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RESEARCH ARTICLE

Induction and characterization of pancreatic cancer in a transgenic pig model

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Abstract

Background

Preclinical testing of new locoregional therapies for pancreatic cancer has been challenging, due to the lack of a suitable large animal model.

Purpose

To develop and characterize a porcine model of pancreatic cancer. Unlike small animals, pigs have similar physiology, drug dosing, and immune response to humans. Locoregional therapy in pigs can be performed using the same size catheters and devices as in humans.

Methods

The Oncopig is a transgenic pig with Cre-inducible $TP53^{R167H}$ and $KRAS^{G12D}$ mutations. In 12 Oncopigs, CT-guided core biopsy of the pancreas was performed. The core biopsy was incubated with an adenoviral vector carrying the Cre recombinase gene. The transformed core biopsy was injected back into the pancreas (head, tail, or both). The resulting tumors ($n = 19$) were characterized on multi-phase contrast-enhanced CT, and on pathology, including immunohistochemistry. Angiographic characterization of the tumors was performed in 3 pigs.

Results

Pancreatic tumors developed at 19 out of 22 sites (86%) that were inoculated. Average tumor size was 3.0 cm at 1 week (range: 0.5–5.1 cm). H&E and immunohistochemical stains revealed undifferentiated carcinomas, similar to those of the pancreatobiliary system in

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humans. Neoplastic cells were accompanied by a major inflammatory component. 1 of 12 pigs only had inflammatory nodules without evidence of neoplasia. On multiphase CT, tumors were hypovascular compared to the normal pancreas. There was no pancreatic duct dilation. In 3 pigs, angiography was performed, and in all 3 cases, the artery supplying the pancreatic tumor could be catheterized using a 2.4 F microcatheter. Selective angiography showed the pancreatic tumor, without extra-pancreatic perfusion.

Conclusion

Pancreatic cancer can be induced in a transgenic pig. Intra-arterial procedures using catheters designed for human interventions were technically feasible in this large animal model.

Introduction

Pancreatic cancer deaths are increasing, and are projected to become the second most common cause of cancer-related death in the United States by 2030 [1]. The 5-year survival rate is 6% [2]. Fewer than 20% of patients are resectable, and 80% of patients have recurrent disease after resection [3]. Better therapies are desperately needed.

Current locoregional therapies for pancreatic cancer are suboptimal. Irreversible electroporation of locally advanced pancreatic cancer can be performed, with a major complication rate of 40%, and median overall survival of 11 months [4]. Liver metastases from pancreatic cancer can be treated using ablation, embolization, or radioembolization, and although many patients respond radiographically, progression is rapid, and overall survival is less than 9 months [5].

Several new locoregional therapies for pancreatic cancer have been proposed, including local drug delivery using ultrasound microbubbles [6, 7], pancreatic transarterial chemoinfusion [8–11], pancreatic chemoembolization using lipiodol [12] or drug-eluting beads [13], intratumoral injection of oncolytic virus [14], and peritumoral injection of siRNA [15]. Translation to human trials has been challenging.

Due to the lack of a suitable large animal model of pancreatic cancer, many new locoregional therapies for pancreatic cancer are initially tested in normal pig pancreas [9, 11], or in nude mice with subcutaneous [6] or orthotopic [15] pancreatic tumor xenografts. Normal pancreas is not an ideal model for pancreatic cancer. Nude mice are immunocompromised, and require much smaller devices and catheters than humans. Furthermore, new cancer therapies developed in rodents have a high failure rate when translated to humans [16]. Presumably this is due to differences in physiology [17, 18], drug dosing [19, 20], and immune response [21–24] between rodents and humans.

Here, we develop and characterize a new immunocompetent pig model of pancreatic cancer. This model allows us to test new locoregional therapies for pancreatic cancer that are not yet ready for human trials.

Methods

Animals

All research procedures were approved by the Institutional Animal Care and Use Committee at Memorial Sloan Kettering Cancer Center. Our animal facility is AAALAC accredited and operates in compliance with the Guide for the Care and Use of Laboratory Animals [25].

