Experiment time: 2025-05-17, 18:00 - 2025-05-22, 21:00

Transwell Migration Assay (I)

I. Objective of the experiment

In this experiment, Transwell was used to detect the migration ability of T24 wild-type cell lines and low, medium and high drug-resistant T24-RC48 cell lines, and the migration characteristics of drug-resistant cell lines were analyzed to provide an experimental basis for elucidating the mechanism of ADC resistance in bladder cancer.

II. Experimental content

2.1 Experimental design

Sample type: T24 wild-type cell line, low/medium/high drug resistance T24-RC48 cell line.

Number of repetitions: 3 per group.

2.2 Measurement principle

The Transwell cell migration assay operates on the fundamental principle of cellular chemotaxis. In this setup, cells are placed in the upper chamber while the lower chamber contains a culture medium with chemokine gradients. When cells detect these concentration differences, they actively migrate through the permeable membrane toward the higher chemokine concentration. The migration capacity is then assessed by visualizing and counting the number of cells that pass through the membrane during the experiment.

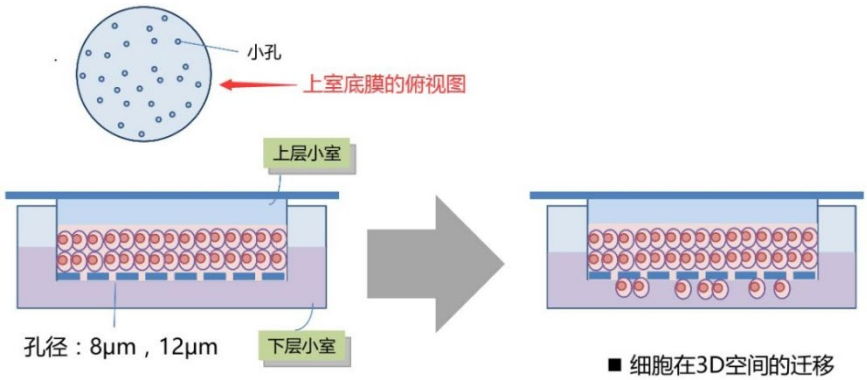


Figure 1 Transwell chamber principle

III. Materials and reagents

3.1 Materials

T25 cell culture bottle

Pipette tips and 15ml centrifuge tubes were purchased from Axygen

Serological pipettes were purchased from a domestic company

Micropipettes: Eppendorf

Transwell chamber (8μm aperture)

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)

4% polyformaldehyde

Crystal violet

IV. Experimental instruments

Clean bench: Suzhou purification equipment factory

Benchtop low-speed centrifuge at room temperature: Eppendorf

Inverted phase contrast microscope

V. Experimental steps

5.1 T24 wild-type cell lines and low, medium and high drug-resistant T24-RC48 cell lines were inoculated into T25 cell culture bottles respectively.

5.2 After 24 hours, the complete medium in each flask was replaced with McCoy's 5A medium containing 1% FBS for serum starvation for 24 hours.

5.3 Place the Transwell chamber into a 24-well plate and add 500μl McCoy's 5A medium containing 10% FBS to the lower chamber.

5.4 Cells digested with trypsin were prepared into a single cell suspension and the cell concentration was adjusted to 3×10^5 cells/ml.

5.5 Take 100μl cell suspension and add it to the upper chamber of Transwell chamber, with 3

replicates in each group.

5.6 Place the 24-well plate in a 37℃,5% CO_2 incubator for 24 hours.

5.7 The Transwell chamber was removed and gently rinsed with PBS to remove non-migrated cells.

5.8 Gently wipe away the unmigrated cells in the upper inner layer with a cotton swab.

5.9 Cells were fixed with 4% paraformaldehyde for 15 minutes.

5.10 Rinse twice with PBS for 3 minutes each time.

5.11 Stain with crystal violet for 10 minutes.

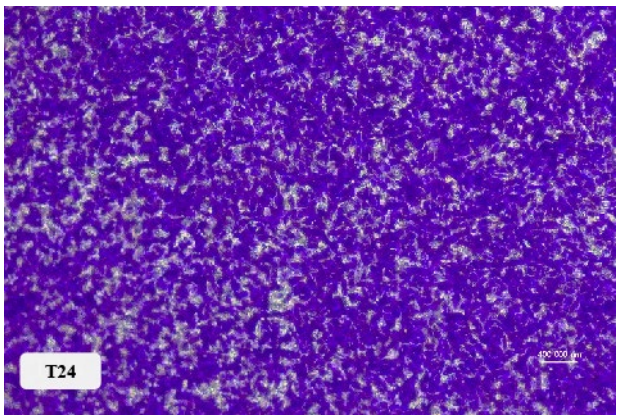
5.12 Rinse off excess stain and air-dry.

