Video Article

Identification, Histological Characterization, and Dissection of Mouse Prostate Lobes for In Vitro 3D Spheroid Culture Models

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Abstract

Genetically engineered mouse models (GEMMs) serve as effective pre-clinical models for investigating most types of human cancers, including prostate cancer (PCa). Understanding the anatomy and histology of the mouse prostate is important for the efficient use and proper characterization of such animal models. The mouse prostate has four distinct pairs of lobes, each with their own characteristics. This article demonstrates the proper method of dissection and identification of mouse prostate lobes for disease analysis. Post-dissection, the prostate cells can be further cultured *in vitro* for mechanistic understanding. Since mouse prostate primary cells tend to lose their normal characteristics when cultured *in vitro*, we outline here a method for isolating the cells and growing them as 3D spheroid cultures, which is effective for preserving the physiological characteristics of the cells. These 3D cultures can be used for analyzing cell morphology and behavior in near-physiological conditions, investigating altered levels and localizations of key proteins and pathways involved in the development and progression of a disease, and looking at responses to drug treatments.

Video Link

The video component of this article can be found at https://www.jove.com/video/58397/

Introduction

The scientific community has been attempting to elucidate the complex mechanism of human cancer development for decades. Whereas identification of potential key players and drug targets begins with patient cells and tissue studies, the translational application of such findings often requires the use of pre-clinical animal models. The use of genetically engineered mice models (GEMMs) to model human cancers has steadily risen since the establishment of the Mouse Models of Human Cancers Consortium (NCI-MMHCC), a committee which sought to describe and unify characteristics of mouse cancer models for scientists worldwide[1,2]. Mouse models fulfill the need for mechanistic studies in pre-clinical studies of most types of cancer, for understanding the development, progression, response to treatments, and acquired resistance[3].

Prostate cancer is the most commonly occurring cancer in men, affecting over 160,000 men every year[4]. Aggressive forms of the disease claim tens of thousands of lives every year. However, the mechanism of disease progression is still poorly understood. This results in a serious lack of effective treatment options for advanced and metastatic prostate cancer, as evidenced by the high mortality rate in advanced prostate cancer patients[4]. Hence, there is a growing need for pre-clinical models to study prostate cancer. However, owing to the inherent differences between the mouse and human prostate, modeling of prostate cancer in GEMMs did not gain popularity until the Bar Harbor Classification system was introduced in 2004, which outlined histopathological changes in the mouse prostate upon genetic manipulation, identification of neoplastic changes, and their relation to stages of cancer progression in humans[5]. One important characteristic of the mouse prostate that must be taken into consideration while studying any prostate GEMM model is the presence of four distinct pairs of lobes: anterior, lateral, ventral and dorsal. The lobes present significant differences in the histopathology and gene expression pattern[6]. Probasin protein expression pattern can vary between lobes in young post-puberty mice[7], which must be considered since Cre-based GEMM models are mostly designed using a probasin-based promotor called Pb-Cre4[7]. The resulting spatial and temporal differences in Cre expression often lead to differences in tumor initiation and progression timelines as well as differences in neoplastic changes between the lobes. Hence, it is important to account for such differences while studying tumor development in the prostate GEMMs, and the individual lobes may need to be evaluated separately to achieve reproducible results. The first part of this article describes the proper methods to dissect a mouse prostate, identify and separate each lobe, and recognize the histological differences between the lobes.

While the analysis of tumor growth and histopathology can provide valuable insights into the tumor development, they do not provide much information about molecular mechanisms. To study the mechanism of tumor development and progression, it is often useful to analyze the

tumor cells *in vitro*. Several methods have been suggested over the years that involve cultures of these cells, including suspension cultures, 3D cultures[8] and recently, regular 2D cultures[9]. Whereas most of these methods result in good cell survival and proliferation rates, the 3D cultures provide an environment that is closest to physiological conditions. In 3D or spheroid cultures grown in a basement membrane extracellular matrix (ECM), the fully differentiated luminal cells usually have very low survival rate; however, the basal and intermediate cells (mostly stem cells) are able to propagate and produce cell clusters called spheroids[10]. This makes it suitable for a cancer study since epithelial cancers are believed to originate from stem cells (popularly known as cancer stem cells)[11]. The second part of this protocol describes a method for culturing the mouse prostate cells in 3D cultures. The resulting spheres can be used for several types of downstream analyses, including the study of organoid morphology and behavior by live cell imaging, immunofluorescence staining for different proteins, and the study of responses to chemotherapeutic treatments.

Overall, the goal of this protocol is to outline optimal methods for using mouse models in prostate cancer by describing the anatomy and dissection techniques of the mouse prostate and the processing of the tissue for spheroid cultures and *in vitro* analysis.

Protocol

All mouse experiments described here were performed according to the guidelines outlined in the institutional IACUC-approved protocols at SUNY Upstate Medical University.

1. The Urogenital System (UGS) Dissection

Note: The schematic is presented in **Figure 1**.