Euthanasia was performed by administering pentobarbital sodium and phenytoin sodium solution (Euthasol, Virbac, Forth Worth, TX) intravenously.

12 female Oncopigs were obtained from the University of Illinois, or the National Swine Resource and Research Center at the University of Missouri. Oncopigs are transgenic pigs with Cre-recombinase-inducible heterozygous $TP53^{R167H}$ and $KRAS^{G12D}$ mutations [26, 27]. R167H is a dominant-negative mutation of the *TP53* tumor suppressor gene, and G12D is an activating mutation of the *KRAS* oncogene.

Animals were maintained in pens with aspen-chip contact bedding (PWI Industries Canada, Quebec, Canada), fed a grower chow (#5081, PMI, St Louis, MO), and provided water ad libitum. Animal room temperature was 21.5±1˚C, relative humidity was 30%– 70%, and light: dark photoperiod was 12:12 hours. All procedures and imaging were performed under general anesthesia, with peri-operative analgesia.

Tumor induction

Tumor induction was performed when the pigs were 12–22 weeks old. An 18 gauge core biopsy of the pancreas was obtained under CT guidance, using co-axial technique (Temno Evolution, Merit Medical, South Jordan, UT). $TP53^{R167H}$ and $KRAS^{G12D}$ expression was induced by incubating the core biopsy with an adenoviral vector carrying the Cre recombinase gene (10^9 pfu Ad5CMVCre-eGFP, University of Iowa Viral Vector Core) for 20 minutes at room temperature, in phosphate-buffered saline containing 15 mM calcium chloride (total fluid volume of 1 ml). Gelatin sponge (Gelfoam, Pfizer) was then added using a 3-way stopcock, and the mixture (virus, core biopsy, gelatin) was injected percutaneously back into the duodenal or splenic lobe of the pancreas, through the biopsy needle, which was kept in place after the biopsy. Note that pigs have a ring-shaped pancreas with 3 lobes: duodenal, splenic, and connecting. In this paper, we will refer to the duodenal lobe as the "head" of the pancreas, and the splenic lobe as the "tail."

Multiphase contrast enhanced CT

Five-phase contrast-enhanced CT was performed 1 week after tumor inoculation. Non-contrast CT of the abdomen and pelvis was obtained. Omnipaque 300 (2 ml/kg, max 150 ml) was power injected at 2–3 ml/sec. The early arterial phase CT scan was obtained when the abdominal aorta reached 150 Hounsfield units. The late arterial phase was obtained 15 seconds after the early arterial phase. The portal venous phase was obtained 25 seconds after the late arterial phase scan. The delayed phase scan was obtained 90 seconds after portal venous phase. All scans were obtained at 120 kVp. Mean Hounsfield units of the tumor, normal pancreas, and aorta were measured on each phase, using elliptical regions of interest (ROI).

Angiography and cone beam CT

Angiography was performed 1 week after tumor inoculation. From femoral access, under fluoroscopic guidance, a 4 F catheter was advanced into the celiac artery, and an arteriogram was performed. A 2.4 F microcatheter (Merit Maestro, Infiniti Medical, Redwood City, CA) was advanced into a pancreatic artery, and an arteriogram was performed. Cone beam CT arteriogram was obtained during a breath hold after administering a paralytic agent (rocuronium 1–1.6 mg/kg IV).

Pathology

Two weeks after inoculation, animals were euthanized, and tumors were macroscopically examined, harvested, and fixed in 10% neutral buffered formalin. Following formalin fixation,

sections of tumor were processed into paraffin blocks, and sectioned at 5 micron thickness. Hematoxylin and eosin (H&E)-stained sections were reviewed by both human (OB, GA) and veterinary (AOM, SM) pathologists. Representative formalin-fixed paraffin-embedded tissue sections were immunolabeled with antibodies against cytokeratin AE1/AE3, cytokeratin 8/18, vimentin, Iba1, and CD31, as described in S1 Table. Masson's trichome stain for collagen was also performed.

Results

Pancreatic tumors developed at 19 out of 22 sites (86%) that were inoculated. Average tumor size was 3.0 cm at 1 week (range: 0.5–5.1 cm). There were no complications from the tumor inoculation procedure, based on daily clinical evaluation and imaging.

