiochemical purity of over 95%, were from JYAMS Ltd. (China). Gemcitabine was obtained from Lilly Ltd. (France). MTT assay was from Sigma Inc. (USA). DMSO was obtained from Duchefa Biochemie B.V. (The Netherlands). Trypsin was from Sigma Inc. (USA).

2.2 Cell Lines

Patu 8988 pancreatic carcinoma cells were maintained in RPMI1640 culture medium supplemented with fetal bovine serum (10% v/v) (GIBCO) and penicillin streptomycin (100 units of each per milliliter).

2.3 Primary experiment of ^{18}F-FDG and ^{18}F-FLT uptake

Patu 8988 cells of 1×10^6 (2 mL) were seeded in 25 cm^2 cell culture flasks, and 24 h later, glucose-free culture medium was changed. After 12 h, the cells were washed twice with 2 mL of ice-cold PBS and incubated for 12 h at 37°C. The glycoprival medium containing 3.7 kBq of ^{18}F-FDG or the regular medium containing 3.7 kBq ^{18}F-FLT per milliliter were added into the culture flasks. After incubated for 100 min at 37°C, the cells were washed twice with 1 mL of ice-cold PBS. The supernatant and PBS were transferred into Eppendorf tubes A. The cells were harvested with trypsin and washed twice with 1 mL of ice-cold PBS. The cell suspension and PBS were transferred into Eppendorf tubes B. Radioactivity of ^{18}F-FDG or ^{18}F-FLT was determined in a well counter. The radioactivities of tubes A and B were defined as *A*. and *B*, respectively, and $B/(A+B)$ was the uptake rate.

2.4 Criterion to determine the uptake rates

The uptake of ^{18}F-FDG or ^{18}F-FLT was measured under the following conditions: the cell number in each flask of 5×10^4, 1×10^5, 5×10^5, 1×10^6, 5×10^6 and 1×10^7, the ^{18}F-FDG or ^{18}F-FLT radioactivity of 1.85, 3.7, 7.4, 14.8 and 29.6 kBq, the glucose concentration of 0, 2.78, 5.55 and 11.1 mmol·L^{-1}, and radioactivity counting at 20, 40, 60, 80, 100 and 120 min after incubation with ^{18}F-FDG or ^{18}F-FLT. The conditions were adjusted according to the result of previous step. The experiment was repeated for 5 times.

2.5 MTT assay in cell line

Individual wells of a 96-well microculture plate were filled with 100 μL 1×10^4 cell suspension. The plates were incubated in an incubator in 5% CO_2 for 12 h at 37°C. The supernatant of experimental group was drawn off and 10 μL Gemcitabine in concentrations of 0.5, 2, 4 or 6 mmol·L^{-1} was added. Six wells with drug-free medium or the cells were used for blanking the plate reader, while six wells with the cells and physiological saline were used for measuring control cell viability. One hour later, each well was added with 90 μL of RPMI1640 culture media, and 24 h later 20 μL of MTT solution in final concentration of 1 mg·mL^{-1} was added, incubated for 4 h at 37°C. Formazan crystals were dissolved with 150 μL of dimethyl sulfoxide. Optical density (OD) of the wells was measured using a microplate spectrophotometer (Sigma Inc., USA) at 492 nm. The OD is linearly related to cell number. The results were calculated by inhibition rate = $1-(OD_{\text{treated wells}}/OD_{\text{control wells}})\times100\%$.

2.6 ^{18}F-FDG or ^{18}F-FLT uptake rate changes after chemotherapy

Patu 8988 cells of 1×10^6 cells (2 mL) were seeded in 30 tissue culture flasks of 25 cm^2. After incubating the flasks in an incubator for 24 h, the supernatant of experimental group was drawn off and 100 μL of Gemcitabine in concentration of 5, 20, 40 or 60 mmol·L^{-1} was added. Flasks of the control group were added with 100 μL of physiological saline. One hour later, each flask was added with 1900 μL of RPMI1640 culture media. Then uptake rate of each flask was determined after 24-h incubation. The results were calculated by inhibition rate = 1–(uptake rate of treated flasks)/(uptake rate of control flasks)×100%.