1. Euthanize a 3-month-old male C57BL/6 mouse using the CO_2 inhalational euthanasia method or another approved technique.
   Note: Mice between the ages of 3 and 12 months can be used successfully for the experiment. Older mice (> 6 months) will most likely have more fat around the UGS, which will need to be cleared. Identification and separation of the lobes is often difficult in mice younger than 3 months. Other strains can be used for the experiment, as well.
2. Place the mouse on its back and secure the legs using pins so that the ventral side of the animal is exposed.
3. Spray 70% ethanol on the mouse's abdomen and wipe it clean.
   Note: Shaving the abdomen hair before dissection is not required.
4. Lift the skin from the abdomen, along with the muscle layer, with a pair of medium blunt forceps and make an inverted Y-shaped incision on the abdomen using scissors.
   1. First, make a straight incision with a pair of sharp scissors from just above the penis to the sternum (**Figure 2a**).
   2. Cut from the base of the incision towards each toe, up to the thighs (**Figure 2b**). Fold back the skin on both sides and at the bottom to view the entire abdominal area (**Figure 2c-e**).
      Note: The cut size varies depend on the mouse age and size. The size of the initial straight incision may range from 1.5 to 4 cm, depending on the size of the mouse.
5. Move over the other organs to expose the UGS. Lift and move the brown-yellow intestines (**Figure 2f**). Pick up the abdominal fat pads (the opaque white spongy tissue) with forceps and move them to the sides (**Figure 2g**).
   Note: The UGS comprises of the seminal vesicles, urethra, prostate, ductus deferens (vas deferens), and urinary bladder. It can be identified by the characteristic pair of opaque white semicircular arches (which are the seminal vesicles), with the fluid-filled bladder sac attached to the base. The translucent tissue located right under the seminal vesicles is the prostate.
6. Locate the UGS, firmly hold the urinary bladder with blunt forceps, and lift the entire UGS upwards from the mouse abdomen.
   Note: If the bladder is full, drain it first with a small syringe to provide a better grip with the forceps and reduce the risk of rupturing the bladder.
7. Continuing to pull up on the bladder, slide a pair of scissors underneath the bladder and prostate all the way to the spine, and make a cut. Cut through any remaining connections to the abdominal cavity (**Figure 2h** and **2i**). Be careful not to cut too close to the UGS to avoid accidental cutting through any prostate tissue.
   Note: While making the cut under the UGS, the scissors should be inserted all the way to the back of the mouse, so that the cut snaps the vertebral column as well. This method results in severing nearly all of the connections between the UGS and the abdominal cavity in one snip, reducing the time needed to extract the tissue from the mouse.
8. Remove the UGS and place it in 2-6 mL (enough to cover the tissue) of phosphate buffered saline (PBS) or Dulbecco's modified Eagle medium (DMEM, high glucose) in a 6 cm Petri dish (**Figure 2j** and **2k**) and move it to a dissection microscope (10X magnification).
   Note: Change the dissecting media as required in the remaining steps.

2. Dissecting the Prostate

1. Clear all the fat from both the dorsal and ventral sides with a pair of fine forceps and microdissection scissors (**Figure 3a**). Use similar surgical instruments for the rest of the dissection protocol.
   Note: The fat is often closely entwined with the UGS (can be identified by its white spongy appearance); hence, this step must be performed carefully to avoid accidentally snipping off any prostate tissue. It can take up to 10-15 min to clear all the fat.
2. Hold and pull the bladder with the forceps and snip it at the base with the scissors (**Figure 3b**).
3. Place the remaining tissue ventral side up. Hold one ductus deferens with forceps, trace it to the base with scissors, and snip it (**Figure 3c**), then repeat on the other side. Remove the ductus deferens, now leaving behind the prostate (translucent and verminous), seminal vesicles (opaque and white, semicircular), and urethra (pink-red and opaque tube) (**Figure 3d** and **3e**).
4. Insert forceps in between the inner arch of the seminal vesicles and prostate tissue anterior lobes. Pry apart the seminal vesicles and prostate and snip any connective tissue as needed (**Figure 3f**). Trace the seminal vesicles to their base at the urethra and remove them (**Figure 3g** and **3h**). Be careful not to puncture them.

5. Proceed to dissect out the individual prostate lobes.

3. Gross Anatomy of the Prostate and Individual Lobe Microdissection (Figures 3i-n, Figure 4)

1. Flip the tissue with a pair of blunt forceps so that the dorsal side faces up, showing the dorsal lobes, which will resemble a butterfly's wings.
2. Collect the dorsal lobes by holding the lobe with forceps and snipping at the base with scissors (**Figure 3j**).
3. Flip over the remaining tissue to the ventral side.
4. Collect the lateral lobes, which are small and usually wrap the urethra on the side, which are wedged between the anterior, ventral, and dorsal lobes (**Figure 3k**).
5. Next, collect the ventral lobes, which are larger than the lateral lobes and lie on the urethra ventrally (**Figure 3l**).
6. Last, harvest the anterior lobes, the largest of the four, by cutting and discarding the urethra (**Figure 3m**).
7. Process the tissue pieces according to experimental needs. Proceed to section 4 for histology, or to section 5 for the spheroid culture.

4. Lobe Identification and Morphology from Hematoxylin and Eosin-Stained Slides

1. Fix the prostate tissue and embed it in paraffin. Proceed with staining the slides with hematoxylin and eosin (H&E) to view and identify differences in prostate lobes based on histology, using the characteristics outlined by Oliveira *et al*.[12] (**Figure 5**).

5. Processing the Tissue for 3D Culture[10]

Note: This is outlined in **Figure 6**.

