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Protocol

Collagen-Embedded Tumor Transplantations in *Xenopus laevis* Tadpoles

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The *Xenopus laevis* tadpole provides a valuable model for studying tumorigenesis and tumor immunity by intravital real-time microscopy. Using well-characterized thymic lymphoid tumor lines (15/0 and ff-2) that are transplantable into their compatible hosts (LG-15 isogenic clones and the F inbred strain, respectively), a system of semisolid tumor engraftment has been designed. Because these lymphoid tumor cell lines are not adherent and grow in suspension, they are first immobilized in a matrix of type I rat tail collagen before transplantation as a semisolid tumor graft under the transparent dorsal skin in the head region of a tadpole. This semisolid tumor engraftment is amenable to manipulation and permits real-time visualization of tumor growth, neovascularization, collagen rearrangements, immune cell infiltration, and formation of the tumor microenvironment.

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MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous materials used in this protocol.

RECIPES: Please see the end of this protocol for recipes indicated by <R>. Additional recipes can be found online at http://cshprotocols.cshlp.org/site/recipes.

Reagents

Amphibian PBS (APBS) <R>
Earle's balanced salt solution (EBSS, 10×)
Iscove-derived tumor media (Robert et al. 1994) (e.g., MSF tumor medium; see Step 8)
Rat tail collagen I (Corning 354249; 100 mg supplied as a liquid in 0.02 N acetic acid)
Tricaine methanesulfonate (TMS, MS-222, Western Chemical, Inc.)
Tumor cell line of interest (1×10^6 cells) and compatible animal recipient
15/0 tumor cells and LG-15 or LG-6 cloned tadpoles
ff-2 tumor cells and MHC homozygous F inbred strain tadpoles

15/0 tumor cells are derived from isogenic X. laevis/gilli LG-15 clones, and are transplantable into LG-15 or LG-6 cloned tadpoles. ff-2 tumor cells are derived from and transplantable into X. laevis tadpoles of the F inbred strain. Culture media and conditions have been described by Du Pasquier and Robert (1992) and Robert et al. (1994).

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Equipment

Centrifuge (benchtop)
Compressed air tank with blood gas mixture (5% CO_2, 21% O_2, 74% N)
Culture plates (six-well, sterile)
Forceps, stainless (small, well-sharpened; e.g., #5 forceps from A. Dumont and Fils, Switzerland)
Incubator at 27°C, with modular incubator chamber
Microcentrifuge tubes (1.5-mL Eppendorf)

METHOD

Preparing the Collagen Setting Solution

Keep all solutions, reagents, and tubes chilled on ice throughout the following section. Higher temperatures will polymerize the collagen.

1. In a chilled microcentrifuge tube, slowly mix 10× EBSS into the collagen at a ratio of 1:5 (v:v). Pipette up and down until the solution is thoroughly mixed. Avoid air bubbles.
2. In 10 µL increments, add more 10× EBSS until the color of the mixture changes to light orange with a ring of light pink on the top.

   This color indicates a neutral pH, which allows the collagen to polymerize at room temperature.
3. Once the required color is achieved, centrifuge the tube briefly to collect the contents. Keep the collagen setting solution on ice.

   See Troubleshooting.

Embedding Tumor Cells into the Collagen Matrix

4. Wash 1×10^6 cells (15/0 or ff-2) in amphibian phosphate-buffered saline (APBS) three times. Centrifuge the cells at 1000 rpm for 10 min at 4°C. Aspirate the APBS.
5. To prepare ten 10-µL grafts of 100,000 cells/graft, add 100 µL of collagen setting solution to the cells. Slowly pipette up and down to evenly mix the cells.
6. For each graft, pipette 10 µL of the cell mixture into one well of a sterile six-well culture plate, forming a single drop in each well. Leave the plates at room temperature for 10 min to allow the collagen to polymerize.

   See Troubleshooting.
7. Transfer the plates into a modular incubator chamber, fill the chamber with blood gas mixture, and incubate for 1 h at 27°C.
8. If the collagen-embedded tumors will not be transplanted immediately, add 2 mL of MSF tumor medium to each well so that the samples are completely covered. Keep samples at 27°C in the incubator.

   To achieve the best results, the grafts should be transplanted within 24 h.

