Analysis of Mice Injected with Green Fluorescence Protein (GFP)-Expressing Tumor Cells

For optimal GFP detection in tissues, it is best to extract and measure levels of fluorescence. If histology is needed, you must first:

1. Perfuse the animal with PBS using the intra-cardiac method
2. Perfuse with ***either* fresh** 4% formaldehyde fixative ***or*** 10% neutral buffered formalin
3. Remove brain and other organs of interest
4. Immerse in 30% sucrose until the organ sinks
5. Blot the tissue to remove the excess sucrose
6. Surround the tissue with OCT and then freeze using a recommended freezing protocol

Preparation

1. Prepare your bench area:
    a. Lay down a piece of clean bench cover before beginning experiments.
    b. Get a small beaker and fill with ~70% ethanol (this will be used to keep surgical instruments clean during the procedure).
    c. Obtain a foam block that is covered with aluminum foil and place a few paper towels on top of it. Make sure that there are at least four needles in the block to stake the appendages of the mouse.
2. Set up the homogenizer. Clean the probe with water 1x and with 70% EtOH 2x.

Note: liquid level should never be at or above the second hole from the bottom of the probe.

3. Add 2 mL lysis buffer to bacterial culture tubes (one tube per mouse to be analyzed). Label tubes with mouse ID and date. Place tubes on ice and set aside.

Lysis Buffer (20 mM Tris, pH 8): make with Milli-Q water. Store at room temperature.

4. Get two sets of surgical equipment (2x scissors, 2x tweezers). Note that during this procedure, one pair of equipment will be used to open the skin and chest cavity and the other pair will be used only for isolating and removing the lungs. This will serve to minimize the amount of carryover between tissue types.
5. Obtain a piece of clean aluminum foil and fold in half to place the lungs following dissection. Also obtain a piece of paper to take notes (mouse ID, appearance of lungs, general health of animal).
6. Obtain a 1cc insulin syringe (with needle attached) and coat with EDTA by pulling 10 mM EDTA solution through the entire syringe. Leave approximately 25 µL 10 mM EDTA in the syringe.
7. Obtain a 500-mL beaker and fill with PBS. Obtain a 10cc syringe and 25G needle to perform heart perfusion.
8. Obtain EDTA-coated collection vials and add 2 µL 10mM EDTA to them. Label vials with mouse ID.

Removal of Lungs for GFP Quantification

1. Anesthetize the mouse with Metofane.
2. Place the mouse on its back (stomach up) on the foam block, and stake each appendage with needles.
3. Open the skin over the belly, and peel it back to expose the peritoneal membrane and chest cavity.
4. Clean the excess blood away and open the chest cavity at the bottom center of the ribcage to expose the heart and lungs.
5. Hold on to the trachea with forceps and remove the lungs carefully.

Quantification of Lung GFP Fluorescence

1. Transfer lungs to labeled bacterial culture tubes that have 2 mL lysis buffer in them.
2. Homogenize each set of lungs, cleaning the homogenizer probe with Milli-Q water and 70% EtOH in between lungs.
3. Return tube to ice after homogenization is complete.
4. Add 50 µL 20% Triton X-100 to each tube. Allow to sit on ice for 30 min.
5. Transfer homogenate to labeled 2 mL Eppendorf tubes.
6. Centrifuge at maximum speed for 10 min at 4°C. Turn on fluorescent lamp so that it can begin warming up (requires ~10-15 min).
7. Transfer clear aqueous layer (under thin oily layer and above larger) to labeled Eppendorf tubes. Put tubes on ice.
8. Obtain the quartz 96-well plate, and create a map for samples. Each sample will be read in a 1:20 dilution and a 1:10 dilution.
9. Pipet the appropriate amount of sample (10 or 20 µL) into the appropriate wells.
10. Pipet the appropriate amount of lysis buffer (180 or 190 µL) into the appropriate wells.
11. Read the fluorescence at gains of 40, 50, and 60 at 485 nm (excitation) and at 530 nm (emission).

Things to note if you want to detect the presence of GFP incorporated into injected neoplastic cells within mouse tissues

1. When attempting to track labeled cells in mouse tissue, plan to extract the tissue per the protocol above. With this, one can more accurately quantify the infiltrating cells. See Borsig et al. 2002.
2. GFP in tissue is fairly soluble in water, PBS, buffers, and in the various reagents that are used to embed tissues into paraffin. If you really want to look at GFP cells, one should plan to perfusion fix the animal. Then, remove the tissues and let them sink at 4°C in 30% sucrose to replace the water. The tissue should then be blotted to remove as much sucrose as possible and frozen in OCT/dry ice/isopentane for frozen sections and subsequent evaluation.
3. One may also try to detect Alu sequences to confirm infiltrating human cells in the mouse milieu. See Borsig et al. 2001.

4. The problem here is one may be trying to look for a “needle in a haystack” in the tissue, since one is using a three-dimensional object and slicing through, and hoping to find those few infiltrating cells – this may or may not work unless you expect to see large numbers of infiltrating cells.
5. One could plan to use a chicken polyclonal to GFP to identify infiltrating GFP cells in tissues that were not perfused.
6. However, before doing that IHC experiment (expensive), one could also take a quick look at the unstained slides to see if any GFP that may have survived the processing steps can be detected.
7. It is recommended to first look at the H&E to see if there are any infiltrating tumor cells because tumor cells are detectable on a simple H&E if they are indeed present on the plane of section.
8. If you have an H&E of the xenotransplant, that will help with identifying infiltrating neoplastic cells within other tissues on the H&E slides.

References

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