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Title: An Orthotopic Resectional Mouse Model of Pancreatic Cancer

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Author Questionnaire

- 1. Microscopy:** Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**
- 2. Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**
- 3. Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one.**

☒ JoVE VO talent delivers statements

- 4. Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length

Number of Steps: 19

Number of Shots: 10 for videographer, all the rest from LAB MEDIA

Introduction

1. Introductory Interview Statements

NOTE to VO talent: Please record the introduction and conclusion statements

REQUIRED:

- 1.1. This protocol describes a robust and reproducible surgical resection model of pancreatic cancer in mice, which may be used for testing adjuvant and neoadjuvant therapy.
 - 1.1.1. [3.1.1.](#)
- 1.2. This technique combines a co-injection orthotopic model, which is effective at recapitulating human pancreatic cancer, with a safe and effective pancreatic resection technique.
 - 1.2.1. [2.3.2.](#)

OPTIONAL:

- 1.3. While an immunodeficient model is described in this protocol, an alternative immunocompetent model using KPC tumour cells implanted into C57B6 mice may be performed. This alternative would be useful for testing immune therapies.
 - 1.3.1. [2.4.1.](#)

Ethics Title Card

- 1.4. Procedures involving animal subjects have been approved by the Animal Care and Ethics Committee of the University of New South Wales (17/109A)

Protocol

2. Orthotopic Pancreatic Cancer Implantation

- 2.1. To begin, place the anesthetized mouse on the sterile field in a supine position and apply povidone-iodine followed by 70% ethanol for skin preparation. Dissect the skin by making a longitudinal incision on the left cranial quadrant of the abdomen [1] and dissect the muscular layers between forceps [2].
 - 2.1.1. LAB MEDIA: implant2.h264. [Video editor play from 0:01:25 to 0:02:46.](#)
OR
implant3.h264. [Video editor play from 0:02:36 to 0:02:59](#)
 - 2.1.2. LAB MEDIA: implant2.h264. [Video editor play from 0:02:47 to 0:03:18](#)
OR
LAB MEDIA: implant3.h264. [Video editor play from 0:03:00 to 0:03:46](#)
- 2.2. After mounting the syringe with the cell suspension on the injection device, place the purse-string swab over the laparotomy incision and exteriorize the spleen and pancreatic tail through the swabs' opening [1]. Tighten the purse-string swab gently to expose the pancreatic tail for injection, making sure that the gauze contacts the pancreas circumferentially but does not constrict it [2].
 - 2.2.1. LAB MEDIA: implant2.h264. [Video editor play video file from 0:07:34 to 0:08:03](#)
OR
LAB MEDIA: implant3.h264. [Video editor play video file from 0:09:55 to 0:10:05](#)
 - 2.2.2. LAB MEDIA: implant2.h264. [Video editor play video file from 0:08:03 to 0:08:17](#)
OR
LAB MEDIA: implant3.h264. [Video editor play video file from 0:10:14 to 0:10:20](#)
- 2.3. Grasp the tail of the pancreas using forceps and gently place lateral tension on it [1]. Puncture the pancreas with the needle bevel up and advance it while the needle tip is lifted slightly upwards until the bevel has completely entered the peritoneum. Inject the peritoneal surface of the pancreas with the cell suspension in a slow and controlled fashion [2-TXT].
 - 2.3.1. LAB MEDIA: implant2.h264. [Video editor play video file from 0:08:27 to 0:08:42](#)
OR
LAB MEDIA: implant3.h264. [Video editor play video file from 0:10:20 to 0:10:35](#)