On multiphase CT, tumors were enhancing (Fig 1A and 1C), but hypovascular compared to the normal pancreas (Fig 2). There was no pancreatic or biliary duct dilation.

In 3 pigs, angiography was performed, and in all 3 cases, the dorsal pancreatic artery supplied the pancreatic tail tumors. In all cases, the dorsal pancreatic artery could be selected

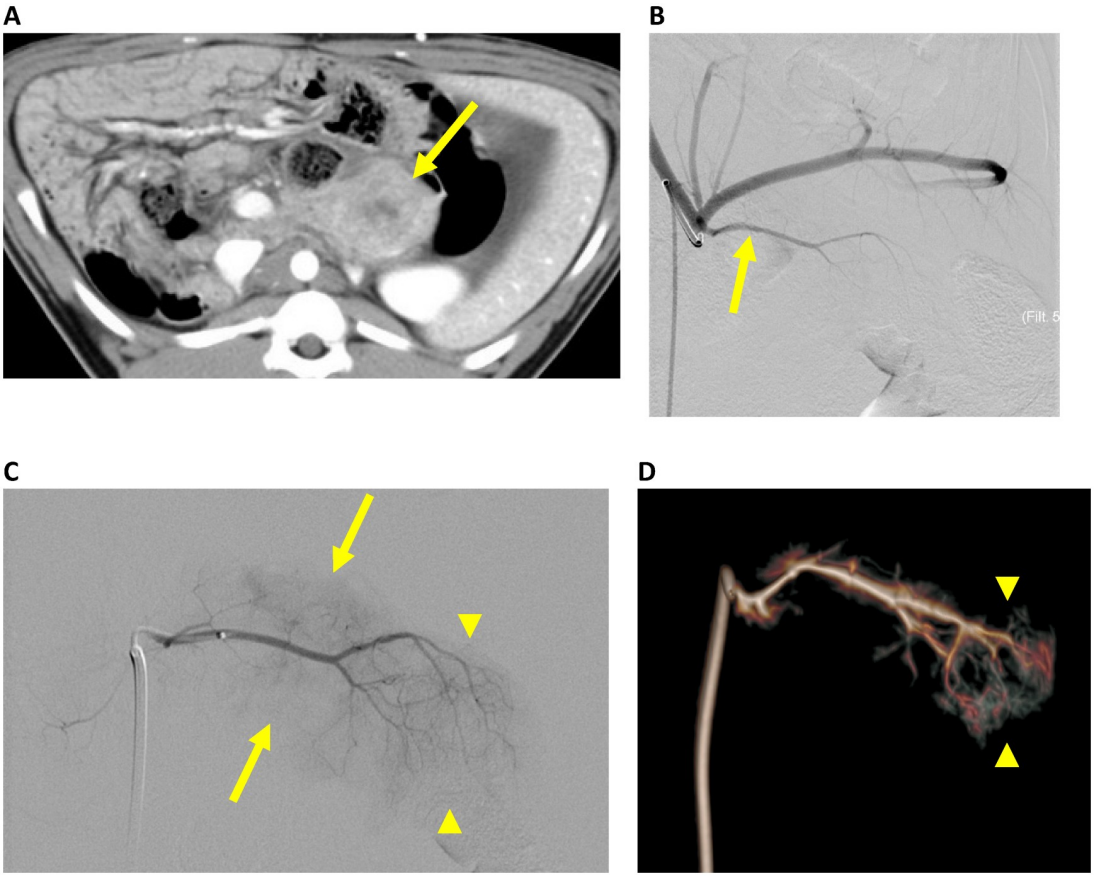

Fig 1. CT and catheter angiography of a pig pancreatic tumor. A. Contrast-enhanced CT shows a tumor in the tail of the pancreas (arrow). **B**. Celiac arteriogram shows the dorsal pancreatic artery (arrow). **C**. Dorsal pancreatic arteriogram shows an enhancing pancreatic mass (arrows) supplied by tiny branches (430 μm or smaller) of the proximal dorsal pancreatic artery, while larger distal branches supply the normal tail of the pancreas (arrowheads). **D**. Cone beam CT arteriogram shows the dorsal pancreatic artery supplying the tumor and the tail of the pancreas (arrowheads), without extrapancreatic perfusion. The pancreatic tumor appears hypovascular, compared to the normal pancreas.