2.7 Immunocytochemical staining

The cells of 1×10^4 were seeded on slides prepared from polylysine. After 24-h incubation, the cells were administrated by 10 μL of gemcitabine (6 mmol·L^{-1}). The slides without adding the gemcitabine were used as the control. All the slides were immobilizated 24 h later by ice acetone for 20 min. Endogenous peroxidase activity was blocked by immersing the slides in freshly prepared 3% H_2O_2 for 5–10 min at room temperature. In order to unmask the TK1 in cells, incubation with digestive solution was done. After incubation with polyclonal anti-TK1 (25 μg·mL^{-1}, in PBS) for 2 h at room temperature, the slides were rinsed in PBS and processed according to the routine streptavidin-biotin-peroxidase complex technique. Biotinylated antirabbit IgG was applied for 20 min at room temperature, and incubated with streptavidin-biotin-peroxidase complex. Diaminohenzidinc (DAB) was used as a chromogen. The slides were slightly counterstained with hematoxylin.

TK1 positively stained cells (at least 100 cells) were counted in at least 10 microscopic fields using a ×100 or ×400 objective. We assessed the expression of TK1 labeling index (TK1-LI) for each slide according to the number of positively stained cells.

2.8 Statistical analysis

The results are expressed as mean±SD. The SPSS16.0 software was used for statistical analysis, taking $P<0.05$ as the significance level, in analyzing the data using the one-way ANOVA test or Student's *t*-test.

3 Results

The ^{18}F-FDG and ^{18}F-FLT uptake rate increased significantly with the cell number (Fig.1). In 100 min incubation time, the ^{18}F-FDG uptake rates(%) were 4.07±1.23, 7.40±3.13, 18.00±6.36, 26.20±2.49, 48.20±2.95, and 48.80±1.64; and the ^{18}F-FLT uptake rates(%)were 4.11±1.46, 8.09±1.81, 16.90±3.17, 27.28±3.65, 43.00±8.37, and 50.57±2.81; at the cell numbers of 5×10^4, 1×10^5, 5×10^5, 1×10^6, 5×10^6 and 1×10^7, respectively.

Also the uptake rate increased significantly with time (Fig.2). Incubating 1×10^6 cells, the ^{18}F-FDG uptake rates(%) were 20.80±7.53, 27.4±6.47, 28.40±7.57, 29.80±10.01, 33.40±10.16, and 34.80±8.44; and the ^{18}F-FLT uptake rates(%) were 13.45±0.61, 17.94±0.63, 19.92±0.34, 23.30±2.71, 29.18± 3.04, and 28.50 ±0.98, at incubation time of 20, 40, 60, 80, 100 and 120 min, respectively.

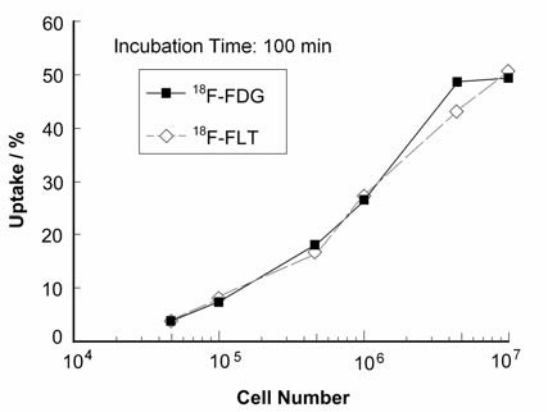

Fig.1 Effect of cell number on uptake of ^{18}F-FDG or ^{18}F-FLT.

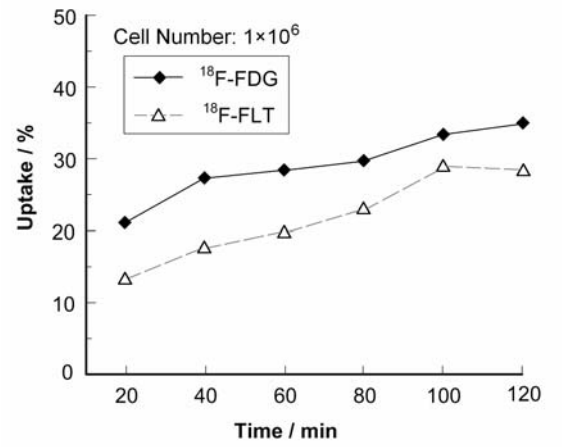

Fig.2 Effect of time on uptake of ^{18}F-FDG or ^{18}F-FLT.