- 2.3.2. LAB MEDIA: implant2.h264. [Video editor play video file from 0:09:01 to 0:10:00](#)
OR
LAB MEDIA: implant3.h264. [Video editor play video file from 0:10:35 to 0:11:18](#)
TEXT: 15-20 seconds
- 2.4. After injection, hold the needle in place for a few seconds before withdrawing to minimize leakage [1]. Use a povidone-iodine-soaked swab to carefully dab the site to absorb any inadvertently leaked cell suspension [2].
- 2.4.1. LAB MEDIA: implant2.h264. [Video editor play video file from 0:10:00 to 0:10:10](#)
OR
LAB MEDIA: implant3.h264. [Video editor play video file from 0:11:18 to 0:11:36](#)
- 2.4.2. LAB MEDIA: implant2.h264. [Video editor play video file from 0:10:23 to 0:10:35](#)
OR
LAB MEDIA: implant3.h264. [Video editor play video file from 0:11:50 to 0:12:00](#)
- 2.5. Replace the spleen and pancreas back to its orthotopic position [1] and close the abdominal wall with a 5/0 (*pronounce as "five-O"*) polyglycolic acid suture [2]. Close the skin with clips [3].
- 2.5.1. LAB MEDIA: implant2.h264. [Video editor play video file from 0:15:26 to 0:15:50](#)
OR
LAB MEDIA: implant3.h264. [Video editor play video file from 0:17:10 to 0:17:20](#)
- 2.5.2. LAB MEDIA: implant2.h264. [Video editor play video file from 0:16:37 to 0:20:04](#)
OR
LAB MEDIA: implant3.h264. [Video editor play video file from 0:18:55 to 0:23:00](#)
- 2.5.3. LAB MEDIA: implant2.h264. [Video editor play video file from 0:20:39 to 0:20:53](#)
OR
LAB MEDIA: implant3.h264. [Video editor play video file from 0:23:34 to 0:23:45](#)

3. Cancer Resection Surgery: Distal Pancreatectomy and Splenectomy

- 3.1. Make a longitudinal incision in the skin of the left cranial quadrant of the abdomen, preferably through the previous incision site [1]. Bluntly dissect the skin off the underlying muscular abdominal wall [2] and place an Alm self-retaining retractor to hold the wound open [3].
- 3.1.1. LAB MEDIA: Mouse pancreas resection(9) edited. [Video editor play video file from 0:00:00 to 0:00:13](#)
- 3.1.2. LAB MEDIA: Mouse pancreas resection(9) edited. [Video editor play video file from 0:00:13 to 0:00:39](#)

- 3.1.3. LAB MEDIA: Mouse pancreas resection(9) edited. [Video editor play video file from 0:00:39 to 0:00:55](#)
- 3.2. Incise the muscular layer between forceps just to one side of the suture line of the previous operation, and then extend the incision to excise the entire previous suture line **[1]**.
 - 3.2.1. LAB MEDIA: Mouse pancreas resection(9) edited. [Video editor play video file from 0:00:55 to 0:02:19](#)
- 3.3. Exteriorize the spleen and pancreas by retracting it cranially **[1]**. The colon may be found attached to the caudal aspect of the pancreas by filmy adhesions. If this is found, bluntly dissect the colon off **[2]**.
 - 3.3.1. LAB MEDIA: Mouse pancreas resection(9) edited. [Video editor play video file from 0:02:19 to 0:02:46](#)
 - 3.3.2. LAB MEDIA: Mouse pancreas resection(9) edited. [Video editor play video file from 0:02:47 to 0:03:00](#)
- 3.4. Expose the segment of pancreas for ligation by carefully passing a pair of forceps dorsal to the splenic vessels at the body of the pancreas **[1]**.
 - 3.4.1. LAB MEDIA: Mouse pancreas resection(9) edited. [Video editor play video file from 0:03:00 to 0:03:07](#)
- 3.5. Ligate the pancreas proximal to the tumor with a titanium ligation clip, and then transect the pancreas distal to this with cautery **[1]**. Alternatively, ligate the pancreas in continuity with 5/0 ("*five-O*") polyglycolic acid suture before transection in order to control the pancreatic stump **[2]**. [Videographer: This step is important!](#)
 - 3.5.1. LAB MEDIA: Mouse pancreas resection(9) edited. [Video editor play video file from 0:03:07 to 0:03:22](#)
 - 3.5.2. LAB MEDIA: Mouse pancreas resection(9) edited. [Video editor play video file from 0:03:22 to 0:03:37](#)
- 3.6. Carefully retract the pancreas caudally and cauterize the gastrosplenic vessels between the cranial pole of the spleen and the stomach, keeping in mind that the vessels are small and may easily bleed if avulsed or inadequately cauterized **[1]**.