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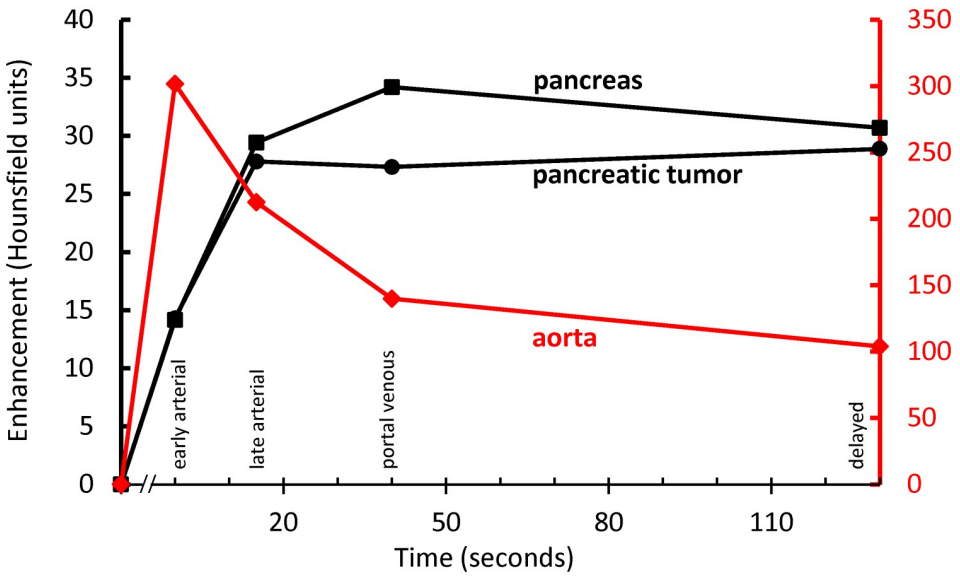

Fig 2. Average enhancement curves of normal pancreas (primary *y*-axis), pancreatic tumors (primary *y*-axis), and aorta (secondary *y*-axis). The pancreatic tumors are hypoenhancing, compared to normal pancreas, in the portal venous phase.

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using a 2.4 F microcatheter, and selective angiography showed the pancreatic tumor, without extra-pancreatic perfusion (Fig 1B–1D).

Grossly, the tumor were soft, poorly demarcated, pale-tan lesions located within the pancreatic parenchyma. In 11 of 12 pigs, H&E (Fig 3) showed undifferentiated carcinomas composed of sheets of epithelioid cells and streams of spindle cells, with or without multinucleated giant cells, resembling undifferentiated carcinomas of the pancreatobiliary tract in humans [28, 29]. Neoplastic cells were accompanied by a major inflammatory component in all tumors. One of 12 pigs only had inflammatory nodules, without evidence of a neoplastic process.

On immunohistochemistry (Fig 3), epithelioid cells were strongly immunopositive for cytokeratin AE1/AE3 and minimally immunopositive for 8/18, indicating epithelial differentiation, and spindle cells were immunopositive for vimentin, indicating mesenchymal differentiation. The spindle cells in these carcinomas are likely due to epithelial-to-mesenchymal transition. Giant cells were negative for cytokeratins and immunopositive for Iba1, confirming histiocytic origin. In the carcinomas, Masson's trichrome stain highlighted a collagen-containing desmoplastic stroma, but the amount of the stroma was significantly less compared to that of human pancreatic ductal adenocarcinoma. Tumor angiogenesis was shown on CD31 stains. The tumors were supplied by tiny vessels, which were seen both on angiography (Fig 1) and immunohistochemistry (Fig 3E).

Discussion

We have developed and characterized a pig model of pancreatic cancer. Tumor inoculation is simple, reproducible, and site-specific, and results in rapidly growing undifferentiated carcinomas with a major inflammatory component, similar to the pancreatobiliary carcinomas seen in humans. Oncopig pancreatic cancer contains both *TP53* and *KRAS* mutations, which are among the most common mutations seen in human pancreatic ductal adenocarcinoma.

