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Title: Orthotopic Transplantation of Breast Tumors as Preclinical Models for Breast Cancer

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Author Questionnaire

1. Microscopy: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **N**

2. Software: Does the part of your protocol being filmed demonstrate software usage? **N**

3. Filming location: Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Xi Chen**: This protocol describes the orthotopic transplantation of breast tumor fragments into the mammary fat pad and provides valuable approaches for investigating tumor biology, drug responses, and mechanisms of drug resistance **[1]**.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

REQUIRED:

1.2. **Xi Chen**: This technique is minimally invasive and ensures proper placement of the tumor tissue within the mammary fat pad, accessibility for caliper measurements, and animal mobility maintenance **[1]**.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

OPTIONAL:

1.3. **Lacey Dobrolecki**: This technique allows the transplantation of dozens of animals from a single patient-derived xenograft for the evaluation of multiple candidate cancer therapies simultaneously in a "preclinical trial" format **[1]**.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

OPTIONAL:

1.4. **Lacey Dobrolecki**: This method is superior to subcutaneous tissue implantation for establishing patient-derived xenograft models and can also be used when orthotopic implants are not possible **[1]**.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

NOTE: This shot 1.4.1 is mislabeled as 1.3.1

OPTIONAL:

1.5. **Xiangdong Lv**: The most difficult steps are applying the appropriate amount of pressure to peel the fat pad from the body wall and making the tissue pocket **[1]**.

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

OPTIONAL:

1.6. **Xiangdong Lv**: Visualization of the fat pad peeling and correct anatomical placement of the tissue fragment will help ensure a successful performance of the procedure **[1]**.

1.6.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

Ethics Title Card

1.7. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at Baylor College of Medicine.

Protocol

2. Cryopreserved Mammary Tumor Fragment Preparation

2.1. To cryopreserve mammary tumor tissue fragments for transplantation, first thaw a cryovial from liquid nitrogen-storage containing the tumor fragments of interest in a 37-degree Celsius water bath **[2]** with occasional gentle flicking **[3]**.

2.1.1. WIDE: Talent placing vial(s) into water bath

2.1.2. Talent flicking vial

2.2. When the fragments have thawed, mix the fragments with gentle inversion **[1]** and dry the outside of the vial **[2]**.

2.2.1. Talent inverting vial

2.2.2. Talent drying vial

NOTE: 2.2.1 and 2.2.2 were captured in one shot and labeled as 2.2.1

2.3. Spray the vial with 70% ethanol **[1]** and place it in a biosafety hood **[2]**.

2.3.1. Talent spraying vial

2.3.2. Talent placing vial into hood

NOTE: 2.3.1 and 2.3.2 were captured in one shot and labeled as 2.3.1

2.4. Transfer the cryovial contents into a 15-milliliter conical tube containing 10 milliliters of cold DMEM (D-M-E-M) **[1-TXT]** and invert the tube to mix **[2]**.

2.4.1. Talent adding fragments to tube, with medium container visible in frame **TEXT: DMEM: Dulbecco's modified Eagle medium**

2.4.2. Talent inverting tube(s)

NOTE: 2.4.1 and 2.4.2 were captured in one shot and labeled as 2.4.1

2.5. Allow the fragments to settle **[1]** and replace the supernatant two times with 10-milliliter aliquots of fresh, cold DMEM **[2]**.

2.5.1. Fragments settling/shot of settled tubes

2.5.1.a Talent aspirating the medium

NOTE: 2.5.1.a shot was added.

2.5.2. Talent adding medium to tube, with medium container visible in frame

2.6. Then place the tube on ice **[1]**.

2.6.1. Talent placing tube onto ice

3. Fresh Mammary Tumor Sample Collection and Preparation

3.1. To collect a mammary tumor tissue sample, spray the tumor region of the tumor-bearing mouse with 70% ethanol **[1-TXT]** and use serrated forceps to pinch and lift the skin surrounding the tumor **[2]**.

