EXPERIMENTAL PROTOCOL:
SYNGENEIC, HETEROTOPIC AND IMMUNOCOMPETENT SMALL CELL LUNG CANCER MOUSE MODELS

Author/s: Miguel Galindo-Campos, Edurne Arriola

Contact information: mgalindo@imim.es, earriola@psmar.cat

Introduction

This SCLC mouse models allow to study tumor growth and the events related to immune response against tumor in a hybrid immunocompetent mouse strain (B6129SF1/J). These models can be used to study therapeutic strategies involving immune checkpoint blockade or other immune interventions.

Reagents

- Complete RPMI + FBS 10% + Penicillin/Streptomycin 1% culture medium.
- Matrigel Basement Membrane Matrix (ref: 354234, Corning)
- Phosphate Buffered Saline
- Topical povidone iodine

Instruments and tools

- Culture flasks
- Plastic pipettes
- Falcon 15- and 50-mL tubes
- Eppendorf 1.5-mL tubes
- Razor machine
- Insuline syringe 50 UI; 25G needle
- Digital calliper
- Surgical scissors and forceps
- Precision balance

- Specific Pathogen Free animal facility
- Mouse gas anesthesia machine
- Mice breeding and crossing:

- **Progenitors:** **C57Bl/6J** (ref: 632C57BL/6J; Charles River, France) females are crossed with **129S1/SvlmJ** (ref: 002448; Jackson Lab, USA) males. The first generation (F1) of this crossing is the strain **B6126SF1/J**.
- **Mice for inoculation:** 6-12 weeks-old first generation (**B6129SF1/J**) mice are used in this model.

Before start

- **KP1** and **5B** cell lines are derived from a ***Rb1$^{lox/lox}$; Trp53$^{lox/lox}$*** genetically engineered mouse model (GEMM) and display neuroendocrine features.
  - KP1: Nat Med. 2011 Oct 9;17(11):1504-8. doi: 10.1038/nm.2473.
  - 5B: not published.
- KP1 cell line grows in suspended cellular aggregates while 5B cell line grows in suspended dense spheroids. KP1 requires several pipette-mediated resuspensions for single-cell suspension, while several passes through a 25G needle is needed to obtain single-cell suspension from 5B cell line.
- KP1 and 5B cell lines show an important variability between size of tumors *in vivo*. Rigorous control in cell counting, management and inoculation is needed.
- 5B generate tumors with multiple necrotic foci and induce ulcers on site of inoculation earlier than KP1.
- To maintain syngeneicity between KP1 and 5B cell lines and mouse recipient **only first generation B6129SF1/J** mice must be used in this model.
- Parental 129S1/SvlmJ strain is only available at Jackson Laboratory (USA).

PROCEDURE

1. KP1 and 5B SCLC cell lines grow in complete RPMI + FBS 10% + Penicillin/ Streptomycin 1% culture medium. Must be thawed and expanded at least one week before inoculation.
2. After washing with PBS 1X, prepare cells for inoculation at a cell density of 10^7 cells/mL:
   a. Resuspend cells in iced-cold PBS (half the final volume). Then add Matrigel to obtain a PBS:Matrigel dilution 1:1.
   b. Matrigel must be thawed in ice at least 2 h before cell suspension preparation and kept ice-cold until inoculation.
   c. Avoid formation of air bubbles.
   d. Be sure to resuspend cells homogeneously.
3. Use the razor machine to expose skin of the lateral dorsal region of the mouse (left or right as preferred).
4. Gas anesthesia is used to improve mouse manipulation and decrease possible variations in the volume of inoculated cell suspension.
5. Once the mouse is anesthetized apply a thin layer of topical povidone iodine on the inoculation area. Wait for the cream to dry.

6. Once in the animal facility cabin, carefully pass the cell suspension to an insulin syringe, avoiding air bubbles formation. Charged syringe must be purged for the first 100 μL and put horizontally on ice.
7. Use small sterile surgery forceps to lift the skin and expose the inoculation area. After puncture and before inoculation, be sure the needle is located subcutaneously and not intradermic or into deep tissues.
8. Inoculate 100 μL subcutaneously. Wait a few seconds before needle withdrawal to avoid inoculated cell suspension leakage.
9. Each needle can be used to inoculate 3 mice. Last 100 μL of each syringe must be discharged.
10. Assure a proper anesthesia recovery and replace mice in their cages.
11. Follow up tumor growth twice a week (or as needed). Inoculation of 10^6 cells allows a 21 days follow up before reaching humane endpoints (tumor volume, ulceration).
12. Follow up mice weight weekly to monitor cachexia/drug toxicity.
13. For tumor analysis, use surgical forceps and scissors to carefully remove tumor right after killing. Tumors can be weighted to complement tumor volume data.