Detection of Precursor Lesions of Pancreatic Adenocarcinoma in PET-CT in a Genetically Engineered Mouse Model of Pancreatic Cancer

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Abstract

BACKGROUND: Pancreatic cancer is among the most dismal of human malignancies. The 5-year survival rate is lower than 5%. The identification of precursor lesions would be the main step to improve this fatal outcome. One precursor lesions are called pancreatic intraepithelial neoplasia (PanIN) and are graduated in grade 1 to 3, whereas grade 3 is classified as carcinoma in situ. Currently, no reliable, noninvasive imaging technique (e.g., ultrasound, computed tomography, magnet resonance imaging) exists to verify PanINs.

METHODS: Recently, a transgenic mouse model of pancreatic cancer was established in which the tumor progression of human pancreatic carcinoma is reproduced. These so-called Pdx-1Cre; LSL-KrasG12D/+; LSL-Trp53R172H/+ mice develop PanINs, which transform to invasive growing pancreatic carcinoma. The pancreata of mice of different ages were immunohistochemically stained using α-GLUT-2 antibodies. Furthermore, mice underwent positron emission tomography (PET)–computed tomography (CT) with 18F-fluorodeoxyglucose (FDG) to evaluate early detection of PanIN lesions.

RESULTS: An expression of GLUT-2 in murine PanINs was found in PanINs of grade 1B and higher. This finding is associated with an elevated glucose metabolism, leading to the detection of precursor lesions of pancreatic cancer in the FDG PET-CT scan. In addition, immunohistochemical staining of GLUT-2 was detectable in 45 (75%) of 60 human PanINs, whereas PanINs of grade 1B and higher showed a very extensive expression.

CONCLUSIONS: In conclusion, we demonstrate for the first time that an elevated glucose metabolism occurs already in precursor lesions of murine and human pancreatic carcinoma. These findings are the basis for the detection of precursor lesions by PET-CT, thereby helping improving the prognosis of this devastating disease.

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Introduction

Pancreatic cancer is a devastating and almost uniformly lethal malignancy that accounts for approximately 33,000 deaths in the United States every year, rendering it the fourth most common cause of cancer-related mortality [1]. Although the past decades have seen intense research efforts aimed at a better understanding of the underlying etiologic and pathophysiological mechanisms, this increased knowledge could not so far be translated successfully into better clinical treatment strategies and improved patient survival. In fact, during the past 30 years, the overall...
handling of dietary sugars [7]. Positron emission tomography (PET) with
the intestine, pancreas, kidney, and liver, which all play key roles in the
function of transporting glucose in proportion to circulating levels.

In the postprandial state, increased plasma levels of glucose as well
as other nutrients stimulate pancreatic β cells to secrete insulin. The
initiating event in this process is the cellular uptake of glucose through
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Materials and Methods

Mice

strains were interbred to obtain Lsl-KrasG12D;Pdcd1-Cre double mutant
animals or Lsl-KrasG12D;Lsl-Trp53R172H;Pdcd1-Cre triple mutant ani-
mals on a mixed 129/SvJae/C57BL/6 background as previously de-
scribed [5]. All mice were generated from the same initial stock. All
experiments were approved by the local committees for animal care
and use. Animals were maintained in a climate-controlled room kept
at 22°C, exposed to a 12:12-hour light-dark cycle, fed standard labora-

tory chow, and given water ad libitum.

Genotyping

For genotyping, genomic DNA was extracted from tail cuttings using
the REDExtract-N-Amp Tissue PCR Kit (Sigma-Aldrich, St Louis,
MO). Three polymerase chain reactions were carried out for each
animal to test for the presence of the oncogenic Kras (using LoxP
primers), p53, and Pdcd1-Cre transgene constructs (using Cre-specific
primers along with Gabra as a positive control), respectively.

Tissue Microarrays

Tissue samples were obtained from the pathology archives of the
Johns Hopkins Medical Institutions (Baltimore, MD). PanIN tissue
microarray blocks were also created as described previously [15]. In
short, 60 PanIN lesions were selected from 33 patients with pancreatic
ductal adenocarcinoma. PanIN lesions were separated into PanIN-1,
PanIN-2, or PanIN-3 grades either at the time of microarray creation
or after a review by A.M.

