Mayo Clinic Brain Tumor Patient-Derived Xenograft National Resource

Serial Passage of Flank Tumor Xenografts

Serial passage of tumors in the flank of nude mice provides a ready supply of tumor cells for use in both in vitro and in vivo studies. While tumor propagation via prolonged cell culture promotes loss of EGFR amplification and gain of MGMT promoter methylation, these and other genetic features are stably maintained even with multiple serial tumor passage in the xenograft model (Carlson, 2009, Giannini, 2005).

In this section, methods are described for propagation of flank tumors through serial passaging in mice. While this method is quite reliable for serial passage, approximately 1% of the time a tumor will not grow after passage. Thus, xenografts are typically passaged into three recipient mice for the first passage, and then a tumor line is subsequently maintained in three mice at any one time.

Typical growth time of an established line is one to two months, depending on the tumor line. The passage of tumors can be staggered to provide a near continuous supply of tumors for use in laboratory studies.

With each tumor passage, tissue samples are routinely archived in liquid nitrogen and/or paraffin, and, at least in early generations, cryopreserved as well to facilitate restoration of early passage tumors as discussed below.

Materials:

- Mouse bearing tumor CO_2 source
- Forceps
- Betadine: 10% povidone-iodine (Carefusion #29906-016)
- Sterile scalpels
- Sterile culture plates
- Tissue Path Disposable Base Molds (Fisher Scientific #22-363-554)
- 20 ml specimen containers (Fisher Healthcare #22-032061)
- Tissue Tek OCT Media (Sakura Finetek#4583; referred to as OCT in this protocol)
- 10% Buffered Formalin (Fisher Scientific)
- 1.8 mL Cryogenic tube (Thermo-Scientific #375418)
- 1 cc syringe
- Hypodermic 16-gauge needles
- Corning™ Matrigel™ GFR Membrane Matrix (Corning #354230; subsequently referred to as Matrigel through the remainder of the protocol)
- Isoflurane (Novaplus: Piramal Enterprises Ltd NDC# 66794-019-10)
- 1.5 mL micro-centrifuge tube (Eppindorf #022363204)
- Dry ice

- Wet ice

Tumor resection and processing:

The description below is for passaging a single tumor measuring 1 to 1.5 cm in greatest dimension into one to three recipient mice.

1) Euthanize the tumor-bearing mouse by CO_2.
2) Swab the skin around the tumor with Betadine to minimize the risk of bacterial or fungal infection. As described above, sterile technique is used but sterile draping is not particularly required.
3) Dissect out the tumor using a sterile scalpel and separate it from the skin.
4) Place tumor into a sterile 100 mm culture dish.
5) Tissue is routinely archived for future studies from each tumor generation using the following techniques, as outlined in steps 6 through 9 below. This archived tissue can be very useful for molecular and histopathological studies. If no tissue is to be archived, proceed to step 10 below.
6) Using sterile scalpels, cut a cross-section of the tumor (~1-2 mm thick slice) for OCT embedding. Add a small amount of OCT to the base mold, and place the tissue section on the OCT bed. Using forceps, press the tissue into the OCT, such that the tumor is lying flat in the base mold, and then encase the rest of the tissue with OCT to ensure that the tissue is not degraded over time in the minus 80^{o}C freezer. Place OCT sample between two blocks of dry ice for freezing and then transfer to a minus 80^{o}C freezer.
7) Cut another cross-section of the tumor (~1-2 mm thick) and place it in a specimen jar filled with formalin. Optimal fixation can be achieved with overnight fixation prior to paraffin embedding.
    - Fixation in formalin for more than two weeks prior to paraffin embedding is suboptimal, since the tissue becomes brittle, sections poorly, and can affect antigen recognition for IHC staining.
8) Place another portion of tumor in a labeled 1.8 mL cryogenic tube for fresh frozen tissue. Immediately place this sample on dry ice or into liquid nitrogen, and subsequently transfer the specimen into a minus 80^{o}C freezer.
9) Additional tumor can be processed for cryopreservation as described in a separate protocol.
10) After the appropriate tissues are archived, thaw Matrigel on wet ice and mince the remaining tissue into small pieces using scalpels in a sterile 100 mm culture dish.
11) Use a 1 cc syringe to break up the tumor into even smaller pieces by repeatedly drawing up and expelling the tissue. Finally, draw up 100 microL of tumor into the syringe, chill on ice, and then draw up an equal volume of Matrigel. Mix the tumor and Matrigel in the syringe by moving the plunger back and forth. Add a 16-gauge needle to the end of the syringe, dispel the air from the syringe (total volume should be approximately 200 microL of a 1:1 solution), recap the needle and place on wet ice until ready to inject.
    - Using a syringe without a needle to break up the tissue reduces the risk of tissue shearing.
    - If there is limited tumor available or it is difficult to draw up in the syringe, the tumor can be moved, after dicing, to a 1.5 mL micro-centrifuge tube, and the syringe can be used as a pseudo pestle to further disaggregate the tumor.

Anesthetize the mice:

12) Mice are anesthetized with isoflurane in a plastic desiccator (Bell jar). Place the desiccator into an externally vented fume hood. If a hood is not available, the biosafety department should test that laboratory personnel are not being exposed to excessive isoflurane fumes using this method.
13) Place a paper towel in the bottom of the desiccator and add 1-2 mL of anesthetic to the towel.
    - Add additional anesthetic as needed to maintain the required effect.
14) Place an individual mouse in the desiccator.
15) Once the mouse is unconscious and not moving, remove it from the desiccator and mark the ear using an ear punch or other method of animal identification. Because the procedure is quite quick, we typically do not confirm depth of anesthesia and we do not warm the animals during anesthesia. We do observe the mice, and if they are unconscious for more than 5 minutes, we will typically group them with other mice to maintain their body temperature.

Inject tumor sample into mouse:

16) Swab the back of the mouse with Betadine or rubbing alcohol over the injection site. Although the operator should wear sterile gown, gloves and mask (typical garb for handling nude mice), an aseptic field with a sterile drape is not necessary.
17) Inject all 200 microL of the tumor/Matrigel mixture into the flank of the mouse.
    - The injection site is typically on the posterior/lateral aspect of the lower rib cage. Insert the needle through the skin into the subcutaneous space to inject. You should lift up the skin with your needle prior to injecting to ensure that you are not in the muscle. The needle should be inserted approximately 5 mm beyond the end of the needle bevel.
18) While removing the syringe, pinch the injection site for 15 to 30 seconds so that the tumor/Matrigel mixture does not leak out of the injection site.
19) Place the animal back in its cage and repeat the process until all animals are injected. Label the cage with the appropriate xenograft number and record the appropriate information in a laboratory book or computer file.
20) Observe mice weekly for presence of tumor growth. Tumors may take up to one year to grow after implantation of a primary tumor. Most tumors will form palpable tumor within 6 months, but some take longer to form tumors. There are no other predictors that we have identified that can be used to predict which tumor samples will form usable xenografts.