SynthImmunol_NMU Notebook No.1

Time: 2024.09.01-2024.09.09

1. **Experiment:** Construction of in situ tumor models
2. **Time:** 2024.09.01-2024.09.09
3. **Member:** Hanyue Liu, Yinran Luo
4. **Material:**

Name	Supplier or Formulation
Animal scales	Shimadzu
High-throughput in vivo optical imaging system for small animals	PerkinElmer
Vetbond Tissue Adhesive	3M
BALB/c Rag2^{KO} IL2rg^{KO} mice (NCG Mice)	GemPharmatech Co., Ltd
PBS	Solabio Biotechnology Co., Ltd.
L-glutamine	GIBCO
FBS (fetal bovine serum)	GIBCO
streptomycin	GIBCO
DMEM medium	GIBCO
disposable 15 mL and 50 mL centrifuge tubes	Corning
cell culture plates and dishes	Corning
an Eppendorf 5804R high-speed centrifuge and mini centrifuge	Eppendorf
FACSCalibur flow cytometer	BD

5. **Method:**

(1) Cell Culture**:** Pancreatic cancer cells ASPC1 were cultured in DMEM medium supplemented with 10% fetal bovine serum, 100 mg/mL penicillin, and 100 mg/mL streptomycin and maintained in a 37 °C incubator with 5% CO_2.

(2) Cell Preparation**:** ASPC1 were seeded in 10 cm culture dishes using DMEM medium and incubated at 37 °C in a 5% CO_2 atmosphere until the cells reached the logarithmic growth phase.

(3) Harvesting Cells with Trypsin**:** 1 mL of 0.25% trypsin was added to each 10 cm dish for digestion for one minute. Afterward, 2 mL of DMEM containing 10% fetal bovine serum was added to terminate the digestion. The cell suspension was transferred to a 15 mL centrifuge tube, and 5 mL of PBS was added to wash the dish. The solution was collected into the 15 mL tube, centrifuged at 1000 r/min for 5 minutes, and the supernatant was discarded. The ASPC1 cells were then resuspended in PBS, centrifuged again at 1000 r/min for 5 minutes, and the wash was repeated twice.

(4) Cell Counting: The harvested ASPC1 cells were resuspended in PBS, counted, and adjusted to a final concentration of 2×10^7 cells/mL.

(5) Anesthesia of Mice: Each mouse received an intraperitoneal injection of the anesthetic pentobarbital (150-200 μL), and the injection site was disinfected with 75% ethanol prior to administration.

(6) Injection of ASPC1 Cells: After anesthesia, the abdomen of the mouse was disinfected with povidone-iodine using a cotton swab. A longitudinal incision of approximately 1 cm was made in the left upper abdomen with ophthalmic surgical scissors. The spleen was gently elevated using a sterile cotton swab to visualize it, followed by careful exploration of the pancreas. Once the pancreas was located, the tip of the tail was grasped with curved ophthalmic forceps and gently pulled to expose the pancreas (care was taken to avoid rupturing

the spleen). A 27-gauge needle was used with a 1 mL syringe to gently insert the needle into the pancreas from the tail end towards the head region, ensuring that it did not penetrate through the pancreas to prevent leakage of pancreatic fluid, which could lead to the death of the mouse. Each mouse received an injection of 50 μL containing 2×10^7 cells/mL of ASPC1 cells (successful injections were indicated by a visible small blister formation; if the pancreas was punctured, leakage of the cell suspension would be observed). After the injection, the direction of the needle was slightly adjusted, and the cell suspension was slowly injected while withdrawing the needle. Applying gentle pressure with a sterile cotton swab at the injection site for a moment effectively prevented leakage. The spleen and pancreas were then gently returned to the abdominal cavity, and the skin and peritoneum were sutured using 3-0 surgical sutures. Post-surgery, the mice were placed in a warm environment illuminated by incandescent light until they awakened naturally.