Histologic Evaluation

After completion of the study, mice were killed, and the pancreas
was removed and inspected for grossly visible tumors and preserved
in 10% formalin solution (Sigma-Aldrich) for histology. Formalin-fixed
paraffin-embedded tissues were sectioned (4 μm) and stained with
hematoxylin and eosin. Six sections (100 μm apart) of pancreatic tis-

sues were histologically evaluated by an experienced gastrointestinal
pathologist (A.M.). Mouse PanIN (mPanINs) lesions were classified ac-

cording to histopathologic criteria as recommended elsewhere [16,17].

Immunostaining

For immunolabeling, formalin-fixed and paraffin-embedded archived
tumor samples and corresponding normal tissues were stained as previously described [18]. Concentrations and sources of primary
antibodies are available on request. Briefly, slides were heated to
60°C for 1 hour, deparaffinized using xylene, and hydrated by a graded
series of ethanol washes. Antigen retrieval was accomplished by micro-
wave heating in 10 mM sodium citrate buffer, pH 6.0, for 10 minutes.
For immunohistochemistry, endogenous peroxidase activity was
quenched by 10 minutes of incubation in 3% H2O2. Nonspecific
binding was blocked with 10% serum. Sections were then incubated
with primary antibodies overnight at 4°C. For immunohistochemistry,
bound antibodies were detected using the avidin-biotin complex pero-
xi
dase method (ABC Elite Kit; Vector Labs, Burlingame, CA). Final
staining was developed with the Sigma FAST DAB peroxidase substrate
kit (Sigma, Deisenhofen, Germany).

FDG-PET-CT In Vivo

A Siemens Inveon small-animal multimodality PET-CT system (Pre-
clinical Solutions, Siemens Healthcare Molecular Imaging, Knoxville,
TN) was used for data acquisition. This PET-CT system is character-
ized by the combination of two independently operating PET and CT
scanner. The PET module has an effective transaxial field of view
(FOV) of approximately 10 cm and an axial FOV of 12.7 cm, whereas
radial, tangential, and axial resolution at the center of FOV is better than 1.5 mm. PET acquisitions were carried out with default settings of coincidence timing window of 3.4 nanoseconds and energy window of 350 to 650 keV. Images were reconstructed using 3D Ordered Subset-Expectation Maximization (OSEM-3D, 2 iterations, 16 subsets) algorithm followed by maximum a posteriori algorithm (18 iterations, 16 subsets, beta smoothing factor 0.1) (Siemens, Germany). Pixel size was 0.431 mm and slice thickness is 0.796 mm based on the size of the image matrix of 256 × 256 × 159. Attenuation was corrected based on the CT measurements. The CT module consists of a cone beam micro x-ray source (50-μm focal spot size) and a 2048 × 3072 pixel x-ray detector. Maximum FOV of the detector is 5.5 cm × 8.4 cm. Our standard micro-CT imaging protocol used 80 kV at 500 μA, 360 degrees of rotation, and 200 projections per bed position. Two bed positions were acquired with an overlap of 43.6%. Exposure time of each projection was 300 milliseconds. Micro-CT images were reconstructed using Shepp-Logan filter and cone beam–filtered back-projection (Modified Feldkamp algorithm) into 256 × 256 × 601 CT data sets with 0.217-mm voxel dimensions. Acquisitions and reconstructions were carried out using Siemens Acquisition workplace (IAW) software version 1.4.3 (Siemens).

After the CT scan was complete, the mouse bed was translated axially and centered within the PET module. The PET data were acquired 40 to 60 minutes after injection of FDG. Because the mouse remains in the same position on the bed for both CT and PET acquisitions and the relative positions of the field of views of PET and CT are known, the CT data were transformed to fuse and coregister the PET and CT data.

Visualization and analysis of the coregistered PET-CT data were performed with the Siemens Inveon Research Workplace (IRW) software version 1.4.3 (Siemens).

