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Title: Generation of Tumor Organoids from Genetically Engineered Mouse Models of Prostate Cancer

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Author Questionnaire:

1. Microscopy: Does your protocol require JoVE to film through your microscope? N

2. Does your protocol include software usage? N

3. Which steps from the protocol section below are the most important for viewers to see?

2.3., 6.2., 6.3.

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

It is difficult to identify the prostate tissue from the rest of the male urogenital region. Keep oriented by knowing where the bladder and urethra are at all times (4.8, 4.9)

5. Will the filming need to take place in multiple locations? N

Section - Introduction

Comment [BC1]: Authors: Each Author can give a maximum of two Introduction statements.

Videographer: Interviewee Headshots are required. Take a headshot for each interviewee.

1. **REQUIRED Interview Statements (Said by you on camera): All interview statements may be edited for length and clarity.**

    1.1. **Kristine Wodosky**: Mouse prostate organoid cultures more closely resemble *in vivo* cell organization than traditional cell cultures **[1]**.

    Comment [BC2]: Authors: Dr. Wodosky will be introduced during this statement and does not need to be introduced with an additional statement.

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

    1.2. **Kristine Wadosky**: In cancer research, organoids model the original tumor better than 2D cell lines and can be used to test potential cancer therapies *in vitro* to develop new drug treatments **[1]**.

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

Ethics title card: (for human subjects or animal work, does not count toward word length total)

1.3. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee (IACUC) at Roswell Park Comprehensive Cancer Center.

Section - Protocol

2. *En Bloc* Male Urogenital System Extraction

2.1. For *en bloc* extraction of the male mouse urogenital system, ~~after dissection of the urogenital system~~ **[1-TXT]**, grasp the fat pad on either side of the bladder **[2]** and pull upward to expose the testicle **[3]**.

2.1.1. WIDE: Talent dissection urogenital system *Videographer: More Talent than mouse in shot* **TEXT: See text for full mouse euthanasia/preparation details**

2.1.2. CU: Shot of bladder, then pad being grasped

2.1.3. CU: Fat pad being pulled/testicle being exposed

2.2. Carefully dissect each testicle away from the rest of the urogenital region **[1]** and gently lift on the bladder so that the urogenital region is elevated together, exposing the urethra underneath **[2]**.

2.2.1. CU: Testicle being dissected

2.2.2. CU: Bladder being pulled/urethra being exposed

2.3. While holding the bladder, orient the scissors against the underside of the dorsal prostate to incise the urethra **[1]**. The entire urogenital region will be released from the abdominal cavity **[2]**.

2.3.1. CU: Scissors being placed against dorsal prostate, then urethra being cut *Videographer: Can split action into separate steps as necessary*

2.3.2. CU: Urogenital region being lifted

3. Pelvic Lymph Node, Spleen, Liver, Kidney, Lung, Tibia, and Femur Extraction

3.1. After removing the urogenital system **[1]**, the pelvic lymph nodes, positioned immediately behind the urogenital system and on either side of the spine, will be exposed **[2-TXT]**.

3.1.1. WIDE: Talent placing system into PBS

3.1.2. CU: Shot of pelvis *Video Editor: please emphasize lymph nodes right behind urogenital system and on either side of spine when mentioned* **TEXT: LN visible only w/ metastatic lesions/inflammation**

Comment [KW3]: We are currently showing extraction of the urogenital region, so this cannot be "after dissection of the urogenital system"

3.2. To remove the lymph nodes, orient straight forceps under the lymph tissue and pull up to remove the lymph node **[1]**.

3.2.1. CU: Forceps being placed under lymph node/lymph node being harvested OR lymph node location being indicated

3.3. Next, grasp the rectum with the straight forceps and cut **[1]**, pulling up on the rectum to unravel the entire colon and small intestine **[2]** to look for metastatic lesions in the mesenteric lymph nodes **[2]**.

