SynthImmunol_NMU Notebook No.2

Time: 2024.08.10-2024.08.13

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1. **Experiment:** Co-culture of NK92 and human pancreatic tumor organoids
2. **Time:** 2024.08.10-2024.08.13
3. **Member:** Xiaoyuan Chen, Hanyue Liu
4. **Material:**
   (1) Organoids: human pancreatic tumor organoids
   (2) Reagent: 10 μM Y-27632, 0.01 μM Prostaglandin E2 (PGE2; R&D Systems)
5. **Method:**

For organoid cytotoxicity assays, organoids were seeded on a Matrigel layer and incubated with the respective NK92 cells for 8 h.

(1) 48-well standard culture plates with a growth area of 1 cm^2 (Greiner Bio-One) were first moistened using culture medium.

(2) Subsequently, each well was evenly covered with 35 μL undiluted Matrigel which was allowed to solidify overnight at RT.

(3) Confluent organoids were collected, mechanically sheared, pelleted, washed, and seeded at a split ratio of 1:2.5. Organoids were resuspended in 150 μL of the respective culture medium supplemented with 10 μM of Y-27632 per assay replicate.

(4) The organoid suspension was carefully added to the center of the Matrigel-covered wells, respectively. Organoids were grown for 24 h before supplement of NK92 cells in 500 μL of medium without Y-27632.

(5) For standard cytotoxicity assays, ~10^5 organoid cells were seeded per well of the 48-well plate (1 cm^2), which was defined as organoid density of 100%. NK92 cells and their CAR-engineered derivatives were pelleted, washed, and resuspended in co-culture medium as indicated below.

(6) Cells were counted using a hemocytometer, and the required number of cells in a total volume of 500 μL (or 1 mL for long-term co-cultures) per well of a 48-well plate was co-incubated with target cells for 8 h at 37 °C.

(7) The following co-culture media were used: For human organoids, the medium contained complete normal or tumor medium lacking nicotinamide. 0.01 μM Prostaglandin E2 (PGE2; R&D Systems) was added to induce a cystic phenotype.