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The In Vivo Invasion Assay: Preparation and Handling of Collection Needles

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Abstract

Analysis of the individual steps in metastasis is crucial if insights at the molecular level are to be linked to the cell biology of cancer. A technical hurdle to achieving the analysis of the individual steps of metastasis is the fact that, at the gross level, tumors are heterogeneous in both animal models and patients. Human primary tumors show extensive variation in all properties ranging from growth and morphology of the tumor through tumor-cell density in the blood and formation and growth of metastases. Methods capable of the direct visualization and analysis of tumor-cell behavior at single-cell resolution in vivo have become crucial in advancing the understanding of mechanisms of metastasis, the definition of microenvironment, and the markers related to both. High-resolution multiphoton imaging of tumors in vivo is a valuable tool in this regard. Because tumor cells have been found to be attracted to blood vessels, the in vivo invasion assay was developed to analyze which factors may stimulate invasion of these cells into the vessels. This protocol describes the preparation and handling of collection needles for the assay. A set of 33-gauge needles is used to create artificial or surrogate blood vessels that are injected into tumors, using a special holding device attached to a micromanipulator to stabilize the needle positions during and after insertion into the anesthetized animal. The needles are filled with Matrigel and various growth factors to determine which of these factors may influence the invading tumor cells.

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.

Reagents

Animal to be injected (rat or mouse), induced for tumor growth

BSA (bovine serum albumin)

DAPI (4',6-diamidino-2-phenylindole)

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WWW RESOURCE <http://www.einstein.yu.edu/biophotonics/> Advanced optical microscope used in studies described in this article are part of the Gruss Lipper Biophotonics Center.

RELATED INFORMATION The setup for the assay is illustrated in Figure 1. A detailed discussion of High-Resolution Multiphoton Imaging of Tumors In Vivo (Wyckoff et al. 2011) is also available.

EDTA (Sigma-Aldrich)

EGF (epidermal growth factor) (Life Technologies) or other growth factor/chemoattractant to be tested

Isoflurane

L15 (GIBCO/Invitrogen)

Matrigel

Oxygen

Equipment

Anesthesia vaporizer with adjustable range from 0% to 5% (Harvard Apparatus; Model 100)

Blocking wires

Coverslips

Micromanipulator with holding apparatus

Multiphoton microscope with a 20× 0.95-NA (numerical aperture) objective

Needles (guide; 25-gauge)

Needles (33-gauge)

METHOD

1. On the day of the experiment, prepare 33-gauge needles by filling them with a 1:10 dilution of Matrigel in L15-BSA (L15-BSA is the isotonic equivalent of 5% fetal bovine serum) or with L15-BSA with a final concentration of 25 nM EGF.

For other chemoattractants and cytokines, it was determined that the optimum concentration in the needle is five times the concentration used for in vitro experiments based on the diffusion gradient from the needle into the tumor (Wyckoff et al. 2004). All needles should contain 0.01-mm EDTA (pH 7.4) to sequester heavy metals that might be released from the needle.

2. Anesthetize a rat or mouse using 5% isoflurane. Lay the animal on its back on the microscope stand. Reduce the isoflurane to 2.5% and lower it during the course of the experiment to maintain breathing.
3. Position three 25-gauge needles into a specially designed holder apparatus held in a micromanipulator for stability (shown in Fig. 1). (The holding device allows the simultaneous insertion of three separate 25-gauge needles into the tumor. At this point, the needles should have guide or blocking wires inside them to keep the

interior of the 25-gauge needles from passively filling with tumor during insertion.) Using the micromanipulator, insert the three 25-gauge needles into the tumor.

4. Remove the guide wires from the 25-gauge needles. Insert the 33-gauge needles containing Matrigel with or without chemoattractant (such as EGF) into the 25-gauge needles.
5. Maintain the animal under monitored anesthesia for 4 h to allow the tumor cells to migrate into the needles.
6. Collect time-lapse observations during the collection of cells using a 20× 0.95-NA objective with a multiphoton microscope.
7. After collection (usually over the 4-h period), expel the needle contents onto a coverslip. Mix 1:1 with DAPI, and count the cells immediately.
8. To create dose–response curves for EGF, fill the 33-gauge needles (as in Step 1) with final concentrations of EGF of 5 nM, 10 nM, 25 nM, and 50 nM. Carry out the experiment as described in Steps 2–7. Use at least three different concentrations of EGF in each experiment to control for differences between/among tumors.

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FIGURE 1.

The in vivo invasion assay can be used to collect invasive tumor cells and accompanying stromal cells from living tumors during intravital imaging. (A) A model for the in vivo invasion assay shows the method for collecting cells from living tumors using needles (ID 102 μm) filled with Matrigel and the ligand of interest when placed in the mammary tumor of an anesthetized animal. (B) Holding apparatus for 25-gauge guide needles and 33-gauge experimental needles is shown attached to micromanipulator used to precisely place needles into the mammary tumor. (C) Living tumor in anesthetized mouse is shown with needles inserted. The needles are left in the tumor for up to 4 h. (D) Movement of tumor cells (*top*, WAP-Cre/CAG-CAT-EGFP/MMTV-PyMT tumor) and macrophages (*bottom*, MMTV-PyMT/lys-GFP^{Ki}) toward EGF-containing needles. The approximate opening of the collection needle is shown in the field as an asterisk. Each image is a 50- μm z projection from a time-lapse series. Images on the *right* were recorded 90 min after images on the *left*. Scale bar, 25 μm .