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Development of Orthotopic Pancreatic Tumor Mouse Models

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Abstract

Genetically engineered mouse models of pancreatic cancer that recapitulate human pancreatic tumorigenesis have been established. However, the cost associated with generating and housing these mice can be prohibitive. Tumor latency and progression to invasive diseases in these models are also highly variable. Xenograft mouse models of human pancreatic cancer including heterotopic and orthotopic have been widely used in preclinical studies for their comparatively low cost and rapid, predictable tumor growth. Of the two, orthotopic tumor mouse models are preferred because they offer tissue site-specific pathology, allow studies of metastasis, and are generally deemed more clinically relevant. Here we describe the procedures of implanting cancer cell lines to generate orthotopic mouse models for pancreatic cancer.

Keywords

Orthotopic; Pancreatic cancer; Xenograft; Mouse model

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¹Implantation of human pancreatic tumors into immunodeficient mice requires the approval of Institutional Review Board as well as Institutional Animal Care and Use Committee (IACUC) before starting the generation of orthotopic human pancreatic cancer mouse models. The procedures of orthotopic pancreatic tumor mouse models must be conducted in accordance with the institutional and national regulations.

²The selection of appropriate types of immunodeficient mice (nude mice, SCID, NOD/SCID, et al.) for orthotopic pancreatic cancer mouse models completely depends on the experimental designs. Athymic nude mice (only T-cell deficient) have been widely used for the establishment of orthotopic and heterotopic human pancreatic cancer xenograft mouse models, especially from established human pancreatic cancer cell lines (10). Athymic nude mice are of low cost and easy to breed. However, if the preservation of the primary pancreatic tumor heterogeneity is the priority of the xenograft mouse model, severe combined immunodeficient mice like NOD/SCID (lack of T, B, and NK cells) are the better option, because there is less immune pressure from these multiple immunodeficient mouse hosts (11). The use of NOD/SCID mice also requires less tumor cell inoculums and offers easy tumor formation (12). The disadvantages of using severe combined immunodeficient mice are the relatively high cost and increased risks of surgical, anesthetic, and infectious complications.

³The cell viability of tumor cell lines must be assessed by trypan blue exclusion before inoculation. More than 95% of the cells for injection should be viable. Importantly, any cell line used for implantation should be routinely tested for mycoplasma contamination to prevent skewed experimental results and animal infection. Cell suspensions should be injected into the head area of mouse pancreas. For human pancreatic cancer cell lines, a single inoculation of 5×10^5 to 1×10^6 cells in serum-free media like PBS is sufficient for tumor formation (13). Typically, you need to prepare at least twice the amount of cell suspension for the experiment.

⁴One critical step of this operation is to minimize leakage of cancer cells from the injection site, which could result in peritoneal spread. Several other approaches have been reported to reduce this occurrence, including using 30-gauge fine needle for injection, reducing the injection volume, mixing tumor cell suspensions with 1% Matrigel (14), and pressing the injection site with a cotton wool tip or with cotton swab immersed with Matrigel for about 1 min after injection (15). In the current procedures, we described one of the best solutions to completely prevent the leakage of injected cell suspension by tying a knot at the injection site.

⁵The development and growth of tumor can be monitored weekly by ultrasound examination. Magnetic resonance imager (MRI) has also been used for monitoring the tumor growth and metastasis in orthotopic pancreatic tumor mouse models (14). However, using MRI to monitor pancreatic tumor development, growth, and metastasis for long-term follow-up in preclinical studies, especially with a large number of mice, is extremely expensive and time-consuming (about 1 h for scanning one mouse). Therefore, if pancreatic cancer cell line is stably transfected with a luciferase-expressing gene, the tumor burden including metastasis could be monitored by measuring bioluminescence emission using IVIS (Xenogen Corp) (16), which would be much simpler and economical (Fig 2).

1. Introduction

Pancreatic cancer is the fourth leading cause of cancer death in the developed countries including the United States. The disease has the worst prognosis in the gastrointestinal malignancies with an overall 5-year survival rate of less than 5%. Unfortunately, this poor survival rate has not improved in the past decades, although dramatic progress has been achieved in understanding the molecular genetics of human pancreatic carcinogenesis (1). Therefore, search for biomarkers of early detection and novel target genes and development of novel therapeutic strategies for this disease are still in urgent demand. Mouse models of pancreatic cancer including genetically engineered mouse models and tumor xenograft mouse models have proved to be very useful in advancing these fronts of cancer research (2–5).