3.3.1. CU: Rectum being grasped and/or cut

3.3.2. CU: Rectum being lifted/gut being unraveled

3.3.3. CU: Shot of lesion(s)

3.4. When the entire ileum has been removed **[1]**, continue to pull on the duodenum to expose the stomach **[2]**.

3.4.1. CU: Ileum being removed

3.4.2. CU: Duodenum being lifted/stomach being exposed

3.5. Cut the esophagus to completely remove the stomach **[1]**. If any metastatic lesions in the lymph nodes are observed, carefully dissect these nodes away from the intestine for storage in PBS **[2]**.

3.5.1. CU: Esophagus being cut/stomach being removed

3.5.2. CU: LN being dissected

3.6. When the stomach has been removed, the spleen can be harvested from the dorsal side of the abdomen for storage in 4% paraformaldehyde **[1]**.

3.6.1. CU: Shot of spleen/spleen being dissected

3.7. Remove the liver for storage in PBS **[1]** and remove the kidneys from either side of the spine **[2]** along with any renal lymph nodes containing metastatic lesions **[3]**.

3.7.1. CU: Liver being removed

3.7.2. CU: Kidney being dissected

3.7.3. CU: LN being dissected

3.8. To expose the thoracic cavity, carefully cut the diaphragm along the ribcage **[1]**. The negative pressure release within the thoracic cavity will expose the heart and lungs **[2]**.

3.8.1. CU: Diaphragm being cut/pierced

3.8.2. CU: Shot of heart and lungs

3.9. Pull up on the sternum to open the thoracic cavity further **[1]** and scan the ventral face of the thoracic cavity along the rib cage for metastatic lesions in the thoracic lymph nodes for dissection if present **[2]**.

3.9.1. CU: Sternum being pulled

3.9.2. CU: Shot of ventral face of thoracic cavity/metastatic thoracic LN lesions OR lymph node location being indicated

3.10. While still holding the sternum, cut away the ventral rib cage to access the heart and lungs **[1]** and pull up on the heart to cut underneath the lungs **[2]**.

3.10.1. CU: Ventral rib cage being cut

3.10.2. CU: Heart being pulled/cutting being performed under lungs

3.11. To fully remove the heart and lungs *en bloc*, cut all of the anterior blood vessels and the trachea **[1]** and carefully transfer the heart without damaging the lung tissue or lung metastatic lesions into a container of PBS **[2-TXT]**.

3.11.1. CU: Blood vessels and/or trachea being cut

3.11.2. MED: Talent placing tissue into PBS **TEXT: Obtain ear/tail cutting for genotyping**

4. Prostate Tumor Dissection

4.1. For dissection of the prostate tumor, place the urogenital region under a dissection microscope **[1]** and use a pair of straight forceps and a pair of curved forceps to flip the urogenital region to its dorsal face **[2]**.

4.1.1. WIDE: Talent placing tissue under microscope

4.1.2. LAB MEDIA: To be provided by Authors: Region being flipped

Comment [BC4]: Authors: Please upload all requested LAB MEDIA files to your project page.

4.2. Locate the proximal prostate region that can be identified by the pink-red color of the urethra **[1]** and grasp the urethra firmly to allow manipulation of the urogenital tissue with the curved forceps **[2]**.

4.2.1. LAB MEDIA: To be provided by Authors: Shot of proximal prostate region

4.2.2. LAB MEDIA: To be provided by Authors:: Urethra being grasped

4.3. Locate the base of the seminal vesicles to allow their careful removal **[1-TXT]**.

4.3.1. LAB MEDIA: To be provided by Authors: Base being located/vesicle being removed **TEXT: Caution: Do not puncture seminal vesicle**

4.4. Then use the curved side of the forceps to remove the vas deferens and as much fatty and connective tissue as possible **[1]**.

4.4.1. LAB MEDIA: To be provided by Authors: Vas deferens and/or fatty tissue being removed

4.5. While still firmly holding the urethra and proximal prostate region, use a pair of fine, pointed scissors to remove the bladder from the urethra **[1]**.

4.5.1. LAB MEDIA: To be provided by Authors: Bladder being **grasped, region being flipped, and bladder being** removed**.**

Comment [KW5]: Describes the shot I got with this particular prostate tumor.