Unlike mice and other small animals, pigs have similar physiology [17, 18], drug dosing [19, 20], and immune response [21–24] to humans. Locoregional therapy in pigs can be performed

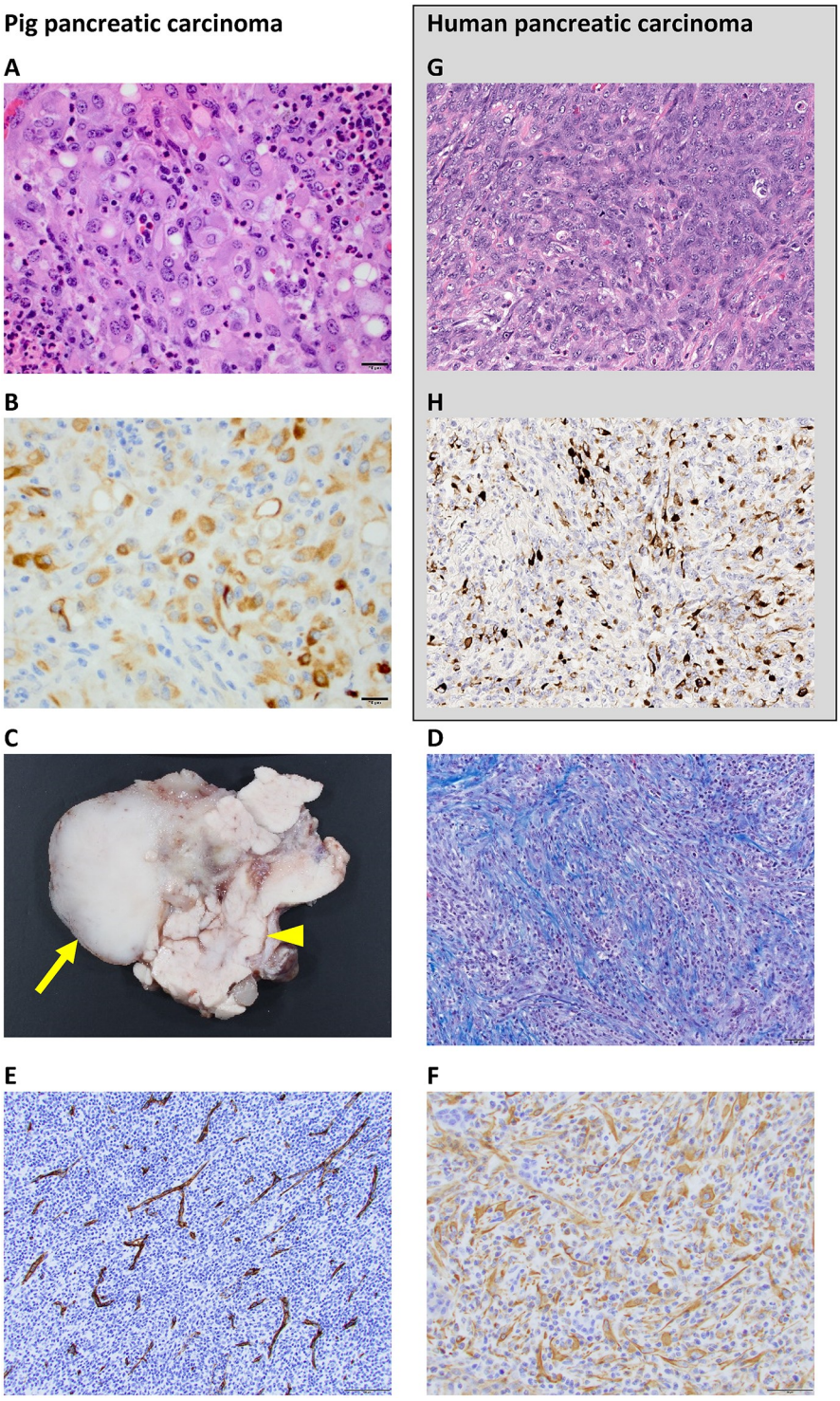

Fig 3. Pathology of pig pancreatic tumors. (**A**) H&E stained section reveals sheets of atypical epithelioid cells with eosinophilic cytoplasm and large round to oval nuclei as well as an associated inflammatory component. (**B**) Cytokeratin AE1/AE3 expression confirms epithelioid differentiation. (**C**) Gross pathology shows a solid mass (arrow) arising from the pancreas (arrowhead). (**D**) Masson's trichrome stain shows collagen bundles (blue) within tumor stroma. (**E**) CD31 immunohistochemistry shows that the tumors contain a high density of microscopic blood vessels. (**F**) Vimentin immunohistochemistry shows that spindle cells are immunopositive, indicating mesenchymal differentiation. (**G** and **H**) For comparison, H&E stain and cytokeratin-19 immunohistochemistry of an undifferentiated carcinoma of the human pancreas shows similar morphologic features.

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using the same size catheters and devices as humans. The artery supplying pig pancreatic cancer could be selectively catheterized using a standard 2.4 F microcatheter. Thus, the Oncopig pancreatic tumor model can be used to develop new image-guided therapies, such as transarterial embolization [30], local immunotherapy [31], and vascular targeted photodynamic therapy [32].

Oncopig pancreatic tumors recreate some of the key challenges for drug delivery in pancreatic cancer: like pancreatic adenocarcinoma in humans, Oncopig pancreatic tumors are hypovascular, and are supplied by tiny feeding arteries [8]. Thus, Oncopig pancreatic cancer could be a promising new model system to test therapies that overcome these barriers to local drug delivery.

One important component of the tumor inoculation protocol is the use of gelatin sponge to retain virus and tumor cells at the site of injection, and to create a receptive microenvironment for tumor growth. Previously, we showed that direct injection of adenoviral vector into the Oncopig, without gelatin sponge, did not result in tumor development [33]. Tumor cells alone are often insufficient for tumor growth, without a receptive microenvironment [34]. For example, in the rabbit VX2 model, tumors must first be grown subcutaneously, prior to transplanting the tumor into the liver or pancreas [35]. Here, we show that direct inoculation of a solid organ is possible, using gelatin sponge, which is made from collagen, an important part of the extracellular matrix in tumors.