Transplanting the Collagen-Embedded Tumors

Use F inbred tadpoles for transplantations with ff-2 tumors, and LG-15 or LG-6 tadpoles for transplantations with 15/0 tumors.

9. Anesthetize a tadpole with 0.1 g/L of TMS solution.
10. Make a small subcutaneous incision on the tadpole's head, posterior to the eyes. Broaden the incision to form a pocket. Using forceps, insert a collagen-embedded tumor into the pocket.

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11. Allow the tadpole to recover from the procedure.

See Troubleshooting.

TROUBLESHOOTING

Problem (Step 3): The collagen setting solution polymerizes before mixing with the tumor cells.
Solution: The reagents were not sufficiently chilled. Keep all reagents and cells on ice before preparing grafts in a six-well plate.

Problem (Step 6): The collagen setting solution does not polymerize.
Solution: The pH of the collagen setting solution was not optimized to neutral. The color should be light orange with a ring of light pink on the top.

Problem (Step 6): Air bubbles are present in the grafts.
Solution: Air bubbles can be introduced into the grafts while mixing the collagen setting solution. Collagen is very viscous, so the collagen setting solution should be pipetted very slowly to avoid air bubbles.

Problem (Step 6): Tumor cells are not dispersed evenly in the graft.
Solution: While mixing the collagen setting solution with the tumor cells, make sure the cells are evenly dispersed in the mixture.

Problem (Step 11): The collagen-embedded tumor transplant does not stay attached.
Solution: For transplantation of collagen-embedded cells, the incision and the pocket under the tadpole skin should be as small as possible.

DISCUSSION

With the aid of intravital microscopy (confocal, two photon), the collagen-embedded tumor graft model offers an opportunity to study the in vivo and real-time processes involved in tumor estab-

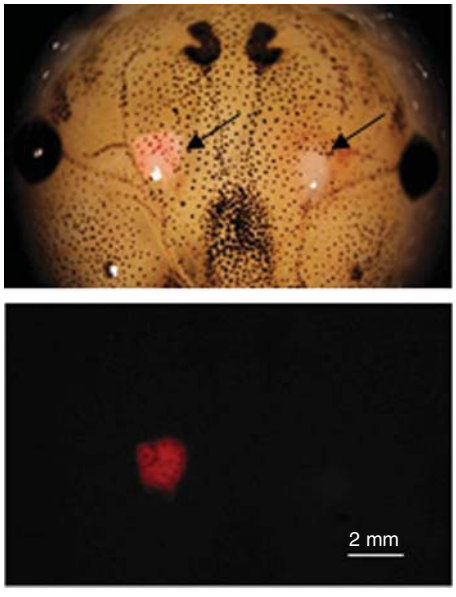

FIGURE 1. Dorsal view of a tadpole of the inbred MHC homozygous F strain grafted with semisolid ff-2 tumors on both sides of the head viewed by a fluorescent dissecting microscope. ff-2 tumor cells (1×10^5) are unlabeled (*right* side) or labeled with PHK-26 (*left* side). Bar size, 2 mm.

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lishment and growth, neovascularization, collagen remodeling, infiltration of immune cells, migration of melanophores, and perhaps metastasis (Haynes-Gilmore et al. 2015).

Collagen-embedded tumor grafts are easily manipulated, for example, using tumor cells fluorescently labeled with CFSE or PKH-26 (Fig.1). The tumors can be harvested at different times post-engraftment, and different techniques (such as microscopy, flow cytometry, or real-time polymerase chain reaction [RT-PCR]) can be applied to study the various infiltrating host cells. Furthermore, different experimental setups can be performed on the same tadpole, for example, by transplanting a wild-type tumor on one side and a tumor mutant on the other side of the tadpole's head.

RECIPES

APBS

25 mL H_2O (sterile; Merck)
100 mL PBS (phosphate-buffered saline; Invitrogen)

In this recipe, PBS is adjusted to amphibian osmolarity (225 ± 5 mOsm/L) by the addition of H_2O.

Earle's Balanced Salt Solution (EBSS, 10×)

100 mL Earl's Balanced Salt Solution (EBSS) (10×) (Sigma E7510)
0.2 M $NaHCO_3$
0.15 M NaOH
42.5 mL APBS (amphibian PBS)

To prepare 150 mL of 10× EBSS, combine the above reagents and sterilize through 0.22-micron filter. Store aliquots at −20°C.

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