SynthImmunol_NMU Notebook No.6

Time: 2024.05.21-2024.05.22

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1. **Experiment:** Secondary flow cytometry sorting
2. **Time:** 2024.05.21-2024.05.22
3. **Member:** Xiaoyuan Chen, Yinran Luo, Song Zhang, Xinyu Zhu
4. **Material:** RPMI-1640 medium (10-15 mL per culture flask), FLAG-APC antibody (BioLegend, 637308) at 10 μL per 2200 μL of PBS (1:200 dilution) for initial labeling and 2 μL per 1 mL of PBS (1:500 dilution) for secondary labeling, Phosphate-buffered saline (PBS).
5. **Method:**
   (1) NK92 cell suspension from the stock is transferred to a tissue culture flask containing RPMI-1640 medium. An appropriate seeding density of 2-5×10^6 cells per 10-15 mL of medium should be ensured.
   (2) The culture flask is placed in a CO_2 incubator set at 37°C with 5% CO_2 for 24 hours. Cell growth should be monitored to ensure that cells are in the logarithmic phase.
   (3) Replace the medium by removing the old medium and adding fresh RPMI-1640 medium.
   (4) When cells reach 70-80% confluency, cells are passaged. The cell suspension is collected, and centrifuged at 300×g for 5 minutes. The supernatant is discarded, and the cells are resuspended in a fresh medium before seeding into new culture flasks.
   (5) Cells should be regularly counted and their morphology checked to ensure healthy growth.
   (6) NK cells suspension was expanded to over 10^7 and centrifuged at 1500 rpm for 5 minutes. 30mL of PBS was used to wash the cells.
   (7) The cells were resuspended by 2200μL of PBS and 10μL of FLAG-APC antibody (BioLegend, 637308) was added to label the cells. The cells were then incubated in the dark for 15 minutes.
   (8) The cells were washed again with 30mL of PBS. The supernatant was discarded, and the cells were resuspended again by 500μL of PBS.
   (9) The positive cells were sorted based on the CAR expression, from the top 10% to 20%.
   (10) The sorted cells were cultured to proliferate to 2x10^7 and FACS detects the CAR-positive cell proportion.
   (11) 1mL of cell suspension was taken. After the three-minute placement on ice, the cells were centrifuged at 3000 rpm for 5 minutes and collected.
   (12) The supernatant was discarded completely and the cells were resuspended in 1mL of PBS.
   (13) 2μL of FLAG-APC antibody (BioLegend, 637308) was added to label the cells.
   (14) The cells were then incubated in the dark at room temperature for 15 minutes. 1mL of PBS was added to terminate the reaction and the cells could be collected after centrifuging at 3000 rpm for 5 minutes.
   (15) The cells were resuspended by 1mL of PBS and the supernatant was discarded. The cells were resuspended in 50μL of ice-cold PBS, and flow cytometry analysis could be carried out.
6. **Result:**

Wild-type NK92 cell lines, CD19 CAR NK92 cell lines, and αCD19 CAR-library NK92 cell lines with the highest transfection rate were selected by flow cytometry, and GFP was used to detect monoclonal positive expression. The control group was NK92 cells, the blue was CD19 CAR NK92 cell line, and the red was αCD19 CAR library NK92 cell line. It can be seen that the expression kurtosis of the experimental group was 10^3-10^5. we built auto-expressed α CD19 CAR NK cell libraries successfully.

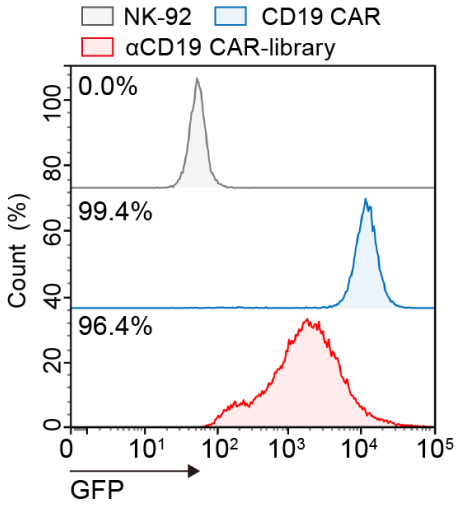

Fig.1 Kurtosis of GFP expression in each cell line