- 3.6.1. LAB MEDIA: Mouse pancreas resection(9) edited. *Video editor play video file from 0:03:45 to 0:04:11*
- 3.7. Remove the specimen and confirm it for hemostasis **[1-TXT]**.
 - 3.7.1. LAB MEDIA: Mouse pancreas resection(9) edited. *Video editor play video file from 0:04:11 to 0:04:17* **TEXT: Confirm by visual inspection.**
- 3.8. Close the abdominal wall with a 5/0 (*pronounce "five-O"*) polyglycolic acid suture in a continuous fashion and close the skin with clips **[1]**.
 - 3.8.1. LAB MEDIA: Mouse pancreas resection(9) edited. *Video editor play video file from 0:04:17 to 0:04:39*

Results

4. Statistical Bifurcation of Observed Parameters during Assessment of Resection Mouse Model for Pancreatic Cancer

- 4.1. Out of 59 consecutive mice who underwent implantation surgery, gross leakage occurred in 8 mice [1].
 - 4.1.1. LAB MEDIA: Representative results v2.pptx. *Video Editor: Emphasize the sentence "59 mice underwent implantation".*
- 4.2. After three weeks of tumor growth, the pre-resection bioluminescence imaging allowed the exclusion of mice with gross metastatic disease from surgical resection. This left 45 mice that proceeded to pancreatectomy [1]. A macroscopic proximal pancreatic margin greater than 5 millimeters was achieved in 43 of these mice [2].
 - 4.2.1. LAB MEDIA: Representative results v2.pptx. *Video Editor: Emphasize the sentence "45 mice underwent distal pancreatectomy".*
 - 4.2.2. LAB MEDIA: Representative results v2.pptx. *Video Editor: Emphasize the sentence 2nd bullet point.*
- 4.3. At the time of resection, local metastasis was found in 9 out of 45 mice [1]. In addition to metastasis to the suture line, isolated metastatic nodules on the greater curve of the stomach were observed in 3 mice and a subcapsular nodule on the liver in 1 mouse [2].
 - 4.3.1. LAB MEDIA: Representative results v2.pptx. *Video Editor: Emphasize the sentence 3rd bullet point.*
 - 4.3.2. LAB MEDIA: Representative results v2.pptx.
- 4.4. Local extrapancreatic invasion occurred in six mice, with direct invasion into the suture line in five mice, and into the liver in 1 mouse [1].
 - 4.4.1. LAB MEDIA: Representative results v2.pptx.
- 4.5. One week post-resection, 32 mice had a maximum radiance ratio of less than 10, indicating minimal or no residual disease [1]. The mean surgery time, from induction of anesthesia to closure, was 22 minutes [2].
 - 4.5.1. LAB MEDIA: Representative results v2.pptx. *Video Editor: Emphasize the sentence 4th bullet point.*

4.5.2. LAB MEDIA: Representative results v2.pptx. *Video Editor: Emphasize the Surgery Time.*

Conclusion

5. Conclusion Interview Statements

5.1. The key step in the resection procedure is recognizing the bloodless embryological plane dorsal to the splenic vessels and pancreas. Dissecting this plane frees up a segment of the pancreas for subsequent ligation.

5.1.1. [2.10](#).

5.2. After resection, the mice may be treated with adjuvant therapies. Progression of disease may then be assessed using bioluminescence imaging, in combination with necropsy findings and animal survival, depending on the local animal ethics protocols.

5.2.1. [2.5.2](#), [2.5.3](#).

