Experiment time: 2025-05-02, 18:00 - 2025-05-07, 17:00

Flow Cytometric Analysis of the Cell Cycle (I)

I. Objective of the experiment

This experiment aimed to determine the cell cycle distribution of T24 wild-type and low-, medium-, and high-drug-resistant T24-RC48 cell lines, both untreated and following treatment with Disitamab vedotin (RC48), using a Cell Cycle Staining Kit. The analysis of cell cycle alterations in the resistant variants provides an experimental basis for investigating ADC resistance mechanisms in bladder cancer.

II. Experimental content

2.1 Experimental design

Sample grouping: blank control group (untreated T24 wild-type cell lines and low, medium and high drug-resistant T24-RC48 cell lines);

Drug treatment group (T24 wild-type cell lines and low, medium and high drug resistant T24-RC48 cell lines were treated with videstizumab (RC48) for 36 hours respectively)

Number of repetitions: 3 samples per group.

2.2 Measurement principle

During the cell cycle, cells in the G0/G1 phase contain 2N of DNA, those in the S phase have DNA levels between 2N and 4N, while cells in the G2/M phase carry 4N DNA. The Cell Cycle Staining Kit utilizes the DNA-binding dye propidium iodide (PI) to stain cells. Flow cytometry is then employed to detect DNA content and generate a cell cycle distribution histogram. By comparing pre-and post-treatment histograms, it enables the analysis of drug-induced effects on cell cycle progression, such as whether they induce specific phase blockages.

III. Materials and reagents

3.1 Materials

Pipette tips and 15ml centrifuge tubes were purchased from Axygen

Serological pipettes were purchased from a domestic company

Micropipettes: Eppendorf

10-cm cell culture dishes (Corning Company)

Human bladder transitional cell carcinoma cell line (T24)

Low, medium, and high drug-resistant cell lines T24-RC48

3.2 Reagents

PBS, trypsin and fetal bovine serum were purchased from Gibco, USA

McCoy's 5A medium purchased from Shanghai Yuanpei Biotechnology Co., Ltd., China

Cell cycle detection kit (Lianke Biology, CCS012)

IV. Experimental instruments

Clean bench: Suzhou purification equipment factory

Benchtop low-speed centrifuge at room temperature: Eppendorf

Flow cytometers (BD, not model C6)

V. Experimental steps

5.1 Seed T24 wild-type and low-, medium-, and high-resistant T24-RC48 cell lines into six 10-cm cell culture dishes per cell line.

5.2 After 24 hours, for each cell line, replace the medium in three dishes with fresh complete medium (untreated control), and the medium in the remaining three dishes with fresh complete medium containing 200μg/mL Disitamab vedotin (RC48). Treat the cells for 36 hours.

5.3 Discard the culture medium and wash the cells with PBS for 3 times.

5.4 Add trypsin to digest the cells and gently tap the culture bottle to detach the cells.

5.5 Collect the cell suspension from each dish and centrifuge at 1000rpm for 5 minutes. Discard the supernatant.

5.6 Wash cells twice with pre-colded PBS and discard the supernatant.

5.7 Resuspend the cell pellet in 1 mL of DNA Staining Solution containing 10μL of Permeabilization Solution. Vortex briefly for 5-10 seconds to mix, and incubate at room temperature in the dark for 30 minutes.

5.8 Analyze the stained cells using a flow cytometer.

5.9 Analyze the flow cytometry data using FlowJo software to determine the percentage of cells

in each cell cycle phase and compare the cell cycle distributions between the different drug-resistant groups and the wild-type cell line.

VI. Experimental results

In this experiment, after PI staining of cell groups (including the T24 wild-type and low-, medium-, and high-drug-resistant T24-RC48 cell lines with blank controls and drug-treated groups), flow cytometry analysis revealed no detectable DNA fluorescence signals in all samples. Only faint background signals were observed, consistent with baseline levels of non-stained samples.

After the staining time was extended to 4h, the machine was tested again. The result showed no improvement, and the fluorescence signal intensity did not increase with the extension of staining time.

VII. Results analysis

The current experiment failed to obtain valid cell cycle distribution data, likely due to excessive cell quantity: each sample used 10-cm culture dish cells (approximately 4×10^6 cells), resulting in suboptimal dye-to-cell ratios. Excessive cells may hinder proper binding of propidium iodide (PI) to DNA or cause uneven dye penetration. For subsequent experiments, the cell number per sample will be significantly reduced, using the yield from a single well of a 6-well plate instead of a 10-cm dish, to ensure proper staining and acquisition of analyzable cell cycle data.