3.1.1. WIDE: Talent spraying mouse *Videographer: More Talent than mouse in shot*
TEXT: Euthanasia: according to institutional guidelines

3.1.2. Skin being lifted

3.2. Use scissors to make a small incision **[1]** and separate the tumor from the skin, taking care to avoid hair contamination **[2]**.

3.2.1. Incision being made

3.2.2. Tumor being dissected

3.3. Trim off any remaining mouse fat pad tissue from the outer surface of the tumor **[1]** and place the tumor in a 15-milliliter conical tube containing 5 milliliters of cold DMEM **[2]**.

3.3.1. Fat being trimmed

3.3.2. Talent placing tumor into tube, with medium container visible in frame

NOTE: 3.3.1 and 3.3.2 were captured in one shot and labeled as 3.3.1

3.4. In a biosafety hood, transfer the dissected tumor into a sterile, 10-centimeter Petri dish containing enough DMEM to prevent drying **[1]** and use a UV-sterilized blade to cut the tumor into 1-cubed millimeter-fragments under aseptic conditions **[2]**.

3.4.1. Talent at hood, placing tumor into dish

3.4.2. Tumor being fragment

3.5. Then transfer the tumor fragments to a 15-milliliter tube containing cold DMEM on ice **[1]**.

3.5.1. Talent adding fragments to tube on ice, with medium container visible in frame

4. Surgical Preparation

4.1. After confirming a lack of response to pedal reflex **[1-TXT]**, apply ointment to the anesthetized recipient mouse's eyes **[2]** and shave the abdomen around the fourth mammary gland **[3]**.

4.1.1. WIDE: Talent pinching toe *Videographer: More Talent than mouse in shot*
TEXT: Analgesia: buprenorphine 1 mg/kg s.c.; Anesthesia: isoflurane 11.25 mL/h

4.1.2. ECU: Ointment being applied

4.1.3. Abdomen being shaved

4.2. Then disinfect the exposed skin three times, using a circular motion and starting in the center of the surgery site while moving toward the outer edges, with povidone-iodine surgical scrub **[1]** followed by scrub removal with a 70% isopropyl ethanol pad **[2]**.

4.2.1. Skin being scrubbed, with povidone-iodine container visible in frame

4.2.2. Skin being wiped, with ethanol container visible in frame

5. Tumor Fragment Transplantation

5.1. For transplantation of the tumor tissue fragments into the fourth, inguinal mammary fat pad, cover the animal with a sterile, fenestrated, surgical drape **[1]** and use serrated forceps to pinch and lift the skin at the fourth nipple **[2]**.

5.1.1. WIDE: Talent placing drape *Videographer: More Talent than mouse in shot*

5.1.2. Skin being pinched and lifted

5.2. With the blunt side of the tips facing down, use scissors to make an approximately 1-centimeter, parasagittal incision from the number 4 nipple toward the head **[1]**.

5.2.1. Incision being made

5.3. Use a cotton tipped applicator to separate the skin from the peritoneum on the medial side of the incision **[1]** and gently peel the number 4 mammary fat pad from the skin **[2]**.

5.3.1. Skin being separated

5.3.2. Fat pad being peeled *Videographer: Important step*

5.4. Use a 27-gauge needle to secure the skin close to the animal's body **[1]** and use serrated forceps to gently extend the fat pad away from the body **[2]**.

5.4.1. Skin being pinned

5.4.2. Fat pad being extended

NOTE: 5.3.1, 5.3.2, 5.4.1 and 5.4.2 were captured in one shot and labeled as 5.3.1

5.5. Locate the inguinal lymph node at the intersection of the major vessels in the gland **[1]** and carefully cauterize the vessels medial to the lymph node and the fat joining the fourth and fifth fat pads **[2-TXT]**.

5.5.1. ECU: Shot of inguinal LN *Videographer: Important step*

5.5.2. Vessel(s) being cauterized **TEXT: Caution: Temporarily turn off O_2 during cauterization**

NOTE: 5.5.1 and 5.5.2 were captured in one shot and labeled as 5.5.1

5.6. Using micro dissecting scissors, cut each cauterized vessel one at a time until the section of the fat pad that contains the lymph node is removed **[1]** and use blunt forceps to grasp the fat pad **[2]**.