In preparation for PET-CT, mice were anesthetized with 1.5% to 2% vaporized isoflurane (DeltaSelect, Dreieich, Germany) in oxygen (1.5 l/min) to prevent animal movement and reduce imaging artifacts. During anesthesia, respiratory frequency was continuously monitored. Hypothermia was prevented by placing the mice on heating pads (36°C). 18F (half-life, 109 minutes) labeled FDG (Eckert & Ziegler, Bad Berka, Germany) with an activity of 9.6 ± 0.5 MBq was injected intravenously into the lateral tail vein of anesthetized mice in a volume of 90 to 110 μl of 0.9% NaCl. Because extended fasting times may profoundly affect outcome of experimental disease in small rodents with approximately 20- to 25-g body weight and high metabolic activity, fasting was completely omitted, and mice had free access to water and food right before FDG administration and imaging.

Results

Development of PanINs in LsL-KrasG12D;Pdx1-Cre and of Pancreatic Cancer in LsL-KrasG12D;LsL-Trp53R172H; Pdx1-Cre Mice

As previously described in the initial reports [4,5], we observed development of low-grade (Figure 1A) and high-grade (Figure 1B) PanINs in LsL-KrasG12D;Pdx1-Cre and fully invasive pancreatic cancers (Figure 1C) in LsL-KrasG12D;LsL-Trp53R172H;Pdx1-Cre transgenic mice. The histologic diagnosis resembled ductal adenocarcinomas of the pancreas or its precursor lesions observed in humans.

Expression of Glut-2 in LsL-KrasG12D;LsL-Trp53R172H; Pdx1-Cre–Derived Murine Precursor Lesions and Pancreatic Cancer

The pancreas was harvested from LsL-KrasG12D;Pdx1-Cre and LsL-KrasG12D;LsL-Trp53R172H;Pdx1-Cre transgenic mice and stained for Glut-2 expression using immunohistochemistry.

In neoplastic mouse tissue, Glut-2 was expressed in 85% of all PanIN lesions graded 1B or higher (Figure 2, A and B). Whereas PanIN 1A epithelium did not show Glut-2 expression (Figure 2A, arrow), higher-graded PanIN lesions had a strong Glut-2 expression. Glut-2 expression was restricted to tumor cells, whereas the stromal compartment of the carcinoma did not show any GLUT-2 expression. These findings suggest that elevated glucose metabolism is an early event in PanIN progression and may help detect precursor lesions during a non-invasive stage. Furthermore, we found expression of Glut-2 in invasive pancreatic carcinoma (Figure 2B). In wild-type mice, Glut-2 expression was detected in islet cells only (Figure 2E).

Expression of Glut-2 in Human Precursor Lesions of Pancreatic Cancer

After the evidence of GLUT-2 expression in murine precursor lesions of pancreatic cancer at stages as early as PanIN 1B, we next sought to translate our findings into the clinics. Therefore, we evaluated a panel of approximately 20- to 25-g body weight and high metabolic activity, fasting was completely omitted, and mice had free access to water and food right before FDG administration and imaging.

Figure 1. (A) Low-grade mPanIN lesion (mPanIN-1A) in a LsL-KrasG12D; Pdx1-Cre mouse. (B) High-grade PanIN lesion after 5 months in a LsL-KrasG12D;LsL-Trp53R172H;Pdx1-Cre mouse. (C) Invasive poorly differentiated ductal adenocarcinoma from a 5-month-old LsL-KrasG12D; LsL-Trp53R172H;Pdx1-Cre mouse.
of human PanINs by tissue microarray by GLUT-2 immunohistochemistry. Remarkably, we found the same expression pattern as in the murine counterparts. GLUT-2 expression was detectable in 45 (75%) of 60 human PanIN lesions graded 1B or higher (Figure 2, C and D). Interestingly, Glut-2 expression was also observed only in tumor but not in stromal cells (Figure 2, C and D).