4.6. Holding the proximal prostate region and urethra firmly with the straight forceps **[1]**, grasp the anterior prostate region with the curved side of the forceps to firmly pull the tissue away from the bladder and the rest of the prostate **[2]**.

4.6.1. LAB MEDIA: To be provided by Authors: Prostate region being grasped

4.6.2. LAB MEDIA: To be provided by Authors: Anterior prostate region being grasped and pulled

4.7. Place the anterior prostate tissue in PBS **[1]** and locate the ventral prostate region **[2]**.

4.7.1. MED: Talent placing tissue in PBS

4.7.2. LAB MEDIA: To be provided by Authors: Shot of ventral prostate region

4.8. Remove the ventral prostate region in the same manner **[1]** and, if the lateral region can be distinguished from the dorsal region, remove the lateral prostate region on both sides **[2]**.

4.8.1. LAB MEDIA: To be provided by Authors: Ventral prostate being removed

4.8.2. LAB MEDIA: To be provided by Authors: Shot of lateral and dorsal prostate regions, then lateral prostate region being removed

4.9. Then remove the dorsal prostate region **[1]** and place the proximal prostate region in 4% paraformaldehyde **[2]**.

4.9.1. LAB MEDIA: To be provided by Authors: Dorsal prostate region being removed

4.9.2. MED: Talent placing proximal prostate region in PFA

5. Tumor Tissue Digestion

5.1. After mincing, place the tumor pieces into a 15-milliliter tube of digestion buffer for a 1.5-2-hour digestion at 37 degrees Celsius **[1-TXT]**.

5.1.1. WIDE: Talent adding pieces to tube **TEXT: Check digestion process every 20 min**

5.2. At the end of the digestion, sediment the digested tissue by centrifugation **[1-TXT]** and discard the supernatant **[2]**.

5.2.1. MED: Talent placing tube(s) into centrifuge **TEXT: 5 min, 175 x g, 4 °C**

5.2.2. CU: Supernatant being removed

5.3. Flick the tube to loosen the cell pellet **[1]** and resuspend the cells in 1 milliliter of pre-warmed trypsin supplemented with 10 micromolar Y-27632 (Y-two-seven-six-thirty-two) Rock Inhibitor for 5 minutes at 37 degrees Celsius **[2]**.

5.3.1. CU: Tube being clicked

5.3.2. MED: Talent adding trypsin to tube, with trypsin container visible in frame

5.4. At the end of the incubation, triturate the tissue slurry 5 times with a standard P1000 pipette tip **[1]** and return the tube to the water bath for an additional 5 minute-incubation and trituration **[2]**.

5.4.1. CU: Tissue being pipetted

5.4.2. MED: Talent placing tube into water bath

6. Matrix Dome Plating

6.1. For matrix dome plating, wash the digested cells in 9 milliliters of cold medium **[1-TXT]** and resuspend the cells in 1 milliliter of fresh medium for counting **[2]**.

6.1.1. WIDE: Talent adding medium to cells, with medium container visible in frame **TEXT: See text for all medium/reagent preparation details**

6.1.2. CU: Shot of pellet, then medium being added to cells, with medium container label visible in frame

6.2. Dilute the tumor cells in the appropriate volume of matrix **[1]** and carefully drop 200 microliters of matrix-cell solution into each well of a 55-degree Celsius warmed, 6-well plate **[2]**.

6.2.1. MED: Talent mixing cells with matrix, with stock matrix container visible in frame

6.2.2. CU: Dome being dropped into well

6.3. Allow the domes to solidify at room temperature for 2 minutes **[1]** before placing the plate upside-down in a 37-degree incubator for 20 minutes **[2]**.

6.3.1. MED: Talent setting timer, with plate visible in frame

6.3.2. CU: Plate being placed upside down in incubator

6.4. When the domes have fully solidified, add 2 milliliters of prostate organoid medium supplemented with androgen R1881 and Rock inhibitor to each well **[1]** and return the plate to the cell culture incubator **[2]**.