1. Proceed with culturing the whole prostate or individual lobes as per the experimental needs. Transfer the prostate tissue to a 10 cm dish containing 2-3 mL of DMEM. With a scalpel, mince the prostate tubules as finely and evenly as possible under the dissection microscope.
2. Continue with the remaining steps under a tissue culture hood, under sterile conditions.
3. Transfer the tissue pieces along with the DMEM to a 15 mL tube and increase the volume to 9 mL with DMEM. Add 1 mL of 10x collagenase stock solution to the DMEM-tissue mixture and vortex to mix.
   Note: Collagenase stock solution is 10 mg/mL in Roswell Park Memorial Institute (RPMI) medium, and the final concentration in the 15 mL tube is 1 mg/mL.
4. Place the tube on a gyratory shaker or tube rotator for 2 h at 37 °C, for the collagenase to degrade the extracellular matrix.
5. Centrifuge the tube at 400 x g for 5 min at room temperature.
6. Discard the supernatant and resuspend in 2 mL of warm 0.05% trypsin-EDTA to cleave the cell-cell and cell-matrix adhesions. Transfer the tube to 37 °C for 5 min.
   Note: If the tissue chunks are too big to mix well, cut the pipette tip with a razor blade to make a wider bore.
7. Break up any tissue clumps by pipetting with a P1000 pipette 8-10 times, then repeating with a P200 pipette.
8. Neutralize the trypsin with 3 mL of complete DMEM. Add 500 U of DNAase I and mix well.
9. Pass through a 5 mL syringe 5-10 times with an 18 G needle. Then pass through 5 times with a 20 G needle.
10. Repeat steps 4-8 once more if the solution still contains big tissue chunks.
11. Filter the cell suspension through a 40 µm filter placed on a 50 mL tube. Rinse the 15 mL tube with 5 mL of DMEM and pass through the same filter. Repeat the rinse.
12. Discard the filter and centrifuge the tube containing the flow-through at 400 x g for 5 min at room temperature.

6. Plating and Culturing the Cells

1. Resuspend the pellet in 0.5 mL of complete Prostate Epithelial Cell Growth Medium (PrEGM). Count the cell density using a cell counter or hemocytometer and dilute the cells to 5 x 10^5 cells/mL in complete PrEGM.
   Note: The yield from a 3-month-old mouse whole prostate can range from 3 x 10^5-10^6 cells. Hence, it is important to initially resuspend the pellet in no more than 0.5 mL of PrEGM (as mentioned above) so that the desired cell density can be achieved, even with low yield.
2. Mix the required volume of cells with the basement membrane ECM in a 2:3 volume ratio (60% basement membrane ECM and 40% cell suspension) and plate in a multi-well plate or chamber slide as per the experimental needs.
   Note: For immunostaining, plate on glass-bottom plates or chamber slides, covering the entire well or middle of the well. Plate around the rim of the well if counting the resulting organoids, or plate as several droplets all over the well if plating higher volumes. Be mindful to not spread it too thick or too thin. Plate at least 100 µL of matrix-cell mixture per 1 cm^2 of plating area; for example, in a chamber slide with a 2 cm^2-well surface area, 200 µL of matrix-cell mixture containing 5 x 10^4 cells should be plated.
3. Transfer the plate/slide to a 37 °C incubator with 5% CO_2 for 30 min for the basement membrane ECM to solidify. Then, add pre-warmed complete PrEGM to cover the well, taking care not to disturb the basement membrane ECM plug.
4. Remove half of the media and add fresh media every 2-3 days. Grow spheroids for 5-10 days.
5. Proceed with harvesting (step 7), immunostaining (step 8), and/or imaging for downstream applications.

7. Harvesting the Spheres

1. To harvest spheres, aspirate the media carefully without disturbing the basement membrane ECM gel plug and add 1 mL of 1 mg/mL dispase solution in PrEGM per 100 µL of basement membrane ECM.
   Note: It is easier to harvest spheroids if they are plated in the middle of the well or the rim (instead of covering the whole well), to ensure that an adequate amount of the dispase solution can be added to the well.

2. Scrape the bottom of the plate with a cell scraper to lift off the basement membrane ECM gel into the dispase-PrEGM solution.
3. Pipette the entire solution up and down once with a wide bore 1000 µL pipette tip or 5 mL pipette to break up the gel plug into smaller pieces.
   Note: Cut the tip of a 1000 µL pipette tip with a razor blade to make a wide bore tip.
4. Incubate in a 37 °C incubator with 5% CO_2 for 1-2 h until the basement membrane ECM has completely dissolved.
   Note: This can be ensured by visual inspection of the well bottom while tilting the plate to confirm that no more chunks of gel remain.
5. Collect the cell suspension in a 15 mL conical tube.
6. Centrifuge spheres at 250 x g for 5 min at room temperature and continue with downstream applications.

8. Immunostaining of the Spheres[13]

Note: After spheroids have grown for the desired amount of time, the basement membrane ECM can be dissolved and spheres can be stained as described in Colicino *et al.*[13].

1. Briefly, rinse the cultures with PBS and add 4% paraformaldehyde (PFA) directly to the basement membrane ECM gel plug. Incubate for 30-90 min at room temperature with slow shaking, until the basement membrane ECM is fully dissolved, replacing fresh PFA every 30 min.
   Note: The PFA will dissolve the gel plug and fix the spheroids during this process.
2. Next, wash with PBS 3 times and add 50 mM ammonium chloride for 10 min to quench any autofluorescence from the PFA. Repeat for 3 washes with PBS.
3. Permeabilize with 0.1% non-ionic detergent for 5 min and block with PBSAT (PBS, 1% BSA, 0.5% Triton X-100) for 30 min.
4. Incubate with a primary antibody in PBSAT overnight at 4 °C, followed by 3 washes with PBSAT and a secondary antibody in PBSAT for 2 h at room temperature.
5. Repeat the washes with PBSAT and stain with DAPI according to the manufacturer's protocol, if desired. Wash 3 or more times with PBS.
6. Add PBS with 200 mM 1,4-diazabicyclo[2.2.2]octane (DABCO) antifade reagent and proceed to imaging.

Representative Results

The mouse prostate lobes can be identified and dissected using their locations with respect to the seminal vesicles and urethra. The mouse prostate is composed of 4 pairs of lobes located dorsally and ventrally to the seminal vesicles and urethra. **Figure 4a** and **4b** (top) show the dorsal and ventral views of the intact prostate, along with the seminal vesicles and urethra. The bottom panels (**Figure 4c** and **4d**) show the different lobes outlined for identification. Lobes can be separated from mice as young as 3 months.

The different prostate lobes differ significantly in histology (**Figure 5**). All mouse prostate lobes are composed of multiple gland profiles composed of a lumen surrounded by secretory epithelial cells; however, the shape of the lobes, organization of the cells, and nature of the secretion vary from lobe to lobe. The identifying characteristics from H&E-stained mouse prostates have been efficiently outlined by Oliveira *et al.*[12], briefly outlined here. Anterior lobes have moderate-to-large acini with frequent infoldings and strongly eosinophilic secretion. Dorsal lobes appear like anterior with eosinophilic secretion but have much smaller acini and less infoldings. Lateral lobes have small to large acini, with characteristic flat luminal borders and eosinophilic secretion. Ventral lobes are structurally like lateral lobes, also with flat luminal borders, but have a unique pale non-eosinophilic luminal secretion.

Mouse prostate cells can be cultured as spheroids in the basement membrane ECM and exhibit epithelial-like characteristics. This is outlined as a schematic in **Figure 6**. Cells were isolated from the mouse prostate as described above and cultured in the basement membrane ECM. The cells start growing into organoids in as early as 4 days of culture. **Figure 7** (top) shows representative fields from 8-day-old cultures showing the organoids in the basement membrane ECM. Under the above-mentioned culture conditions, most of the cells grow into solid spheres, with a fraction having a partial or full lumen inside. These organoids usually have an even spherical morphology. Organoids were harvested and immunostained as described in Colicino *et al.*[13]. **Figure 7** (bottom panel) shows an organoid without a lumen (left) and with a lumen (right), stained with phalloidin (F-actin marker, green) and DAPI (nucleus, blue). β-catenin is a cell-cell adhesion marker strongly expressed at cell-cell interfaces in epithelial cells. **Figure 8** demonstrates strong β-catenin staining (green) at the cell-cell junctions in spheroids that co-localize with F-actin (red). The spheroids also express cytokeratin 5, cytokeratin 8, p63, and androgen receptor proteins, which provide evidence that the spheroids are indeed derived from cells originating in the prostate[10].

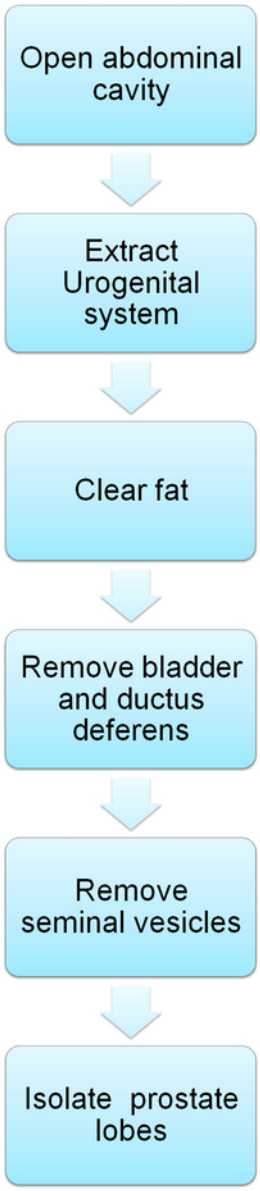

Figure 1: Schematic flowchart demonstrating dissection of the mouse urogenital system and isolation of the prostate. Flow chart for the protocol steps described in sections 1-2. Please click here to view a larger version of this figure.

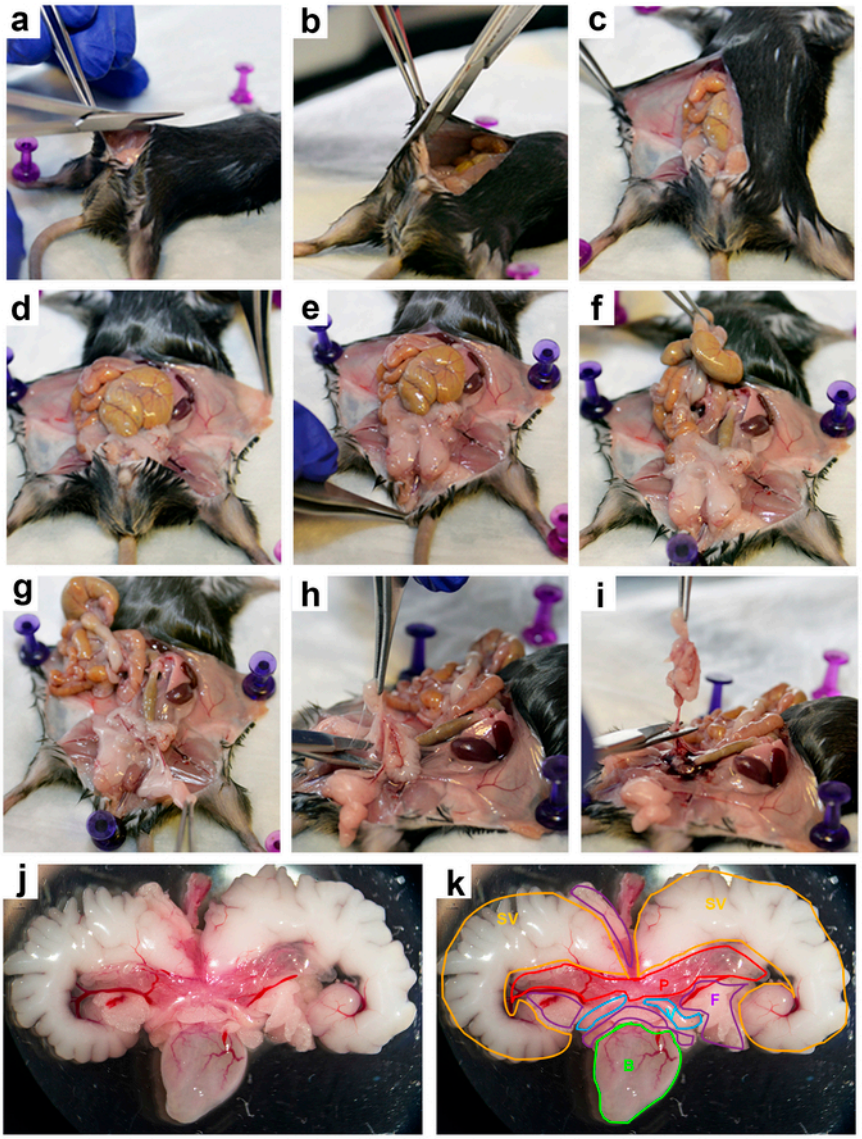

Figure 2: Dissection of the mouse UGS. Step-by-step images from the dissection of the UGS from a 3-month-old mouse: (a and b) making the incisions, (c-e) pulling back the skin to expose the abdominal area, (f) shifting the intestines, (g) moving the abdominal fat pads, (h and i) extracting the UGS from the abdominal cavity, (j) the extracted UGS, (k) the extracted UGS with the different organs and tissues marked as follows: SV (yellow) = seminal vesicles; P (red) = prostate; F (purple) = fat; V (light blue) = vas deferens; and B (green) = bladder. Please click here to view a larger version of this figure.

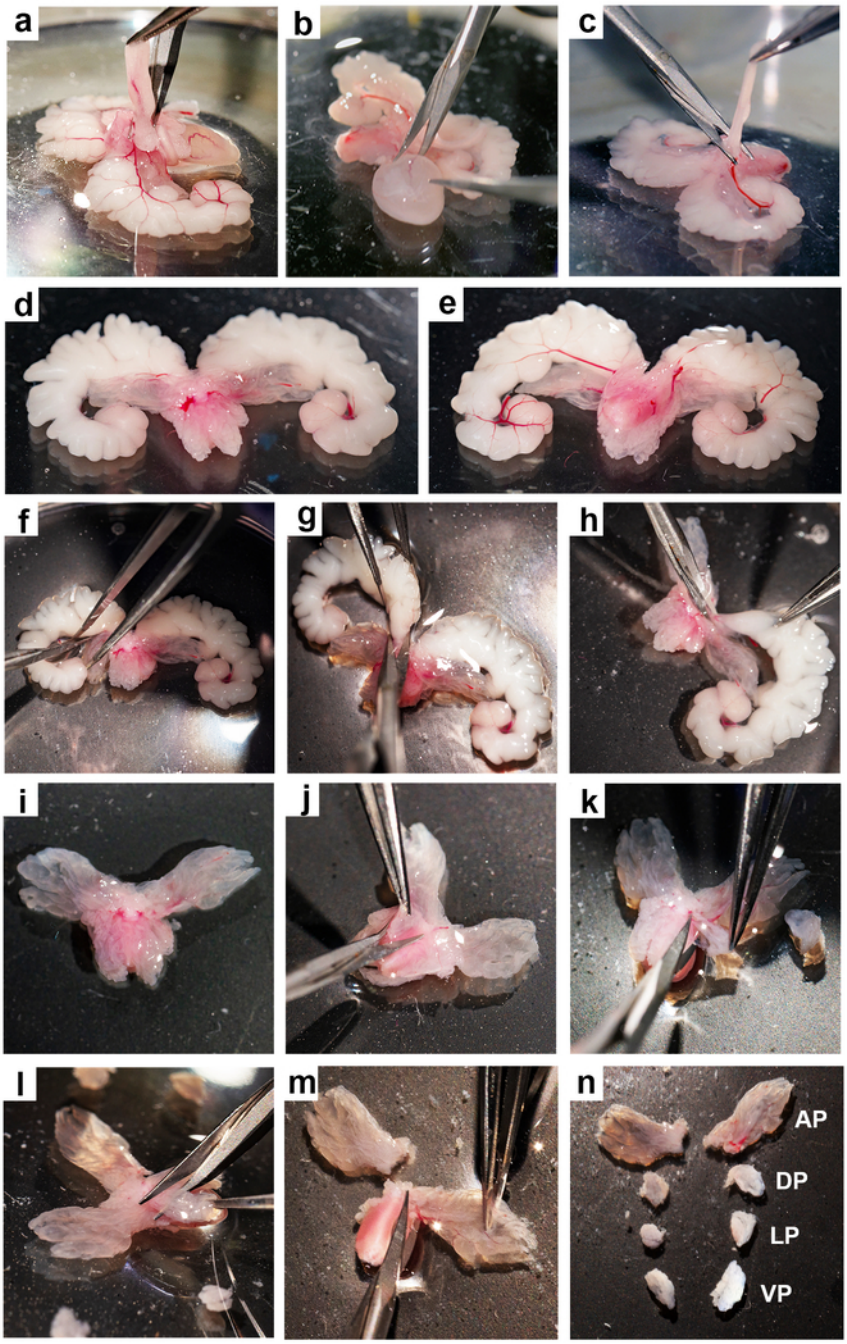

Figure 3: Dissection of the mouse prostate. Step-by-step images for the dissection of the mouse prostate from the UGS: (a) removing fat, (b) removing the urinary bladder, (c) removing the vas deferens, (d) ventral view of the prostate with the urethra, (e) dorsal view of the prostate with the urethra, (f-h) removing the seminal vesicles, (i) ventral view of the prostate, (j) dissecting a dorsal lobe, (k) dissecting a lateral lobe, (l) dissecting a ventral lobe, (m) dissecting an anterior lobe, and (n) the dissected prostate lobes: anterior = AP, dorsal = DP, lateral = LP and ventral =VP. Please click here to view a larger version of this figure.

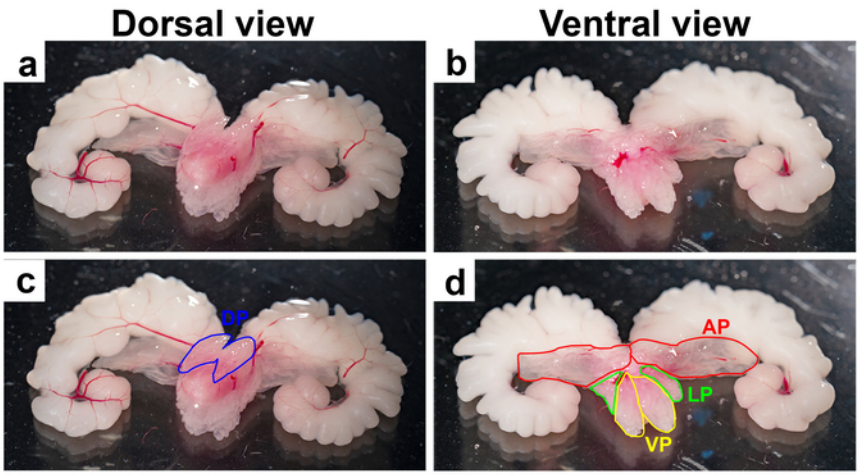

Figure 4: Anatomy of the mouse prostate lobes. Representative images from 3-month-old mouse UGS after removal of the fat, bladder, and vas deferens. Images show prostate lobes with seminal vesicles and urethra, with dorsal (a and c) and ventral (b and d) views. Top panels (a and b) show untouched images, while bottom panels (c and d) show the lobes outlined in blue (dorsal), red (anterior), yellow (ventral), and green (lateral). Please click here to view a larger version of this figure.

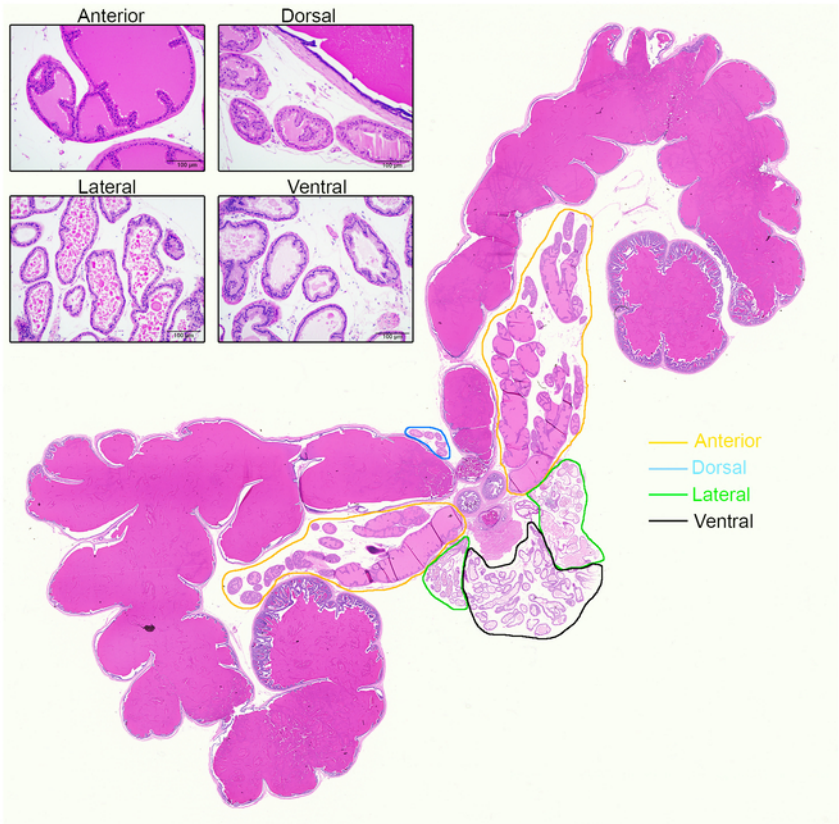

Figure 5: The different prostate lobes vary significantly in histology. Here is an H&E-stained whole prostate image showing the seminal vesicles, urethra, and the four prostate lobes. Lobes are outlined in orange (anterior), blue (dorsal), green (lateral), and black (ventral). Inset: representative images from each lobe, taken under a 10x objective. Scale bar represents 100 µm. Please click here to view a larger version of this figure.

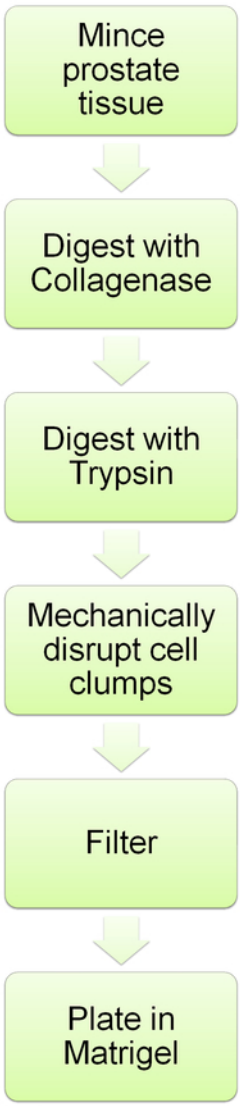

Figure 6: Schematic flowchart demonstrating digestion of the prostates and subsequent spheroid cultures. Flow chart for the protocol steps described in sections 5 and 6. Please click here to view a larger version of this figure.

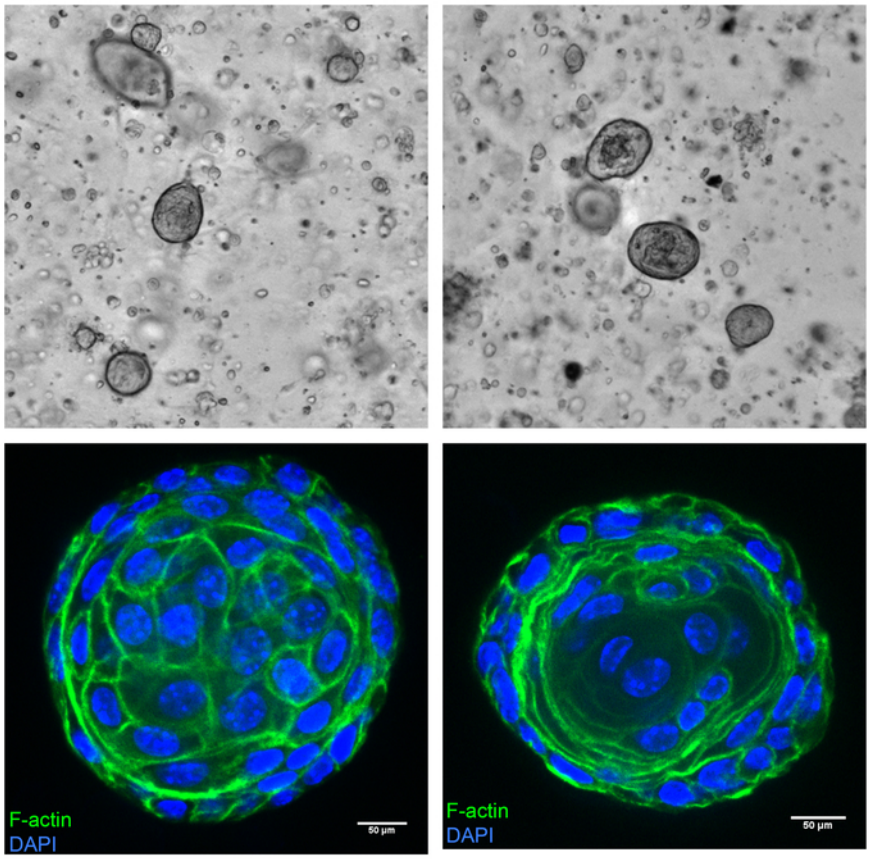

Figure 7: The prostate cells grow into even spherical spheroids with or without a lumen. Prostate tissue harvested from a wild-type 3-month-old mouse was cultured as per the protocol. Cultures were imaged on a plate imager under a 4X objective, at 8 days post-culture, showing the formation of spheroids (top). The spheroids were harvested and stained as described in the protocol with a fluorescent dye-conjugated phalloidin for F-actin (green) and DAPI for nuclei (blue), then imaged on a spinning disk confocal microscope under a 40X water objective (bottom). The spheroid on the right shows evidence of a lumen. Scale bars represent 50 µm. Please click here to view a larger version of this figure.

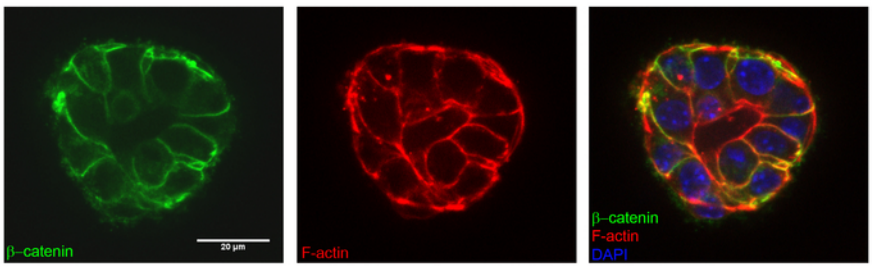

Figure 8: The mouse prostate organoids show junctional localization of β-catenin. Spheroids harvested from 6-day-old 3D cultures were stained, using a primary antibody against β-catenin and a fluorescent dye-conjugated secondary antibody, and imaged on a spinning disk confocal microscope under a 40X water objective. Representative images show immunostaining for β-catenin (green, left), F-actin (red, middle), and DAPI (blue). The right panel shows all 3 channels merged. Scale bar represents 20 µm. Please click here to view a larger version of this figure.

Discussion

This paper outlines the methods for dissection of the mouse prostate and identification of individual lobes. Also described is the protocol for culturing mouse prostate cells in a 3D culture for *in vitro* analysis.

A critical step in the dissection protocol is (1) harvesting the entire UGS out of the mouse and separating the individual organs under a dissection microscope. The prostate tissue is very small and surrounded by the rest of the UGS; thus, it is practically impossible to harvest the organ directly from the body cavity while procuring all four pairs of lobes intact. Hence, it is important to harvest the entire UGS from the mouse and then proceed to extract the prostate. It is also critical in this protocol to (2) perform the steps in a timely fashion but remain meticulous. Time is of the essence during dissection in order to minimize tissue degradation. The specific technique described here for extracting the UGS from the

mouse is faster compared to alternatives and is highly recommended. The entire dissection and lobe isolation procedure usually takes 35-40 min, which can be lowered to 25 min with more practice. The most challenging part of the dissection is cleaning the fat surrounding the UGS, which takes up a large fraction of total dissection time. The fat must be meticulously removed to make sure none of it remains with the prostate, but it is important to be extremely careful not to accidentally lose any prostate tissue in the process. Another critical step is (3) to not remove the urethra before separation of the prostate lobes. The urethra essentially forms the base on which all four pairs of prostate lobes are arranged. It is easiest to identify the lobes if they are still attached to the urethra, based on their spatial distribution with respect to the urethra.

A critical step in the spheroid culture protocol is (1) mincing the tissue well. Mincing the prostate tissue with a scalpel is a tedious step, especially since the prostate tissue has a sticky nature. However, mincing the tissue as small as possible is essential to obtain maximum yield. Another essential step is (2) pipetting after trypsinization and passing through syringes of the sizes specified post-neutralization. Both these steps must be performed correctly and repeated, if required, to achieve optimum cell yield. Finally, it is important (3) not to plate the basement membrane ECM too thick or too thin. If the basement membrane ECM is plated too thin (*i.e.*, less than 100 µL/cm^2), cells will not grow into proper organoids in the middle of the well, where the gel will be the thinnest. Plating it too thick (*i.e.*, more than 250 µL/cm^2) will result in the basement membrane ECM not solidifying properly and sliding off during media changes. Plating in the middle of the well will help to achieve the most even gel thickness.

The protocol described can be slightly modified according to experimental needs. The technique for the extraction of the UGS from the abdominal cavity requires a firm grip on the urinary bladder with forceps. In case the bladder is too full, it is important to drain the bladder with a 1 mL syringe and thin (26G or similar) needle, to ensure a proper grip before starting to extract the tissue. The tissue should be kept moist with PBS or DMEM throughout the dissection process. Using DMEM is preferred if the tissue is going to be used for spheroid cultures. The harvested cell suspension can be sorted by flow cytometry to isolate sub-populations of cells before plating, if desired, as described by Lukacs *et al.*[10].

Histological characteristics of the different prostate lobes have been outlined in this protocol, and one should be able to identify the lobes in healthy prostate tissue by following these guidelines. However, there are certain limitations of the process. In cases of hyperplasia or aggressive tumors, the epithelial morphology is disrupted, which can make it significantly harder to distinguish between lobes based on the H&E staining. In such instances, separation of lobes (as outlined in this protocol) before embedding and staining is necessary if lobe-specific information is needed. Even in healthy tissue, it is often necessary to analyze lobes separately since they present wide variability in anatomy and histology and have differential expression signatures[6]. However, the translational relevance of separating lobes in mouse prostate may be debated, since the human prostate is not divided into lobes. In spite of this, the mouse remains the best model to study PCa *in vivo*, and several mouse models have been developed[14,15]. The 3D culture method especially is a key technique that can be used to investigate mechanisms of the disease. However, one caveat of the spheroid culture method arises from the differential expression pattern of probasin, which is the commonly used promoter for Cre recombinase in prostate cancer mouse models. The probasin promoter is predominantly activated in luminal and intermediate cells but not in basal cells[7]. The culture conditions described here promote spheroid formation predominantly from basal and intermediate cells, but not from luminal cells[10]. Hence, the resulting cultures actually produce a mixture of Cre-expressing and non-Cre-expressing spheroids (*i.e.*, a mixture of control and knock-out spheroids). As a result, during analysis, it is important to immunostain for the protein of interest to identify knock-out organoids and draw conclusions on organoid behavior based on gene knock-outs.

The significance of these methods lies in the multi-faceted applications of using GEMMs in prostate cancer studies. The dissection method described has detailed steps which will enable researchers to extract prostates faster and more effectively. The sequence of the dissection process is designed to ensure that individual lobes can be identified and extracted, even by those with minimal experience in prostate dissections. The culture method outlined can be used to further analyze GEMM models of prostate cancer. The culture conditions should allow for growth and survival of both control and tumor cells. In our experience, we have successfully used GEMM prostates that exhibit mPIN[16]. Mouse prostates with higher grade tumors have also been used for spheroid culture analysis[17]. The control mouse spheroids exhibit an even spherical morphology; however, spheroids derived from prostate tumor cells may demonstrate differences in morphology and behavior due to their neoplastic potential. Hence, studying organoid behavior *in vitro* will provide an extended look into the characteristics of these cells, and possibly observations of spheroid behavior in real-time through live-cell imaging.

In conclusion, the prostate dissection and culture methods described in this protocol can be incorporated into various types of studies involving GEMMs to continue providing information on prostate cancer in all downstream applications.

Disclosures

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