**Invasive Ductal Carcinomas Show High FDG Uptake in PET-CT**

To determine whether FDG PET-CT could detect invasive carcinoma of \( \text{LsL-Kras}^{G12D};\text{LsL-Trp53R172H};\text{Pdx1-Cre} \) mice, we first studied four 5-month-old transgenic mice bearing palpable tumors and age-matched nontransgenic littermates. All four control wt mice failed to display an abnormal abdominal FDG uptake in the pancreatic region (Figure 3, A and D). As expected, no tumor could be found in the pancreas of wt mice (Figure 3 G). On the contrary, all transgenic littermates showed a strong FDG uptake in the pancreatic area (Figure 3 C), correlating with a huge tumor burden in PET-CT scan (Figure 3 F) and confirmed macroscopically in the abdomen of the transgenic mice (Figure 3 I).

**Precursor Lesions of Pancreatic Cancer Show FDG Uptake in PET-CT**

After proving the detection and visualization of invasive pancreatic carcinoma in the \( \text{LsL-Kras}^{G12D};\text{LsL-Trp53R172H};\text{Pdx1-Cre} \) mouse, we next evaluated if it would be possible also to visualize precursor lesions of pancreatic cancer. Therefore, we analyzed four \( \text{LsL-Kras}^{G12D};\text{Pdx1-Cre} \) mice, which do not develop invasive pancreatic carcinoma but only PanIN lesions [4,5]. Again, we studied 5-month-old transgenic mice and age-matched nontransgenic littermates, and we were able to find a weak but clear visible signal in the pancreatic area (Figure 3, B and E). After laparotomy, no tumor was palpable or visible macroscopically (Figure 3 H). To underline our findings, we show a series of acquired images of PET-CT in a wild-type (Figure 4 A) and a \( \text{LsL-Kras}^{G12D};\text{Pdx1-Cre} \) mouse (Figure 4 B), demonstrating that the signal was detectable throughout the series of images. According to our immunohistochemistry results, where Glut-2 expression was found only in tumor but not stromal cells, these signals arise from PanIN lesions and not from surrounding stroma or extracellular tissue.

**Discussion**

PET uses radiolabeled FDG, a glucose analog, to detect enhanced metabolism of glucose by malignant cells causing selective uptake of the radiotracer. FDG enters the cell through the glucose transporter protein (GLUT) transporter system and accumulates there because it is not completely metabolized. In normal pancreatic tissue, one of these transporters, GLUT2, is expressed only in pancreatic \( \beta \) cells [7]. PET has demonstrated efficacy in the evaluation of metastatic disease in different types of cancer, such as pancreatic cancer [19]. Overall sensitivity of PET scan alone for pancreatic adenocarcinoma has been reported as 85% to 96% [20]. Although useful, the lack of anatomic localization limited the overall utility of the test. More recently, with improved computing abilities and technology, PET has been fused with CT to add precision anatomic and functional localization [20]. This colocalization has the potential benefit of improving diagnosis, staging, treatment planning, and evaluation of response to treatment in patients with pancreatic cancer. PET-CT could improve diagnosis by detection of the primary cancer. In addition, PET-CT could improve staging by identifying local regional disease and occult metastatic disease and
confirming suspicious metastatic lesions found on standard CT. Con-
cerning the usefulness of FDG-PET in early stage pancreatic neoplasia,
encouraging results have recently been reported in the diagnosis of
intraductal papillary mucinous neoplasms of the pancreas with better
sensitivity than conventional imaging [21].

In our study, we now show for the first time that GLUT-2 is
expressed in precursor lesions of pancreatic cancer at an early non-
invasive stage, making it a possible target for early detection of pan-
creatic cancer. This would be especially important for patients with
familial pancreatic cancer that accounts for 3% to 5% of all pan-
creatic cancer cases [22]. Using immunohistochemistry, we found
strong expression of GLUT-2 in murine PanINs grade 1B in a gen-
etically engineered mouse model of pancreatic cancer but also in
human PanINs using a tissue microarray. This shows at which stage
in tumor progression of pancreatic cancer change in glucose metab-
olism seems to be detectable. Our results close a gap left behind by
a recently published study by Abasolo et al. [13]. Using Ela1-myc
mice, they showed that mixed acinar-ductal tumors could be iden-
tified by FDG PET several weeks before they could be detected by
hand palpation. To gain insight into the biologic basis of the differ-
ential FDG uptake, glucose transporter expression was studied in
microdissected tumor areas enriched for acinar or ductal cells. They
found that GLUT2 messenger RNA levels were up to 20-fold higher
in ductal than in acinar tumors. Besides, GLUT2 protein overexpres-
sion was found in ductal neoplastic cells but not in the surrounding
stroma. Because the Ela1-myc model does not recapitulate the pro-
gression of PanIN progression model associated with human PDAC,
it was not possible for the authors to establish when—during the pro-
gression of intraductal lesions—GLUT2 overexpression takes place.
The authors stated that their work did not have direct implications
regarding the early detection of pancreatic cancer in humans. How-
ever, their findings support the notion that the study of the FDG
PET imaging properties of pancreatic ductal precursor lesions may
contribute to develop better imaging methods to detect preneoplastic