The uptake rate demonstrated no significant correlation with the intensity of radioactivity. The ^{18}F-FDG uptake rates(%) were 48.60±5.81, 48.60±4.39, 47.00±3.54, 48.80±2.28, and 48.60±2.70, at the^{18}F-FDG radioactivities of 1.85, 3.7, 7.4, 14.8 and 29.6 kBq, respectively, without significant difference among the groups ($F=0.177$, $P>0.05$). The ^{18}F-FLT uptake rates(%) were 29.22±3.28, 27.46±5.22, 29.36±2.42, 29.18±3.04, and 27.77±2.95, at the ^{18}F-FDG radioactivities of 1.85, 3.7, 7.4, 14.8 and 29.6 kBq, respectively, without significant difference among the groups ($F=0.298$, $P>0.05$).

Twelve hours after the culture medium of different glucose concentration was changed, the uptake rates were detected (Fig.3). The ^{18}F-FDG

uptake rates (%) were 50.00±2.35, 52.40±4.72, 60.60±3.05 and 11.80±5.02, at glucose concentrations of 0, 2.78, 5.55 and 11.1 mmol·L^{-1}, respectively. The uptake increased with the glucose concentration below 5.55 mmol·L^{-1} (F=151.803, P<0.01). But the uptake of 11.1 mmol·L^{-1} was the lowest of all groups (P<0.01). The ^{18}F-FLT uptake rates (%) were 35.84±11.69, 35.13±5.61, 35.40±7.51 and 35.01±7.50 at the glucose concentrations of 0, 2.78, 5.55 and 11.1 mmol·L^{-1}, without significant difference between the two groups (F=0.013, P>0.05).

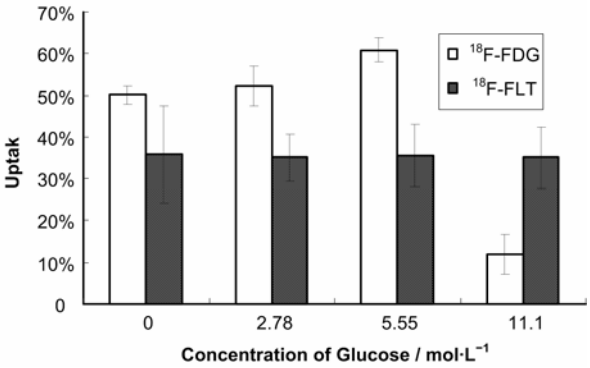

Fig.3 Effect of glucose on uptake of ^{18}F-FDG or ^{18}F-FLT (100 min, 1×10^6 cells).

Incubating 1×10^6 cells, the cell growth was inhibited 24 h after the gemcitabine administration. The proliferation inhibition rates(%) detected by MTT were 19.76±8.12, 31.20±5.56, 43.06±5.37 and 57.81±5.64, 24 h after administration of 5, 20, 40 and 60 mmol·L^{-1} gemcitabine, respectively. The difference among the groups was significant (F=40.46, P<0.01). The proliferation inhibitions were positively correlated with the gemcitabine dose (r=0.942, P<0.01).

One day after administration of gemcitabine in concentration of 0, 5, 20, 40 and 60 mmol·L^{-1}, the ^{18}F-FDG uptake rates (%) were 58.35±2.19, 56.34±1.56, 48.92±5.91, 39.14±7.40 and 29.67±4.41, respectively. The uptake rates decreased significantly with the gemcitabine dose. The uptake rates were negatively correlated with the gemcitabine dose (r=–0.928, P<0.05). The ^{18}F-FLT uptake rates(%) were 50.02±4.45, 37.70±8.02, 12.42±5.62, 4.60±0.83 and 2.57±0.56, respectively, negatively correlated, too, with the gemcitabine dose (r= –0.876, P<0.01).

One day after administration of gemcitabine in concentration of 5, 20, 40 and 60 mmol·L^{-1}, the ^{18}F-FDG uptake inhibition rate(%) were 3.36±3.47, 16.13±9.78, 33.15±11.02 and 49.25±6.33, respectively. The uptake inhibition rates were positively correlated with the proliferation inhibition (r=0.697, P<0.01). The ^{18}F-FLT uptake inhibition rates(%) were 25.12±10.50, 75.76±8.76, 90.86±0.91 and 94.91±0.67, respectively (Fig.4).

