Preclinical MRI and US to detect and monitor the development of early pancreatic alterations and pancreatic tumors in a transgenic mouse model of pancreatic ductal adenocarcinoma (PDA)

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Aims and objectives

Infiltrating ductal adenocarcinoma of the pancreas (PDA) accounts for over 85% of all pancreatic malignancies and has a poor prognosis as less than 5% of patients survive 5 years after diagnosis with a median survival period of 4-6 months\(^1\).

During the last few years there have been important advances to better understand the molecular mechanisms regulating the development of PDA; however, progress in prevention, early diagnosis and treatment needs major advances\(^4\). Some of the recent advances have been possible by employing mouse models which have provided an important model system to better understand the molecular mechanism underlying pancreatic cancer\(^1\). Currently, there are several different genetically modified mouse tumors and xenograft models available that offer the possibility of experimental and preclinical model systems to evaluate different strategies for targeting this disease, early detection, chemoprevention, treatment and finally improve the outcome for pancreatic cancer patients\(^2\).

A specific GEM Pdx1-cre/LSL-KrasG12D/LSL-p53R172H (triple mutant) develops PDA with a partially understood stepwise process, as seems to occur in many cases in humans. This mouse model was generated by Hingorani et al.\(^3\), based on the previously described PDX-1-Cre, LSL-KrasG12D mouse\(^4\), adding a conditionally expressed point mutant allele of the Li-Fraumeni human ortholog (Trp53R172H). Four to six weeks old triple mutant mice present early PanIN lesions similar to what it is observed in single PDX-1-Cre, LSL-KrasG12D mice. A significant disease burden is observed in animals by ten weeks of age at the earliest and the full spectrum of pre-invasive lesions is apparent; histological analyses of primary pancreatic carcinomas in triple mutant mice reveal a predominant moderately well-differentiated to well-differentiated morphology organized in a glandular architecture, as observed in the human disease\(^3\). Triple mutant mice have dramatically shortened median survival of approximately 5 months, significantly less than wild type and Trp53 or Kras double mutant and all of them die before 12 months\(^3\).

As the development of PDA in this specific triple mutant GEM is totally asynchronous, the age of the mice is not a reliable parameter to predict the effective clinical stage of pancreatic disease especially in the early phases of carcinogenesis, when a wide spectrum of preinvasive lesions is variably observed.
As a result we needed to validate an accurate method for disease staging, based on the non-invasive detection and characterization of preneoplastic and neoplastic pancreatic alterations.

Using preclinical dedicated MR and US imaging we described malignant and premalignant pancreatic alterations compared to post-mortem histopathological analyses and we defined an age-independent 4-class staging system of the pancreatic disease for this specific GEM.
Methods and materials

Eight 5 week old, triple mutant transgenic mice were included in this preliminary study and were delivered to the facility of preclinical imaging at our institution.

All of the 8 transgenic mice underwent longitudinal in-vivo abdominal imaging (4 with US and 4 with 7T MRI) and post-mortem histopathological analyses with different timing according to pancreatic findings at imaging (table 1), as schematically represented in figure 1. Four different stages of pancreatic disease that were histologically proven (table 1 and figures 1-11) were defined.

In vivo examinations with both modalities were carried out under inhalational anesthesia (Isoflurane, 5% for induction and 2% for maintenance in 2liter/minute oxygen) laying on a dedicated temperature control apparatus to prevent hypothermia, having breathing rate and body temperature continuously monitored.

MRI studies were performed on a 7T preclinical scanner (Bruker, BioSpec 70/30 USR, Paravision 5.1), equipped with 450/675 mT/m gradients (slew-rate: 3400-4500T/m/s; rise-time 140µs) and a circular polarized mouse body volume coil with an inner diameter of 40 mm. Axial fat-saturated T2-weighted images (TurboRARE-T2: TR = 3000 ms, TE = 27 ms, voxel-size = 0,128 x 0,088 x 1.0 mm, averages = 4, gap size 0 mm) were acquired covering the body region between the diaphragm and the bladder apex.

US examination were performed using a VEVO 2100 system (FUJIFILM, VisualSonics, Toronto, Canada), equipped with high-frequency solid-state transducers with a 40 MHz center frequency that provides axial resolution down to 30 µm.

US examinations and MR images analysis were performed by a resident radiologist with the supervision of a radiologist with high experience in clinical and preclinical MR and US imaging.
Fig. 1: A schematic view of the experimental design: mice were included in the study after at least 5 weeks of life and underwent US and MRI examinations at different time points; based on imaging findings animals were sacrificed for post-mortem histopathological proof of the imaging detected pancreatic disease. A 4-class staging system was defined.

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Results

According to imaging findings and histopathological data a 4-class staging system for the pancreatic disease was defined, as schematically described in table 1.

At ultrasound, at 6 weeks, 4/4 mice presented a normal pancreas. At 8 weeks only 1/4 mice showed normal pancreas; 2/4 mice presented cystic lesions and heterogeneous hypo-echogenicity of the pancreas with no detectable nodules and they were followed up; 1/4 mouse presented a small head-of-the-pancreas tumor (stage 3) that was confirmed at post-mortem histopathology (Figure 4 and 6). At 10 weeks 1/4 mice still presented normal pancreas (stage 1) and was sacrificed for histopathological analyses that confirmed US diagnosis (Figure 2 and 6). At 12 weeks 1/4 mice was identified as stage 2, confirmed at histopathology (Figure 3 and 6), while a stage 4 mouse, presenting a large tumor of the pancreatic tail proven at histopathology, was sacrificed at 14 weeks (Figure 5 and 6).