Figure 3. (A–C) PET scans. (D–F) Overlay of PET scan and CT acquired simultaneously. (E–I) Macroscopical evaluation of the pancreas
and abdominal region. Pictures in one column were generated from an individual mouse. (A, D) Control wt mice displaying normal
abdominal FDG uptake without enrichment in the pancreatic region (circle in A). As expected, no tumor could be found in the pancreas
of the wt mice (circle in G). (B, E) LsL-KrasG12D;Pdx1-Cre mice demonstrated a weak but clear visible signal in the pancreatic area (circle
in B). After laparotomy, no tumor was palpable or visible macroscopically (circle in H). (C, F) All LsL-KrasG12D;LsL-Trp53R172H;Pdx1-Cre
littermates showed a strong FDG uptake in the pancreatic area (circle in C), which correlates to a huge tumor burden in PET-CT scan (F).
As shown in I, a huge invasive pancreatic cancer was found in the abdomen of the transgenic mice (circle in I).
lesions [13]. The conditional LSL-KrasG12D;Pdx1-Cre mice [4] used in our study is considered a very valuable tool to study PanIN biology because it mimics rather a slow progression from PanIN 1 over PanIN 2 and 3 lesions to invasive cancer in around 12 to 15 months. Furthermore, LSL-KrasG12D/+-LSL-Tpp53R172H/+-Pdx1-Cre [5] mice manifest widely metastatic pancreatic ductal adenocarcinoma that recapitulates the human spectrum. The use of these models now provides the opportunity to conduct chemopreventive studies on pancreatic cancer.

After we found an impressive GLUT-2 expression in 85% of murine PanINs graded 1B or higher, we stained more than 60 human PanINs from a tissue microarray for GLUT-2 and found similar expression pattern. Of 60 human PanINs, 45 (75%) revealed expression of GLUT-2, and virtually all PanINs graded 1B or higher were GLUT-2-positive. As mentioned in the study of Abasolo et al. [13], the expression was restricted to tumor cells but not to the surrounding stromal compartment.

The next step of our study was to evaluate LSL-KrasG12D;Pdx1-Cre mice, which do not develop invasive pancreatic carcinoma but develop PanIN lesions [4,5]. We studied 5-month-old transgenic mice and age-matched nontransgenic littermates and were able to find a weak but clear visible signal in the pancreatic area, whereas in wild-type mice, no such signal was detectable. Because GLUT-2 expression was restricted to neoplastic but not stromal cells, this signal is most likely based on real glucose uptake in PanIN lesions. Because only PanIN 3 are classified as carcinoma in situ, detecting noninvasive PanIN stages in PET-CT would have a major effect on improving the prognosis of this fatal disease and would help to detect precursor lesions in patients with familial pancreatic history in a curable stage [22]. Furthermore, we found a strong FDG uptake in the pancreatic area in LSL-KrasG12D/+-LSL-Tpp53R172H/+-Pdx1-Cre mice, which correlates to a huge tumor burden in the PET-CT scan, and this was verified macroscopically in the abdomen of the transgenic mice.

In conclusion, we demonstrate for the first time that an elevated glucose metabolism occurs already in precursor lesions of murine and human pancreatic carcinoma. These findings are the basis for the detection of precursor lesions by PET-CT, thereby helping improving the prognosis of this devastating disease.

References


