Mayo Clinic Brain Tumor Patient-Derived Xenograft National Resource

Intracranial Tumor Implantation

Large numbers of animals can be implanted with tumor in the flank or intracranially in a single session. Several different methods can be used for generating a uniform population of cells for implantation, including direct isolation of a single cell suspension from flank tumors or short-term explant cultures of cells.

While direct isolation of a single cell suspension eliminates the use of cell culture and may have some theoretical advantages, the PDX National Resource at Mayo Clinic has used short-term explant cultures for establishing our large-scale tumor studies.

Short-term explant cultures can provide a relatively pure population of tumor cells with reproducible intracranial growth characteristics. Moreover, when implanting a large number of mice, this technique allows cells to be trypsinized throughout the day, so that isolated tumor cells are not kept on ice for longer than three to four hours. This method ensures optimal viability of cells throughout the implantation procedure.

While the methods described for direct flank-to-flank tumor injection for serial passaging can be scaled up to inject large numbers of animals with flank tumors, we have found that injection of flank tumors using short-term cultured cells provides more reproducible and uniform tumor growth than does direct tumor passaging (unpublished data), which can be important for therapy evaluation experiments.

Here are the techniques for both flank and intracranial tumor implantation from short-term explant cultures.

Materials:

- Short-term explant cultured tumor cells
- Trypsin-EDTA (Corning #25-052-CI; 0.05% trypsin/0.53 mM EDTA in HBSS)
- Complete DMEM (10% FBS and 1% P/S)
    - DMEM (Corning #10-013-CV)
    - Penicillin/Streptomycin (Corning #30001CI; 5000 I.U./mL Pen, 5000 ug/mL strep (P/S))
    - Fetal Bovine Serum (FBS) Premium (Atlanta Biologicals #S11150)
- Centrifuge
- Sterile PBS
- 1.5-mL sterile microcentrifuge tubes
- Wet ice
- Ketamine (100 mg/ml)
- Xylazine (20 mg/ml)
- Children's liquid ibuprofen
- 0.5-cc tuberculin syringes

- Dremel drill with a #7 or #8 bit
- Betadine
- Scalpels
- Alcohol
- 10-µL Hamilton syringe with a 26-G needle
- Spore-Klenz or similar disinfectant
- Stereotactic frame (ASI Instruments) with a neonatal rat adaptor (Stoelting)
- 50 mL sterile conical (Falcon)
- 4-0 vicryl with rb-1 needle (Ethicon J30 4H)
- Artificial tears (Petrolatum opthalmic ointment, Puralub Vet Ointment, Dechra)
- Triple antibiotic (Bacitracin, Neomycin, Polymyxin B sulfate, G&W Laboratories)
- Heating pad (optional)
- Radiofrequency identification (RFID) chips and reader (optional; Datamars Companion Animal ID; www.datamars.com).

Preparation of cells:

1. Prepare cells for injection as described for flank tumor injection but resuspend the cells in sterile PBS at 100,000 cells/µL in a 1.5-mL sterile microcentrifuge tube. For most cell lines, a total of 300,000 cells in 3ul are injected.
    • Some cell lines can be difficult to draw into the syringe, so test this prior to anesthetizing the animals. If the cells are difficult to draw up, discard the cells and trypsinize a back-up flask(s) of cells.
2. Place the tube containing the cell suspension on wet ice.
    • Cells are viable for up to four hours when kept on wet ice.

Anesthesia and skull preparation for injection:

3. Provide children's ibuprofen in the animal drinking water (final concentration 0.01X) starting 24 hours prior to the procedure, and maintain the ibuprofen in the water for at least 48 hours after the procedure.
    • Monitor the water for signs of bacterial or fungal growth associated with the flavoring of the ibuprofen and change water if necessary.
4. Prepare the anesthetic mixture by adding 2 mL ketamine and 1 mL of xylazine to 17 mL of normal saline. For a 20-g mouse, use approximately 200 µL of the ketamine/xylazine mixture injected intraperitoneal (IP) with a 0.5-cc syringe for a dose of 100 mg/kg ketamine and 10 mg/kg xylazine.
    • This dose usually provides 30 minutes of anesthesia. The amount of anesthetic will vary based on the strain and size of the animals being used. For example, SCID mice require less anesthetic for effective anesthesia. Adequate anesthesia is assessed by toe pinch of the hind leg. If the mouse withdraws from this pinch, then either wait longer for the anesthetic to take effect or consider providing an additional dose of anesthetic.
5. Use a disinfectant regularly throughout the injection process (e.g. Spore-Klenz) to ensure that your hands remain as sterile as possible to reduce the risk of infection in the mice.