At MRI, at 9 weeks 2/4 mice had normal pancreas and 1 of them was sacrificed for histopathological analyses that confirmed a stage 1 disease with few PanIN1-2 foci (Figure 7 and 11). 1/4 mice presented a large head-of-the-pancreas tumor, confirmed at histopathology (stage 4, Figure 10 and 11). 1/4 mice presented multiple cystic lesions with heterogeneous slight signal hyperintensity of the pancreas. The same mouse at 13 weeks demonstrated the persistence of stage 2 disease and was sacrificed for histopathological analyses, confirming the presence of premalignant alterations and the absence of pancreatic ductal adenocarcinoma (Figure 8 and 11). 1/4 mice with normal pancreas at 9 weeks and few alterations at 13 weeks, at 19 weeks presented 2 small slightly hyperintense nodules at the pancreatic head and tail surrounded by an edematous pancreas with multiple cysts (stage 3, Figure 9 and 11); at histopathology the nodules resulted moderately-differentiated pancreatic ductal adenocarcinomas, with dilated branch pancreatic ducts and "pancreatitis-like" surrounding parenchyma.
Table 1: Findings at US, MRI, gross pathology and histopathology are schematically shown in this table.

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Fig. 2: Appearance of normal pancreatic tissue at US: homogeneously slightly hyperechoic to the liver and spleen, and slightly hypoechoic to the kidney.

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Fig. 3: Heterogeneous pancreatic tissue with sporadic hypoechoic areas, small cysts or dilated branch ducts (PanIN 1-4, acinar-to-ductal metaplasia at histopathology).

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Fig. 4: Small tumor of the pancreatic head with heterogeneous appearance of the surrounding parenchyma.

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Fig. 5: Large infiltrative tumor of the pancreatic tail with mucinous and necrotic cystic components; the rest of the pancreatic parenchyma is heterogeneously hypoechoic.

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Fig. 6: Post-mortem histopathology of stage 1 sacrificed mouse confirmed normal pancreatic parenchyma with islets of Langerhans. The stage 2 mouse presented diffused preneoplastic alterations such as PanIN 1-4 and acinar-to-ductal metaplasia. The stage 3 mouse presented a moderately-differentiated pancreatic ductal adenocarcinoma of the pancreatic head with diffuse preneoplastic alteration in the rest of the parenchyma, showing a "pancreatitis-like" aspect, probably due to pancreatic main duct obstruction. The pancreas of the stage 4 mouse was involved by a large poorly differentiated ductal adenocarcinoma of the pancreatic tail associated with multinodular disease in the rest of the edematous pancreas.
**Fig. 7:** Normal pancreas at MRI with homogenous slightly hyperintense signal to the liver.

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**Fig. 8:** The pancreas of the stage 2 mouse at MRI presented heterogeneous signal intensity with cystic lesions, segmentally dilated main and branch ducts and peripancreatic ascites; no pancreatic nodules were detected.

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**Fig. 9:** The pancreatic tissue of the stage 3 mouse presented heterogeneous signal intensity with at least two detectable nodules (PDA confirmed at histopathology) in the pancreatic head and tail.

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Fig. 10: The stage 4 mouse presented a large tumor of the pancreatic head with other small nodules in the surrounding pancreatic parenchyma with heterogeneous signal intensity; also enlarged celiac lymph nodes were detected along with ascites and signs of bowel obstruction.

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Fig. 11: Histopathological analyses revealed results similar to those reported in Figure 6 with the exception of stage 1 disease at MRI that presented sporadic foci of preneoplastic PanIN1-2.

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Fig. 12: The graph shows the good correlation between amylase levels and imaging detected disease stage. Lower levels detected in stage 4 mice are probably due to an extensive damage to pancreatic parenchyma progressing toward atrophy.

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Conclusion

Imaging modalities such as US and MR offer the possibility to detect and monitor noninvasively the development of pancreatic disease in genetically engineered mouse models with high degree of accuracy. This is particularly useful dealing with asynchronous GEMs in which time from birth is not a reliable tool to predict the degree of disease development.

In our preliminary study we demonstrated that pancreatic alterations detected both with preclinical dedicated US and MRI were histopathologically confirmed and we defined a 4-class staging system to separate the four principal phases of pancreatic disease development in the triple mutant mouse model of PDA. Interestingly this staging system presented good correlation with amylase blood levels, a biomarker indicative of pancreatic damage.

To our knowledge an accurate description of imaging findings in this mouse model during the early phases of pancreatic carcinogenesis lacks in the literature and opens to the possibility of further investigating the mechanisms and timing of disease progression. Moreover, with our non-invasive staging system, the efficacy of new therapeutic agents would be tested and monitored at different phases of disease development.

Although any findings in these GEMs will need to be verified in PDA patients, the accurate mouse models of PanIN and PDA should spur innovation and discovery of novel detection methods and new therapeutics for patients suffering from this malignancy.
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