By the locations of implanted tumor or tumor cells, human xenograft mouse models are majorly classified into two types: heterotopic and orthotopic, which both are commonly used in the cancer research field. Subcutaneous heterotopic mouse modeling is rapid, is of lower cost, requires easily manageable techniques, and is best suited for expanding human tumor specimens, a means to convert human tumor specimens into cell lines, angiogenesis studies, etc. However, promising results of therapeutic regimens demonstrated in the subcutaneous heterotopic mouse models often have little effects on human patients; preclinical drug testing now inclines toward employing genetically engineered or orthotopic mouse models. In orthotopic xenograft models, tumors or tumor cells are either implanted or injected into the equivalent organ from which the cancer originated. The orthotopic xenograft models have similar tumor microenvironment as the original tumor and are therefore deemed to more closely resemble the natural tumorigenesis in human. In addition, orthotopic mouse models are better suited for metastasis studies because subcutaneous xenograft mouse model rarely develops metastasis (6). However, the generation of orthotopic pancreatic cancer mouse models is labor intensive, technically challenging, and needs more complex imaging methods to monitor the growth of the implanted tumors. Orthotopic implantation of tumor cells or mass into the pancreas by surgery can also inflict significant physical trauma to the recipient animals and the animals may require lengthy postoperative recovery. Thus, an ultrasound-guided method of injection of tumor cells into pancreas for the development of orthotopic pancreatic cancer mouse model has recently been established in order to minimize the surgical wound to the recipient animals (7).

Immunodeficient mouse models of orthotopic implantation do not completely replicate the process of natural tumorigenesis in human due to the lack of comparable tumor microenvironment. It is commonly accepted that the host immune cells in the tumor microenvironment play critical roles in the progression and metastasis of pancreatic tumor. However, xenografting human cancer cells directly into an immunocompetent murine host would only result in graft rejection. Recently several genetically engineered mouse models have been validated to recapitulate the full spectrum of pancreatic tumorigenesis in human (2, 3, 8). Invasive pancreatic tumors from these mouse models can be a good source for the generation of syngeneic orthotopic pancreatic mouse models in an immunocompetent mouse host (9). Syngeneic orthotopic pancreatic mouse models encompass both the advantages of orthotopic mouse models and genetically engineered mouse models and are a lower cost

alternative to genetically engineered mouse models. Here, we focus on the procedures for surgical generation of orthotopic pancreatic cancer mouse models using cancer cell lines.

2. Materials

2.1. Reagents

1. RPMI-1640 or DMEM medium for tumor cell culture.
2. Phosphate-buffered saline (PBS).
3. 70% (vol/vol) ethanol.
4. Betadine solution (10% providone-iodine).
5. Trypsin–EDTA, 0.05% (Gibco, cat. no. 15400).
6. Ketamine HCl (100 mg/ml).
7. Xylazine hydrochloride (Sigma, cat. no. X1251-5G).
8. Matrigel (BD Biosciences, cat. no. 35620).
9. Antibiotic Enrofloxacin (Enzo life science, lot: L22571).
10. Immunodeficient mice (athymic nude mice or NOD/SCID mice, female, 4–8 weeks) (see Notes 1 and 2).

2.2. Equipment

1. Scale.
2. Luminar flow hood.
3. 1.5 ml sterile Eppendorf tubes.
4. 50 ml centrifuge tubes (Corning, cat. no. 430828).
5. Sterile petri dishes, 10 cm dishes.
6. 26G needles.
7. 100 µl Hamilton Syringe (Hamilton Syringe Co., Nevada, USA).
8. Electric clipper (if mouse hosts have hair).
9. Microscissor.
10. Curved forceps.
11. Splinter forceps.
12. Homostatic forceps.
13. Gauze pads, 10 × 10 cm.
14. Sterile Cotton tipped applicator (Puritan medical, Maine, USA).
15. Surgical tape (Fisher).
16. 4-0 polysorb sutures.

