

Time: 2024.06.27-2024.06.29

1. **Experiment:** Co-culture with tumor cells
2. **Time:** 2024.06.27-2024.06.29
3. **Member:** Xiaoyuan Chen, Song Zhang, Yaqi Gao, Xudong Tang
4. **Material:** NK cell media (Gibco, supplemented with 5% FBS and 500 IU/mL IL-2), IL-2 (Gibco), 10 $\mu\text{g}/\text{mL}$ Mitomycin C, 5% hAB serum (Gibco), Trypan Blue
5. **Method:**
 - (1) Cell preparation and initial co-culture:
 - ① Prepare NK92 cell library and Mitomycin-treated K562 cells. Treat K562 cells with 10 $\mu\text{g}/\text{mL}$ Mitomycin C for 2 hrs, then wash thoroughly three times to remove the residual drug.
 - ② Co-cultivate NK92 cells and Mitomycin-treated K562 cells in three 15 cm culture dishes (labeled A, B, and C), with each dish containing 5×10^6 NK92 cells and 5×10^6 K562 cells. Add 20 mL RPMI-1640 medium (supplemented with 10% FBS, 1% Penicillin-Streptomycin, and 100 IU/mL IL-2) and incubate at 37°C with 5% CO₂.
 - (2) Co-culture conditions and cell addition:
 - ① Dish A: After 36 hrs of incubation, sort and collect EGFP+ NK92 cells using flow cytometry.
 - ② Dish B: After 36 hrs of incubation, add an additional 5×10^6 Mitomycin-treated K562 cells to Dish B. Continue incubation for another 36 hrs (total 72 hrs), then sort and collect EGFP+ NK92 cells.
 - ③ Dish C: After 36 hrs of incubation, add 5×10^6 Mitomycin-treated K562 cells to Dish C, and add another 5×10^6 K562 cells at 72 hrs. Continue incubation for a total of 108 hrs, then sort and collect EGFP+ NK92 cells.
 - (3) Distribution and fluorescence detection:
 - ① After sorting, distribute the NK92 cells from dishes A, B, and C into three groups of ten 96-well plates each (30 plates total). Ensure that cells are evenly distributed among the wells.
 - ② Incubate the plates for 24 hrs at 37°C with 5% CO₂.
 - ③ Perform fluorescence detection by photographing the wells in a dark box equipped with a fluorescence filter. Capture images of each well to assess EGFP expression levels based on fluorescence intensity.

6. Result:

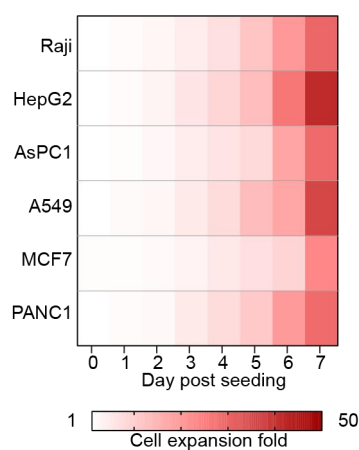


Fig.1 Tumor cell stimulation without cytokine