5.6.1. Vessel(s) being cut

5.6.2. Fat pad being grasped

NOTE: 5.6.1 and 5.6.2 were captured in one shot and labeled as 5.6.1

NOTE: The angle can not change so shots from 5.6.1 to 5.13.1 were captured in sequence.

5.7. Using the other hand and a curved motion, insert the closed tip of a pair of angled fine forceps into the middle of the fat pad above the remaining vessel close to the wall of the peritoneum **[1]**.

5.7.1. Tips being inserted *Videographer: Important/difficult step*

5.8. Retract the tips **[1]** and reinsert the tips into the original hole **[2]**, opening the forceps to stretch the hole into a small pocket **[3]**.

5.8.1. Tips being retracted *Videographer: Important/difficult step*

5.8.2. Tips being reinserted *Videographer: Important/difficult step*

5.8.3. Tips being opened *Videographer: Important/difficult step*

5.9. Remove the forceps from hole **[1]** and use them to insert a piece of tumor fragment into the pocket **[2]**.

5.9.1. Tips being removed

5.9.2. Tumor being inserted *Videographer: Important/difficult step*

5.10. Slowly open the tips to release the tumor fragment into the fat pad pocket **[1]** and carefully withdraw the forceps **[2]**.

5.10.1. Tips being opened

5.10.2. Tips being withdrawn

5.11. After confirming that the fragment has been deposited into the fat pad pocket **[1]**, starting from the base of the incision, collect the skin on each side of the incision with serrated forceps **[2]** and lift the skin slightly **[3]**.

5.11.1. ECU: Shot of fragment in fat pad

5.11.2. Skin being collected

5.11.3. Skin being lifted

5.12. Uncurl the edges of the incision with claw forceps **[1]** and pinch the edges together to form a continuous surface at the top **[2]**.

5.12.1. Edge(s) being uncurled

5.12.2. Edge(s) being pinched together

5.13. Holding the two sides with the serrated forceps, use a wound clip applicator to place a clip in the center of the incision **[1-TXT]** and place the animal in a clean cage on a warming surface with monitoring until full recumbency **[2]**.

5.13.1. Clip being applied **TEXT: Apply tissue adhesive to ends of incision as necessary**

5.13.2. Talent placing mouse into cage *Videographer: More Talent than mouse in shot*

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see?
5.3., 5.5., 5.7., 5.8., 5.9.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success?

5.7., 5.8., 5.9. Ensuring that the pocket is in the correct anatomical location and that the tissue stays in the pocket

Results

6. Results: Representative Transplant Tumor Characterization

6.1. Typically, patient-derived xenograft or genetically engineered mouse model tumors can be palpated from about 2-8 weeks post transplantation **[1]**.

6.1.1. LAB MEDIA: Figure 2A *Video Editor: please emphasize data line*

6.2. H&E staining can be performed to analyze the tumor pathology **[1]**, while immunohistochemistry can be used to monitor markers for specific cell lineages of interest **[2]** or cell viability and functional status **[3]**.

6.2.1. LAB MEDIA: Figures 2B-2D *Video Editor: please emphasize Figure 2B*
6.2.2. LAB MEDIA: Figures 2B-2D *Video Editor: please emphasize Figure 2C*
6.2.3. LAB MEDIA: Figures 2B-2D *Video Editor: please emphasize Figure 2D*

Conclusion

7. Conclusion Interview Statements

7.1. **Xiangdong Lv**: It is important to make sure that the tumor fragment is placed securely in the pocket so that it doesn't fall out and grow within the subcutaneous space **[1]**.

7.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (5.9.2)

7.2. **Xiangdong Lv**: Once the tumors have grown, drug treatments can be administered to assess tumor responses. The tumors can also be removed to determine the metastatic potential of the cancer cells **[1]**.

7.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

7.3. **Lacey Dobrolecki**: This basic technique has been used since the 1950's. Our modification provides a more efficient and less invasive manner in which to conduct larger scale developmental biology and cancer studies **[1]**.

7.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera