

Video Article

An Orthotopic Model of Murine Bladder Cancer

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Abstract

In this straightforward procedure, bladder tumors are established in female C57 mice through the use of catheterization, local cauterization, and subsequent cell adhesion. After their bladders are transurethrally catheterized and drained, animals are again catheterized to permit insertion of a platinum wire into bladders without damaging the urethra or bladder. The catheters are made of Teflon to serve as an insulator for the wire, which will conduct electrical current into the bladder to create a burn injury. An electrocautery unit is used to deliver 2.5W to the exposed end of the wire, burning away extracellular layers and providing attachment sites for carcinoma cells that are delivered in suspension to the bladder through a subsequent catheterization. Cells remain in the bladder for 90 minutes, after which the catheters are removed and the bladders allowed to drain naturally. The development of tumor is monitored via ultrasound. Specific attention is paid to the catheterization technique in the accompanying video.

Video Link

The video component of this article can be found at <https://www.jove.com/video/2535/>

Protocol

1. Animals

1. Female C57BL/6J mice (*Mus musculus*) are verified free of contagious ectoparasites, helminth endoparasites, and antibodies to 17 murine viruses by the vendor prior to shipping. Mice are housed in the AAALAC-accredited animal facility of Tulane University in accordance with *Guide for the Care and Use of Laboratory Animals*.¹
2. Specifically, mice are group housed (4-5 mice/cage) in individually ventilated polycarbonate cages with microisolation filter tops on hardwood bedding. They are provided ad libitum pelleted rodent food and tap water.
3. Mice are allowed 7 days to acclimate before the start of the study.

2. Cells

1. The murine transitional cell carcinoma cell line MB49 is used to produce the bladder tumors.
2. Cells are cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 units/ml streptomycin.
3. Cultures are maintained at 37°C and 5% CO₂ with media changes every two to three days.

3. Animal Orthotopic Tumor Model

The instillation of the bladder tumor model described here is a modification of the procedure described by Gunther et al.²

1. Anesthesia is induced with 2% isoflurane (MWI/VetOne, Meridian, ID, Cat# 501017) in oxygen and maintained at 0.75-1.25%. (Multiple animals can be treated concurrently, which would necessitate an increase in isoflurane levels.) To prevent corneal drying, eyes are lubricated with an artificial tears ophthalmic ointment. Body heat is maintained with a heated pad under a paper drape.
2. The fur of the lower back is clipped to promote contact with the metal grounding plate, and the shaved area is coated with electroconduction gel.
3. Each mouse is then individually placed in dorsal recumbency onto a metal grounding plate, and the bladder is catheterized transurethrally using a lubricated 24-gauge, 3/4 inch, over-the-needle IV catheter with the stylet removed.
4. The urinary bladder is drained of urine, and a platinum wire is inserted through the catheter into the urinary bladder leaving exposed wire on each end of the catheter - approximately 2 mm on the luminal end of the catheter. The wire is made of platinum because of the resistance of the material to oxidation.

5. A 2.5 W current is then applied to the bladder for approximately 0.5 second by touching the exterior portion of the wire with an electrocautery unit set to coagulation.
6. The wire is then moved to a second location in the bladder and another 2.5 W charge applied.
7. The wire is removed, and 100 μ l of MB49 cells at a concentration of 1×10^6 cells/ml is instilled through the catheter into the bladder via a 1ml syringe.
8. The syringes are left attached to the catheters to prevent efflux of the cell suspensions, and the catheterized mice are maintained under anesthesia for 90 minutes to allow the cells to attach to the burn sites.
9. 100-200 μ l of sterile normal saline is administered by IP injection to each mouse prior to recovery.
10. At the end of the incubation period the catheters are removed and the animals are allowed to recover on the heated pad until righting reflexes return, at which point the mice are returned to their home cages.

4. Ultrasound

1. Tumors are allowed to grow for 3-5 days before the mice undergo ultrasound examination of the urinary bladder to monitor tumor growth.
2. For the initial ultrasound examinations, animals are anesthetized as described above. Mice are placed in dorsal recumbancy, and abdominal skin is prepared by clipping the fur.
3. A 24 gauge IV catheter is inserted transurethraly into the urinary bladder. At this point, the bladder can be drained and the urine collected for further analysis.
4. 100 μ l of sterile normal saline is instilled into the bladder through the catheter to improve bladder visibility on ultrasound.
5. Ultrasound conduction gel is applied to the abdomen, and ultrasonography is performed to assess the bladder wall and lumen.

5. Representative Results:

Tumor tissue will begin to appear 3-5 days after the innoculation. In our experience, in a group of 12 mice, 50% or more routinely have a detectable tissue mass within the bladder (0.5 - 1.0 μ l, using $V = \pi/6 \cdot L \cdot W \cdot H$) by day five. When using MB 49 cells, tumors will continue to grow and euthanasia will be required by day 24 if the mice go untreated. Euthanasia is recommended when mice become lethargic or cachectic, display ruffled fur, hunching, and non-responsive behavior, or when they lose 20% of their initial body weight.

The tumors grow quickly and will have necrotic centers after they reach a mass of 200 mg or more,³ ostensibly because the cells in the developing tumor proliferate faster than what can be serviced by the angiogenic blood supply. One can also indirectly monitor tumor progression via the level of hematuria, which is presumably related to rapid angiogenesis within developing tumors. It was consistently noted that the urine of untreated tumor-bearing mice contained blood by day 14. The concentration of red blood cells appeared to increase over time in these mice, and the volumes of urine collected diminished. Tumors in excess of 50 mg can also be palpated with relative ease, typically by 14 days post innoculation.

Discussion

This minimally invasive method of establishing orthotopic bladder tumors in female mice can also be applied for cell types other than MB49, although differing results should be anticipated. When less-aggressive tumor cells are used, increasing the wattage slightly (using 0.5 W increments to establish the new optimum) will yield an area more amenable to tumor cell attachment, and longer bladder incubation times for the instilled cells will yield a greater amount of cell attachment at the burn sites (unpublished data). Although some protocols recommend delivering 10 W via electrocautery,² this amount of power should be considered an upper limit, and perhaps be avoided altogether. Increasing the concentration of cells delivered can also increase the chances of success when other cell types are used.

Disclosures

Experiments on animals were performed in accordance with the guidelines and regulations set forth by the Institutional Care and Use Committee of Tulane University.

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