6. Swab the head with Betadine and lubricate the eyes with artificial tears. Make a 1-cm midline incision extending from just behind the eyes to level of the ears using a sterile scalpel while applying pressure to the skin so that the skin separates as you are making the incision.
7. Using your fingers, push apart the skin to expose the skull. From the bregma, identify a point 1 mm anterior and 2 mm lateral, and drill through the skull using a #7 or #8 bit in a Dremel drill.
    - The bregma is the junction of the sagittal and coronal sutures.
    - There is a tactile sensation of a slight popping when the bit penetrates the skull. Keep a firm grip on the drill and the animal to prevent the drill bit from skipping across the skull and to prevent the mouse from moving as the drill bit establishes purchase on the skull.
    - A Dremel drill can be purchased from any local hardware store.

Stereotactic injection:

8. Clean the Hamilton syringe/needle assembly thoroughly by drawing up alcohol into the syringe and dipping the plunger into the alcohol. Rinse the syringe and needle by drawing PBS into the syringe multiple times. Sterilize the injection jig by wiping it down with Spore-Klenz and drape it with a sterile towel.
9. Using a neonatal rat adaptor in association with the stereotactic device, place the mouse in the jig by their front teeth.
    - Using the ear pins is not necessary.
    - Placing the mouse on the jig prior to loading the needle will help to minimize the risk of a needle stick.
10. Mix the cells by flicking the tube with your finger several times and draw up 3 μL of cells in a 10-μL Hamilton syringe with a 26-G needle. Make sure that you visualize the cells being drawn up into the syringe. There is usually a small air bubble that is noticeable in the syringe, and it is a good indicator that the cells are loaded in the syringe.
    - Confirming that cells have been pulled up into the syringe is critical, since failure to inject the appropriate number of cells will have an obvious impact on the formation of intracranial tumors and can lead to significant variability in the time to reach a moribund state.
11. Insert the needle and syringe into the holder and place the needle just within the drill hole. Using the stereotactic controls, drive the needle 3 mm into the brain (for large mice) or 2 mm (for particularly small mice).
12. Inject 1 μL of cells per min over 3 minutes. This can be done either manually or with a syringe pump. Allow the needle to remain in the brain for an additional minute (total injection time is 4 min). There is no need to use cement or other material to seal the drill hole in the skull.
13. Remove and clean the needle and syringe with alcohol followed by PBS.
14. Remove the mouse from the jig and suture the wound with 4-0 vicryl with rb-1 needle (Ethicon J304H).
    - Use two to three sutures depending on the size of the wound for the head.
    - If applicable, use one suture for closing the wound from inserting the RFID chips (see step 15).
    - Apply triple antibiotic liberally to the incision and stitches to prevent infection.

15. Insert the RFID chips (if appropriate) while the mouse is under anesthetic. This can be done either before or after stereotactic injection. The RFID chip typically comes preloaded in 13-G trocar. Insert the trocar under the skin in the subcutaneous space and depress the plunger to push the chip out under the skin.
    - Turn the chip approximately perpendicular to the surgical wound to ensure that the chip does not get extruded from the injection site.
16. Place a paper towel in a clean cage on the bedding chips on the opposite side from the water bottle.
    - Hypothermia can be a major problem for mice as they recover from anesthesia. Therefore, lay 5 mice per cage next to and on top of each other to ensure adequate body temperature is maintained. Alternatively, place a portion of the cage over a heating pad. If you use this approach, make sure the heating pad is not too hot otherwise the mice will die from overheating.
    - Make sure the animals are not getting water dripped on them as this also can lead to hypothermia.
17. Observe the animals to make sure that they wake up from the anesthetic. Once mice have fully recovered from anesthetic (walking around and normally active), their cages can be moved to the housing unit.
18. Mice with intracranial tumors must be observed daily, as neurologic decline can occur rapidly, at which point mice should be euthanized. Typical signs of neurologic decline include a hunched posture with an arched back, circling, walking on their tip-toes, balance issues, eyes that are not opened, weight loss, hyperactivity and seizures.