A major inflammatory component was seen in all of the Oncopig pancreatic tumors. Undifferentiated carcinomas in humans can also contain significant inflammation [28, 29]. Subcutaneous and intramuscular tumors in the Oncopig contain a significant inflammatory component, which is due to an antitumor T-cell response [36]. Future experiments should address whether these inflammatory pig tumors serve as a good model for the anti-tumor immune response in humans.

Several other animal models of pancreatic cancer are available [37]. The KPC mouse model of pancreatic adenocarcinoma [38] can be used to test drugs, but mice are too small to use human ablation probes or catheters. VX2 tumors can be implanted in rabbit pancreas, and the GDA can be catheterized, but selective angiography of a pancreatic artery has not been reported in rabbits [35]. A previously reported Oncopig pancreatic cancer model used a surgical (not percutaneous) inoculation technique, and required 1 year for growth of small pancreatic tumors that were not visible on computed tomography [39]. The prior Oncopig paper also reported development of large tumors (described as leiomyosarcomas) 16 days after inoculation, but these tumors were not characterized radiographically. In this paper, we solve some technical challenges with solid organ tumor induction in the Oncopig, and report the first large animal pancreatic cancer model that enables testing of new intra-arterial therapies.

One limitation of the Oncopig model is that there is no pancreatic duct dilation. Another limitation is that the inflammatory, poorly differentiated, rapidly growing tumors might not be a good model for well differentiated or slowly growing tumors.

In conclusion, Oncopig pancreatic tumors are rapidly growing, immunogenic, hypovascular undifferentiated carcinomas that can be used to test new percutaneous and intra-arterial therapies for pancreatic cancer.

Supporting information

S1 Table.
(DOCX)

S2 Table.
(DOCX)

S3 Table.
(DOCX)

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