3. Procedures

1. Purchase sufficient number of female athymic nude or other type of immunodeficient mice such as SCID for the experiments (see Note 2).
2. Administer antibiotic Enrofloxacin (5 µg/mg) daily by subcutaneous injection for 2 days before surgery.
3. All surgical instruments should be autoclaved freshly and allowed to cool to room temperature before use.
4. Prepare ketamine/xylazine solution containing 10 mg/ml ketamine and 2 mg/ml xylazine by a combination of 1 ml ketamine stock (100 mg/ml), 0.2 ml xylazine stock (100 mg/ml), and 8.8 ml distilled water. This freshly made solution should be filter-sterilized and could be stored at room temperature for continuous use up to 2 months.
5. Prepare cell suspension (from human pancreatic cancer cell lines) to a concentration of 500,000–1,000,000 cells in 50 µl PBS solution, and leave on ice with occasional agitation (see Note 3).
6. Weigh the recipient nude mouse for orthotopic surgery, and anesthetize the animal by intraperitoneal administration of the prepared ketamine/xylazine solution at a dose of 10 µl/mg. Before starting the operation, make sure that the mouse completely lose consciousness by stimulating the abdominal skin with a pair of splinter forceps.
7. Sterilize the left side of abdominal skin by painting the area with betadine solution from the neck level to the tail, and then completely remove the stain of the betadine with 70% ethanol.
8. Cover the animal with a gauze sponge previously cut with an ~2 cm diameter hole at the center to expose the left abdominal flank, and fix the animal to the platform with surgical tapes (Fig 1a).
9. The site of incision is chosen at left abdominal flank, which is slightly medial to the splenic silhouette. Lift the abdominal skin with forceps and make a 1-cm incision with sterile microscissors (Fig 1b).
10. Grasp and lift the underlying muscle with forceps and incise to enter the abdominal cavity without injury to the underlying organs. Extend the muscle incision to 1 cm. At this point, you should be able to note the spleen organ.
11. Gently pull out and expose the entire pancreatic body together with spleen to the outside of the peritoneal cavity by using a pair of blunt-nose forceps (Fig 1c).
12. Make a knot surrounding the pancreas tail at ~2 mm from the end of the tail using a string of 4-0 polysorb suture, but do not tight the knot (Fig 1d).
13. Remove the syringe containing the cell suspension from the ice and agitate to disrupt the cell clumps or clusters.

14. While gently retracting the pancreas laterally, insert the needle through the knot into the tail of the pancreas and pass into the pancreas head (Fig 1e). Slowly inject 50 μ l of cell suspension while withdrawing the needle. A fluid-filled region should be formed within the pancreatic parenchyma.
15. Remove the needle from the pancreas and immediately tight the knot (see Note 4) (Fig 1f).
16. Leave the pancreas externalized and untouched for 1 min to inspect for any hemorrhage and any leakage.
17. The pancreas and spleen are carefully returned back to the peritoneal cavity with blunt forceps, and the abdominal muscle layer and the skin layer are sequentially closed with interrupted 4-0 polysorb sutures (Fig. 1g, h).
18. After tumor implantation, all mice should be monitored at least twice weekly for the tumor growth and general signs of morbidity such as ruffled fur, hunched posture, and immobility. Eight weeks after surgery, mice will be sacrificed to examine the growth of pancreatic tumors. The tumor mass will be removed from the normal pancreatic tissues. The weight and size of tumor should be documented (measurement of tumor volume = $1/2 \text{ length} \times \text{breath} \times \text{width}$) (see Note 5).

