Subcutaneous Injection of Tumor Cells

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[Abstract] Growth of cells in the subcutaneous space of immunocompromised mice is a common method for assaying tumorigenic potential *in vivo*. This technique is also used to assess the effects of therapeutic interventions on cancer cell lines.

Materials and Reagents

1. Tumor cells
2. CB17 scid/scid mice
3. Trypsin (Life Technologies, Invitrogen™, catalog number: 25300-054)
4. DMEM (Life Technologies, Invitrogen™, catalog number: 11965-092)
5. Fetal bovine serum (FBS) (Life Technologies, Invitrogen™, catalog number: 16000-044)
6. Phosphate buffered saline (PBS)
7. Trypan blue (Life Technologies, Invitrogen™, catalog number: 15250-061)
8. Isoflurane (usually purchased through animal facility at institution)
9. Matrigel (BD Biosciences, catalog number: 356234)
10. DMEM cultural medium (see Recipes)

Equipment

1. Centrifuges
2. Insulin syringe
3. Hemocytometer
4. Cell culture hood
5. Incubator
6. Microscope
7. Small gauge
8. Eppendorf tube

Procedure

1. Remove growth medium from cells and wash with 5 ml of PBS.
2. Aspirate PBS, add 2 ml of trypsin and incubate for 5 min, or until cells have detached, at 37 °C.
3. Quench trypsin by adding at least 3 volumes of 10% FBS containing medium.

4. Pellet cells by centrifugation for 5 min at 1,100 rpm and 37 °C.
5. Aspirate medium, wash cells with 10 ml sterile PBS, mix well with pipette and save 50 µl aliquot of cells for counting.
6. Pellet cells by centrifugation for 5 min at 1,100 rpm and 37 °C.
7. While cells are spinning, add 50 µl of trypan blue to saved aliquot, mix well and count cells using a hemocytometer.
   Note: Dark blue cells are dead and should not be counted.
8. Aspirate PBS, resuspend cells in fresh PBS to a concentration 1 x 10^6 cells/100 µl and transfer cells to a sterile Eppendorf tube.
   Note: The cell number required depends upon the aggressiveness of the tumor cells and can vary by an order of magnitude.
9. (Optional) Add an equal volume of thawed Matrigel to cells and mix carefully with pipette. Important, Matrigel should be thawed on ice because it will solidify at room temperature.
   Note: Matrigel provides a favorable environment for less aggressive cells to grow and is often used.
10. Slowly pull up 100 µl of cells alone or 200 µl of cell/matrigel mixture using an insulin syringe.
    Note: Cells can be damaged by the small gauge of the insulin needle; however, insulin syringes provide a more accurate volume measurement. If significant death is observed, 1 ml syringes with 22 gauge needles can be substituted. Place cell containing syringes on ice to prevent matrigel from polymerizing.
11. Inject 1 x 10^6 cells into the flanks of immune deficient mice, preferably CB17 scid/scid. To do this, pinch the skin of the mouse between your index finger and thumb and pull the skin away from the body of the mouse.
12. Inject slowly and evenly into the pouch created by your fingers, creating a single bubble of cells beneath the skin and avoiding too much spread of the cells.
13. Anesthetizing the mice using isoflurane makes the injection process significantly less stressful for the both the mice and the researcher.

Recipes

1. DMEM cultural medium
   Supplemented with 10% FBS