

Stereotactic Orthotopic Xenograft Injections into the Mouse Brain

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Abstract

Xenografting of human cancer cell lines injected subcutaneously in nude mice has become the standard testing platform for dissecting mechanistic aspects of tumorigenesis and for pre-clinical drug development. However, subcutaneous xenografts do not model tumors in their tissue of origin and thus may have some clinically relevant limitations. Xenografted human tumor cells directly injected into their relevant organ may present a more accurate model of human tumors. Here, we describe a rapid protocol for the injection of tumor cell lines into the mouse brain for the establishment of orthotopic tumors.

Introduction

Subcutaneous growth of human tumor cells in immunodeficient mice is a mainstay of cancer research and anti-cancer drug development. However, it is known that the microenvironment influences tumor growth as well drug response. To better model human tumors in immunocompromised mice, cancer cells can be injected into the organ of origin (orthotopically), allowing organotypical interactions between cancer cells and the surrounding microenvironment. Orthotopic tumors may more properly recapitulate human tumor biology, as the interactions of tumor cells with normal tissue cells as well as stromal, vascular, and immunologic cells could have effects on cell survival and migration, and even developmental potential. Similarly, orthotopic tumors may recreate some physiologic barriers and could reveal important issues in drug penetration and metabolism. Located within the microenvironment of the highly specialized and protected central nervous system, orthotopic brain tumor models may have higher clinical relevance than conventional subcutaneous xenografts for understanding tumor behavior and for predicting drug efficacy.

Reagents

Anesthetic (Ketamine) Analgesic (Buprenorphine) 1cc syringes and 30g needles Sterile phosphate-buffered saline Ethanol 70% Sterile water Hamilton syringe and cleaning solution Betadine Alcohol wipes Sterile cotton swabs Disposable scalpels Sterile ocular lubricant (Puralube vet ointment) Forceps Autoclip applier and 9mm clips Clip remover Cells on ice: enough for 100,000 cells per 3ul injection Immunocompromised mice: We use typically either use female SCID (B and T cell deficient; Taconic # ICRSC-F) or Nude (T-cell deficient , Taconic # NCRNU-F) mice between the ages of 6 to 20 weeks.

Equipment

Micromotor drill and bits (Freedom 1070) Stereotaxic device (Stoelting Elliptic Lab Standard™ Stereotaxic Instrument) Heating pad Microscope light Timer

Procedure

The procedure described below details how to perform intracranial tumor cell injection in mice. This is a major surgery and all procedures must be approved by IACUC or other animal use committee. Prepare cells for injection: In the tissue culture room, trypsinize cultured cells. Wash cells with sterile PBS, and then resuspend in sterile PBS to an adjusted final volume of at least 100,000 cells per 3ul injection. Cells should be transported to the injection area on ice. Set up: Assemble the stereotaxic device according to manufacturer's instructions. Lay out the drill and drill bit. Turn on the heating pad and monitor its temperature with a thermometer. Load Hamilton syringe with cells: Prepare the Hamilton syringe by rinsing in 70% Ethanol and then sterile PBS. Gently resuspend trypsinized cells in PBS. Draw cells to 3uL in the Hamilton syringe, carefully avoiding bubbles. Position the syringe in the stereotaxic device, and tighten the screw to lock the syringe in the correct position. Surgical procedure: -Preoperative preparation: Anesthetize mouse by intraperitoneal injection of Ketamine 100 mg/kg. Confirm anesthetic effectiveness by toe pinch. Wipe the mouse's head 3 times with sterile cotton swab dipped in betadine, then swab with an alcohol wipe. Protect the mouse's eyes by coating with sterile ocular lubricant. Make a 1.5 cm sagittal incision in the mouse's scalp from the front to the back. -Stereotaxic immobilization: Position the mouse in the stereotaxic apparatus: first displace the tongue to the side with the wooden end of a sterile cotton swab, then position the front teeth in the tooth holder, next tighten the nose holder until firm, and finally place both ears in the ear holders. Once the mouse is in the correct position, tighten the ear holders until firm. Wipe the skull with a sterile cotton swab to remove the shiny membranes and to aid identification of the bregma suture. -Cell injection: Drill a hole 2 mm right and 1 mm anterior to the bregma suture. Lower the Hamilton syringe needle to the edge of the hole. Lower the needle 3.5 mm, into the brain. Raise the needle 1mm and wait 1 minute. Inject 1 uL of cells, raise the needle 1 more mm, and wait 1 minute. Inject 1 uL, raise 1 mm, and wait 1 minute. Then inject the final 1 uL. Completely remove the needle from the mouse's brain. Remove the mouse from the stereotaxic device. -Recovery: Close the skin incision by holding the edges with forceps and applying one clip. Place the mouse on the heating pad until the effects of anesthetic wear off, at which time the mouse can be returned to its cage. Follow up: Clean the Hamilton syringe with Hamilton cleaning solution, then 70% Ethanol, then sterile water. Administer analgesic twice a day for the first 3 days after surgery. Remove sutures after 10 days. Monitor mice daily for signs of neurologic compromise.

Timing

After proper setup of an efficient system, 3-4 mice injected per hour can be passed through the procedure, which includes, anesthesia, injection, and recovery.

Troubleshooting

Some typical problems include injection of cells in the sub-cutaneous areas of the skull or into the membranes (dura, pia) covering the brain, rather than the brain itself. The ideal location is in the frontal cortex, with tumor cells located within the brain parenchyma near the lateral ventricles. As with all mouse surgical procedures requiring deep anesthesia, death from hypothermia can be an issue, which

must be minimized by appropriate supportive care including monitoring and the use of a heating pad. It is of course advisable to include a positive control when first performing this procedure. Many established glioma and non-glioma cell lines rapidly and consistently form intracranial tumors when injected in this manner, including U87 cells. A negative control would consist of normal, immortalized but non-transformed human astrocytes.

Anticipated Results

The tumor latency is dependent on both the cell line and the number of cells injected. The typical time to onset of neurological symptoms is about 3 months but can be as long as 6 months, or even longer. Volumetric measurement of intracranial tumors by MRI is possible but not practical, and is cost-prohibitive when using a large number of animals. Therefore, neurological symptoms, specifically abnormalities of gross motor function, are used as an endpoint for tumor latency. Neurological symptoms are indicative of a tumor that is sufficiently large to have displaced or infiltrated the mouse brain or to have caused obstruction of cerebrospinal fluid flow with resultant increased intracranial pressure. Dormant, non-tumor forming cells can remain alive in the cerebrospinal fluid for a prolonged time after injection: using anti-human NUMA (Epitomics 3402-1) that specifically detects human but not mouse cells, we have observed residual, non-tumor forming cells as long as 6 months after orthotopic injections.

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