6.4.1. CU: Shot of solidified dome, then medium being added to well

6.4.2. MED: Talent placing plate into incubator

Section – Results

7. Results: Representative Solid and Fluid-Filled Tumor Characterization

7.1. Fluorescent dissection images reveal green fluorescent protein, or GFP (G-F-P), expression by solid prostate tumors **[1]**, indicating that the tumors cells express Cre (cree) **[2]**.

7.1.1. LAB MEDIA: Figure 2C: JoVE Video Editor please emphasize green signal in GFP Prostate image

7.2. The liver and lungs from the same animal exhibit metastatic tumors expressing GFP **[1]**, demonstrating that these tumors originated from the primary prostate tumor **[2]**.

7.2.1. LAB MEDIA: Figure 2C: JoVE Video Editor please emphasize green signal in GFP Liver and Lung images
7.2.2. LAB MEDIA: Figure 2C

7.3. The pelvic lymph node from this mouse expresses GFP and not Tomato **[1]**, indicating that this metastatic tumor has overtaken the lymph node and no normal tissue remains **[2]**.

7.3.1. LAB MEDIA: Figure 2C: JoVE Video Editor please emphasize green signal in GFP Lymph Node image
7.3.2. LAB MEDIA: Figure 2C: JoVE Video Editor please emphasize lack of red signal in Tomato Lymph Node image/Tomato Lymph Node image

7.4. At Day 1 of culture, small organoids generated from a solid prostate tumor begin to form **[1]**, with both Tomato- and GFP-expressing cells present in the tumor organoid culture **[2]**.

7.4.1. LAB MEDIA: Figure 4 Day 1 images: JoVE Video Editor please emphasize pink/white cells in Phase image
7.4.2. LAB MEDIA: Figure 4 Day 1 images: JoVE Video Editor please add/emphasize white arrows in Tomato and GFP images

7.5. By Day 7 and beyond, when the prostate tumor organoids have fully formed **[1]**, however, the organoids express GFP but not Tomato **[2]**, suggesting that the organoids have originated from Cre-expressing tumor cells and not from normal epithelial cells **[3]**.

7.5.1. LAB MEDIA: Figure 4 Day 7, Passage 1, and Passage 2 images: JoVE Video Editor please emphasize organoids in Phase images
7.5.2. LAB MEDIA: Figure 4 Day 7, Passage 1, and Passage 2 images: JoVE Video Editor please emphasize green signal in GFP images

7.6. On Day 1 of culture of small organoids generated from fluid-filled prostate **[1]**, both Tomato- and GFP-expressing cells are present within the organoid culture **[2]**.

7.6.1. LAB MEDIA: Figure 5 Day 1 images: JoVE Video Editor please emphasize pink/white cells in Phase image
7.6.2. LAB MEDIA: Figure 4 Day 1 images: JoVE Video Editor please add/emphasize white arrows in Tomato image and emphasize green signal in GFP image

7.7. Organoids from fluid-filled prostate tumors express either GFP or Tomato at Day 7 and beyond **[1]**, however, indicating that the organoids formed from cells that do not express Cre **[2]**.

7.7.1. LAB MEDIA: Figure 5 Day 7, Passage 1, and Passage 2 images: JoVE Video Editor please emphasize green signal in GFP images
7.7.2. LAB MEDIA: Figure 5 Day 7, Passage 1, and Passage 2 images: JoVE Video Editor please emphasize red signal in Tomato images

Section - Conclusion

Comment [BC6]: Authors: Each Author is limited to two Conclusion statements.

8. Conclusion Interview Statements: (Said by you on camera) - All interview statements may be edited for length and clarity.

8.1. **Kristine Wadosky**: To stay oriented when harvesting the prostate tissue, be aware of the bladder and urethra positions at all times (Step: 4.8.) **[1]**.

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

8.2. **Kristine Wadosky**: After their generation, the organoids can be used in imaging applications, molecular biology analysis, and drug screens **[1]**.

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