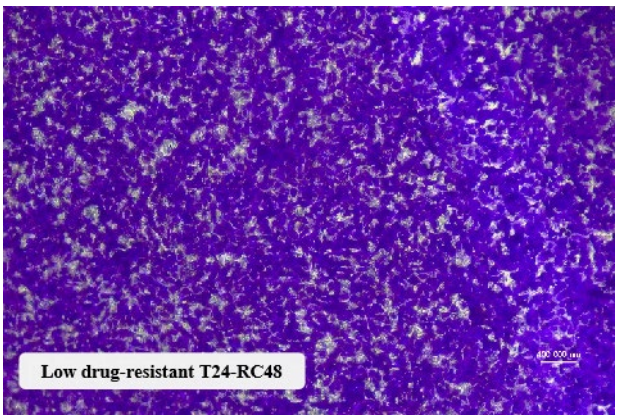
5.13 Place the stained chamber on a moist glass slide and take pictures along the cross line to record the cell migration.

5.14 Use the image analysis software Image J to measure and calculate the number of migrating cells.

VI. Experimental results

In the Transwell cell migration experiment, each chamber contained 30,000 plated cells. Staining results indicated excessive cell density. The cells formed dense clusters on the lower chamber surface of the Transwell microchamber, with severe overlapping that obscured individual cell boundaries. This compromised the accuracy and reliability of cell migration quantification. Although the overall staining was well-performed with uniform coverage and clear background, the high cell density caused extensive overlap that significantly compromised the quantitative analysis of experimental outcomes.





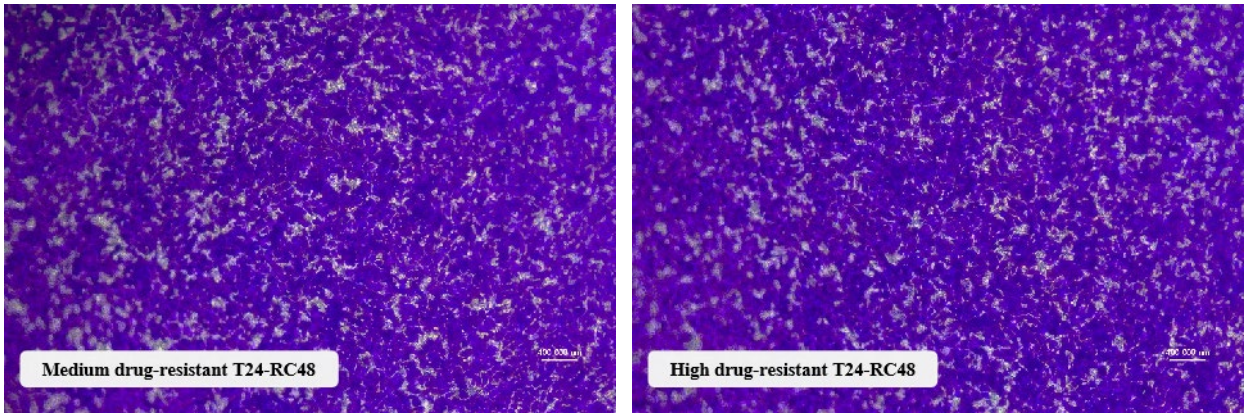


Figure 2 Transwell chambers stained with crystal violet under inverted phase contrast microscopy

VII. Results analysis

The primary cause of the aforementioned issues may stem from excessive cell density during plate seeding. To address this, the next experiment will adjust the cell count per chamber to 10,000 cells to reduce overlapping and achieve a more uniform distribution, thereby enabling clearer identification of cell boundaries. By lowering the cell density, we expect to significantly improve cell distribution patterns, enhance the accuracy of migrating cell count statistics, and provide more reliable experimental data for subsequent studies.