Mayo Clinic Brain Tumor Patient-Derived Xenograft National Resource

Implantation of Patient Tumor Samples Into Nude Mice

Processing of patient tumor samples for implantation into mice is the first step toward developing primary xenografts. For development of our model, we have exclusively relied on direct implantation of primary patient tumor samples into the flank of nude mice.

Prior to initiating a xenograft, the appropriate institutional approvals must be in place for use of human tissue and animals in research. From a laboratory safety point of view, tumors derived from patients with blood-borne viral infectious diseases, such as HIV or hepatitis, should not be used to establish tumor lines.

After implantation, tumors can take up to one year to develop. But on subsequent tumor passage, the growth rates of the tumors typically increase. Experience in multiple labs suggests that approximately one-third of tumors implanted will establish viable xenografts.

Materials:

- Tumor sample
- NOTE: Ideally 1 cm^3 of tumor tissue is used for implantation, although tumor samples as small as 0.125 cm^3 have been used to implant a single mouse. Tissue obtained in the operating room using a Cavitron Ultrasonic Surgical Aspirator (CUSA) works well for tumor implantation.
- Sterile 50 mL conical tube (Falcon)
- Hanks Balanced Salt Solution (HBSS, Irvine Scientific)
- 4- to 5-week-old female athymic nude mice (Envigo #Hsd:Athymic Nude-Foxn^{nu})
- Centrifuge
- 1 cc syringe
- 16-gauge 1½ inch needles
- Isoflurane (Novaplus: Piramal Enterprises Ltd NDC# 66794-019-10)
- Bell jar-type desiccator for anesthesia
- Fume hood
- Corning™ Matrigel™ GFR Membrane Matrix (Corning #354230; subsequently referred to as Matrigel through the remainder of the protocol)
- Betadine: 10% povidone-iodine (Carefusion #29906-016)
- Scalpels
- Petri dish
- Wet ice

Process patient tumor sample in preparation for injection:

1. Transport tumor sample from pathology to laboratory in HBSS at room temperature and record relevant patient information (e.g., age, sex, date of resection, medical record number, etc).
    - While the time from resection to implantation is not critical, we implant all tumors within eight hours of resection. However, tumors remain viable for 24 to 48 hours, so in theory, they could be shipped at room temperature via overnight courier and implanted the next day.
2. Upon receipt of the tumor sample, spin down the sample in a centrifuge at 320 RCF for three minutes. All subsequent steps are performed under aseptic conditions. Aspirate off the transport media and move the specimen to a sterile Petri dish. Because GBM tumors are typically quite soft, mechanical disaggregation or other homogenization steps are not performed. Using a 1 cc syringe without a needle, pull up approximately 100 microL of the tumor into the syringe and then cap the syringe with a 16-gauge needle. Place the syringe with capped needle on wet ice. If injecting more than one mouse, make up individual syringes for each mouse.
    - The tumor tissue typically is quite soft and does not require mechanical disaggregation. However, if necessary, the tumor sample can be broken up in a sterile Petri dish using a sterile scalpel blade.
3. Thaw out enough Matrigel on ice for all the injections (100 microL per syringe).
    - In step 4, you will add Matrigel to the tumor tissue. Since Matrigel solidifies at room temperature, the syringes and tumor material that contacts the Matrigel must be kept as cold as possible to avoid solidification prior to injection.
    - Matrigel that has not been used can be refrozen for use at a later time, provided that it is kept on ice and not contaminated with cells. Matrigel that has solidified is unusable.
4. Draw up 100 microL of Matrigel through the needle and mix the tumor and the Matrigel by moving the plunger back and forth. 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.

Anesthetize the mice:

5. 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.
6. 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.
7. Place an individual mouse in the desiccator.
8. 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:

9. 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.
10. 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.
11. 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.
12. 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.
13. Observe mice weekly for presence of tumor growth. Tumors may take up to one year to grow following 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.