At the same gemcitabine dose of 20, 40 or 60 mmol·L^{-1}, the ^{18}F-FLT uptake inhibition rate was larger than that of ^{18}F-FDG (P<0.01).

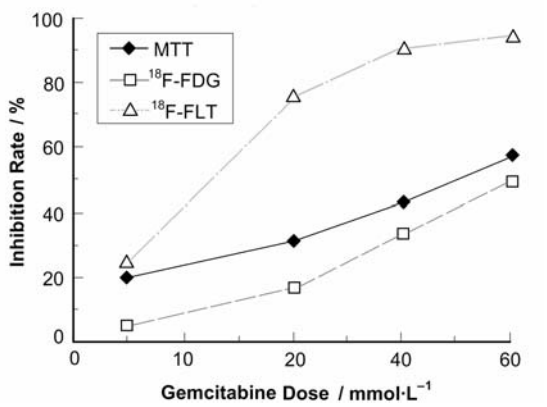

Fig.4 Inhibition rate detected by MTT and inhibition rates of ^{18}F-FDG and ^{18}F-FLT.

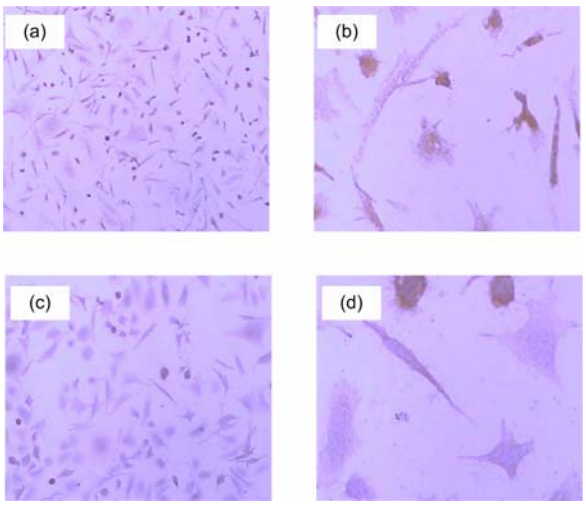

Fig.5 (a) Magnification, ×100 and (b) Magnification, ×400, TK1-stained cells without administration of gemcitabine. (c) Magnification, ×100 and (d) Magnification, ×400, TK1-stained cells 24 h after administration of gemcitabine.

Typical control and gemcitabine-treated Patu 8988 cells immunocytochemically stained for TK1 are shown in Fig.5. Before the administration of gemcitabine, TK1 immunocytochemistry indicated a proliferation fraction in Patu 8988 cells of (42.23±2.91)%. The TK1-LI decreased significantly (P<0.01) after administration of gemcitabine, being (10.12±2.67)% 24 h after treatment.

4 Discussion

^{18}F-FDG is an analog of glucose. It is transported into the cell via Glut 1, phoshporylated to 2-DG-6-P and trapped in cells. It accumulates preferentially in the cells with high glucose uptake, such as pancreatic cancer cells. After chemotherapy, metabolic alterations in tumor cells, indicative of tumor response to therapy, may occur before alterations in tumor size.

^{18}F-FLT is phosphorylated by thymidine kinase 1, the key enzyme of the salvage pathway of DNA synthesis, and trapped in the cell with little further metabolism[9]. Thymidine kinase 1 is selectively up-regulated in the S phase of the cell cycle. Therefore, ^{18}F-FLT uptake is dependent on cell proliferation. Gemcitabine cytotoxic activity has been correlated with dFdCTP formation, its incorporation into DNA, and its inhibition of DNA synthesis. Gemcitabine can induce an S-phase arrest, and trigger apoptosis in human leukemic cells and in solid tumors[10].

In this study, we investigated the method to determine the uptake of ^{18}F-FDG and ^{18}F-FLT systematically. Ren *et al*. [11] reported that transfecting 6×10^6 cells by spatial carrier, implanting pSUPER in 48 h, and incubating with 0.74 kBq/μL ^{18}F-FDG for 1 h, the intra-cellular radioactivity to extracellular radioactivity was (7.2±5)%. By changing the glucose levels and other conditions, we got a much higher ratio. Seitz U *et al*. [12] added 0.07 MBq/mL ^{18}F-FDG and ^{18}F-FLT into flasks and found ^{18}F-FDG uptake rates(%) of 0.55±0.04 (SW-979) and 0.28±0.13 (BxPc-3) after 240 min of incubation, and ^{18}F-FLT uptake rates(%) of 18.42±3.59 (SW-979) and 5.22±1.43 (BxPc-3), and attributed the low ^{18}F-FDG uptake to elevated glucose levels. We found that the ^{18}F-FDG uptake could increase within certain levels, though it decreased when glucose concentration was too high.