References

1. Kern SE. Molecular genetic alterations in ductal pancreatic adenocarcinomas. *Med Clin North Am.* 2000; 84(3):691–695. xi. [PubMed: 10872425]
2. Hingorani SR, et al. Preinvasive and invasive ductal pancreatic cancer and its early detection in the mouse. *Cancer Cell.* 2003; 4(6):437–450. [PubMed: 14706336]
3. Hingorani SR, et al. Trp53R172H and KrasG12D cooperate to promote chromosomal instability and widely metastatic pancreatic ductal adenocarcinoma in mice. *Cancer Cell.* 2005; 7(5):469–483. see comment. [PubMed: 15894267]
4. Fu X, Guadagni F, Hoffman RM. A metastatic nude-mouse model of human pancreatic cancer constructed orthotopically with histologically intact patient specimens. *Proc Natl Acad Sci USA.* 1992; 89(12):5645–5649. [PubMed: 1608975]
5. Rubio-Viqueira B, et al. An in vivo platform for translational drug development in pancreatic cancer. *Clin Cancer Res.* 2006; 12(15):4652–4661. [PubMed: 16899615]
6. Killion JJ, Radinsky R, Fidler IJ. Orthotopic models are necessary to predict therapy of transplantable tumors in mice. *Cancer Metastasis Rev.* 1998; 17(3):279–284. [PubMed: 10352881]
7. Huynh AS, et al. Development of an orthotopic human pancreatic cancer xenograft model using ultrasound guided injection of cells. *PLoS One.* 2011; 6(5):e20330. [PubMed: 21647423]
8. Qiu W, et al. Disruption of p16 and activation of Kras in pancreas increases ductal adenocarcinoma formation and metastasis in vivo. *Oncotarget.* 2011; 2(11):862–873. [PubMed: 22113502]
9. Tseng WW, et al. Development of an orthotopic model of invasive pancreatic cancer in an immunocompetent murine host. *Clin Cancer Res.* 2010; 16(14):3684–3695. [PubMed: 20534740]
10. Gray MJ, et al. Neuropilin-1 suppresses tumorigenic properties in a human pancreatic adenocarcinoma cell line lacking neuropilin-1 coreceptors. *Cancer Res.* 2005; 65(9):3664–3670. [PubMed: 15867361]
11. Ito M, et al. NOD/SCID/gamma(c) (null) mouse: an excellent recipient mouse model for engraftment of human cells. *Blood.* 2002; 100(9):3175–3182. [PubMed: 12384415]
12. Quintana E, et al. Efficient tumour formation by single human melanoma cells. *Nature.* 2008; 456(7222):593–598. [PubMed: 19052619]

13. Trevino JG, et al. Inhibition of SRC expression and activity inhibits tumor progression and metastasis of human pancreatic adenocarcinoma cells in an orthotopic nude mouse model. *Am J Pathol.* 2006; 168(3):962–972. [PubMed: 16507911]
14. Partecke IL, et al. In vivo imaging of pancreatic tumours and liver metastases using 7 Tesla MRI in a murine orthotopic pancreatic cancer model and a liver metastases model. *BMC Cancer.* 2011; 11:40. [PubMed: 21276229]
15. McNally LR, et al. KISS1 over-expression suppresses metastasis of pancreatic adenocarcinoma in a xenograft mouse model. *Clin Exp Metastasis.* 2010; 27(8):591–600. [PubMed: 20844932]
16. Kunnumakkara AB, et al. Zylamend suppresses growth and sensitizes human pancreatic tumors to gemcitabine in an orthotopic mouse model through modulation of multiple targets. *Int J Cancer.* 2012; 131(3):E292–E303. [PubMed: 21935918]

Fig. 1.

Orthotopic implantation of pancreatic cancer cells. **(a)** Let the mouse sleep on its right side. **(b)** Make about 1-cm incision with sterile microscissors beside the splenic silhouette. **(c)** Expose the entire pancreas and spleen by using a pair of blunt-nose forceps. **(d)** Make a loose knot at the tail of pancreas using a 4-0 polysorb suture. **(e)** Insert the needle into the tail of the pancreas via the knot, and pass into the pancreatic head area. **(f)** Immediately tighten the knot after the administration of cancer cell solution. **(g)** Suture the abdominal muscle layer first as putting back the pancreas and spleen into the abdominal cavity. **(h)** Close the skin with interrupted 4-0 polysorb sutures.

Fig. 2.

An example of dynamic and quantitative monitoring of tumor burdens by measuring bioluminescence emission using IVIS (Xenogen Corp). **(a)** Tumor burden displayed as ROI 2 = 3.151×10^9 , 3 weeks after pancreatic orthotopic implantation with half million human pancreatic cancer cells. **(b)** and **(c)** The growth of pancreatic tumor can be precisely measured by bioluminescence emission using IVIS.