This may be due to better growth situation of Patu 8988 cells in culture medium with more glucose. We changed the culture medium of different glucose concentration 12 h before the uptake rates were detected. The cells in culture medium of more glucose proliferated more than those in glucose-free medium, and more ^{18}F-FDG was needed. However, the ^{18}F-FLT uptake was not affected by the glucose levels. It was proved in our experiment that the uptake was correlated with the number of cells while the uptake had no relationship with radioactivity of ^{18}F-FDG or ^{18}F-FLT in the range of 1.85–29.6 kBq. It can be hypothesized that there may be a limit to the uptake of ^{18}F-FDG or ^{18}F-FLT on human pancreatic carcinoma cell Patu 8988. The uptake increased with time, with the ^{18}F-FDG and ^{18}F-FLT uptake rates being respectively 1.23 times and 1.43 times higher at 120 min than those at 60 min. This result verified the importance of delayed imaging. There was some deviation between different groups, which may be related with the growth situation of cells implanted into flasks in different phases.

In our study, the uptake of ^{18}F-FDG and ^{18}F-FLT on Patu 8988 human pancreatic carcinoma cell decreased 24 h after the administration of gemcitabine. It suggested that response to gemcitabine might be predicted early by ^{18}F-FDG and ^{18}F-FLT PET scans. The response to chemotherapy is usually predicted after 1 cycle of chemotherapy[13]. Nahmias *et al*. [14] proposed to perform ^{18}F-FDG PET 1 to 3 weeks after the initiation of chemotherapy, so as to allow prediction of the response to therapy. In this study, however, we found that the uptake of ^{18}F-FDG and ^{18}F-FLT decreased as early as 24 h after the administration of gemcitabine.

For a typical lesion, a parameter change of more than 20% is outside the 95% range for spontaneous fluctuations, therefore this reflects true changes in glucose metabolism of the tumor mass[15]. In this study, we found 49.2% decrease of ^{18}F-FDG uptake and 94.9% decrease of ^{18}F-FLT uptake between control group and 60 mmol·L^{-1} groups 24 h after administration. We think that the change could be the criteria to assess tumor response.

The uptake decreased with increasing gemcitabine, which was correlated with the result of MTT assay. And there was a significant decrease in TK1-LI 24 h after administration of gemcitabine. The uptake inhibition was positively correlated with the proliferation inhibition 24 h after administration of gemcitabine. We concluded that response to gemcitabine could be predicted early and sensitively by the uptake of ^{18}F-FDG and ^{18}F-FLT.

Leyton J *et al*. [16] suggested ^{18}F-FLT PET be superior to ^{18}F-FDG PET for imaging changes in

proliferation associated with early response. Other studies reported that ^{18}F-FLT uptake correlated better than ^{18}F-FDG uptake with the proliferation activity and the decrease in ^{18}F-FLT uptake after treatment was more rapid than that in ^{18}F-FDG uptake in preclinical models[17,18]. We found that ^{18}F-FLT uptake decreased more than ^{18}F-FDG uptake after cells were administrated with equal gemcitabine for 24 h. These results agree with the findings that the uptake of ^{18}F-FLT provided more accurate assessments of the early response to anticancer therapy than that of ^{18}F-FDG.

5 Conclusion

We investigated the method to determine the uptake of ^{18}F-FDG and ^{18}F-FLT systematically and proved that the inhibitation effect of gemcitabine on Patu 8988 human pancreatic carcinoma cell could be observed by this experiment. Our work strongly supports the use of ^{18}F-FDG and ^{18}F-FLT PET or PET/CT for imaging response to gemcitabine on pancreatic cancer as early as 24 h after the treatment. We also found that ^{18}F-FLT was more sensitive than ^{18}F-FDG to predict the response to therapy in cellular level. Further research should be made on the effect mechanism of gemcitabine on the uptake of ^{18}F-FDG and ^{18}F-FLT and the effect on animal models.

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