Experiment time: 2025-05-24, 18:00 - 2025-05-29, 21:00

Transwell Migration Assay (II)

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.

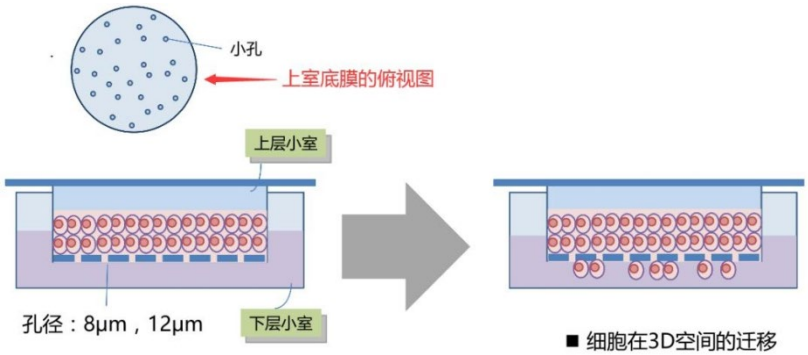

Figure 1 Transwell chamber principle

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

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 1×10^5 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

After adjusting the cell density (10,000 plated cells per chamber), the distribution of cells on the lower surface of the Transwell chamber was significantly improved. Crystalline violet staining revealed that migrating cells were uniformly dispersed with clear individual boundaries and no significant overlap (Figure 2). Quantitative analysis using ImageJ showed that the number of T24 wild-type cells migrating per field was (752±31.6), while low-drug resistance T24-RC48 cells migrated (894±144.3) per field, medium-drug resistance T24-RC48 cells (978±133.9) per field, and high-drug resistance T24-RC48 cells (1252±338.3) per field (Figure 3). Statistical analysis demonstrated no significant differences between wild-type and low-drug resistance groups ($p=0.6057$) or wild-type and medium-drug resistance groups ($p=0.2394$), but a highly significant difference between wild-type and high-drug resistance groups ($p<0.01$).

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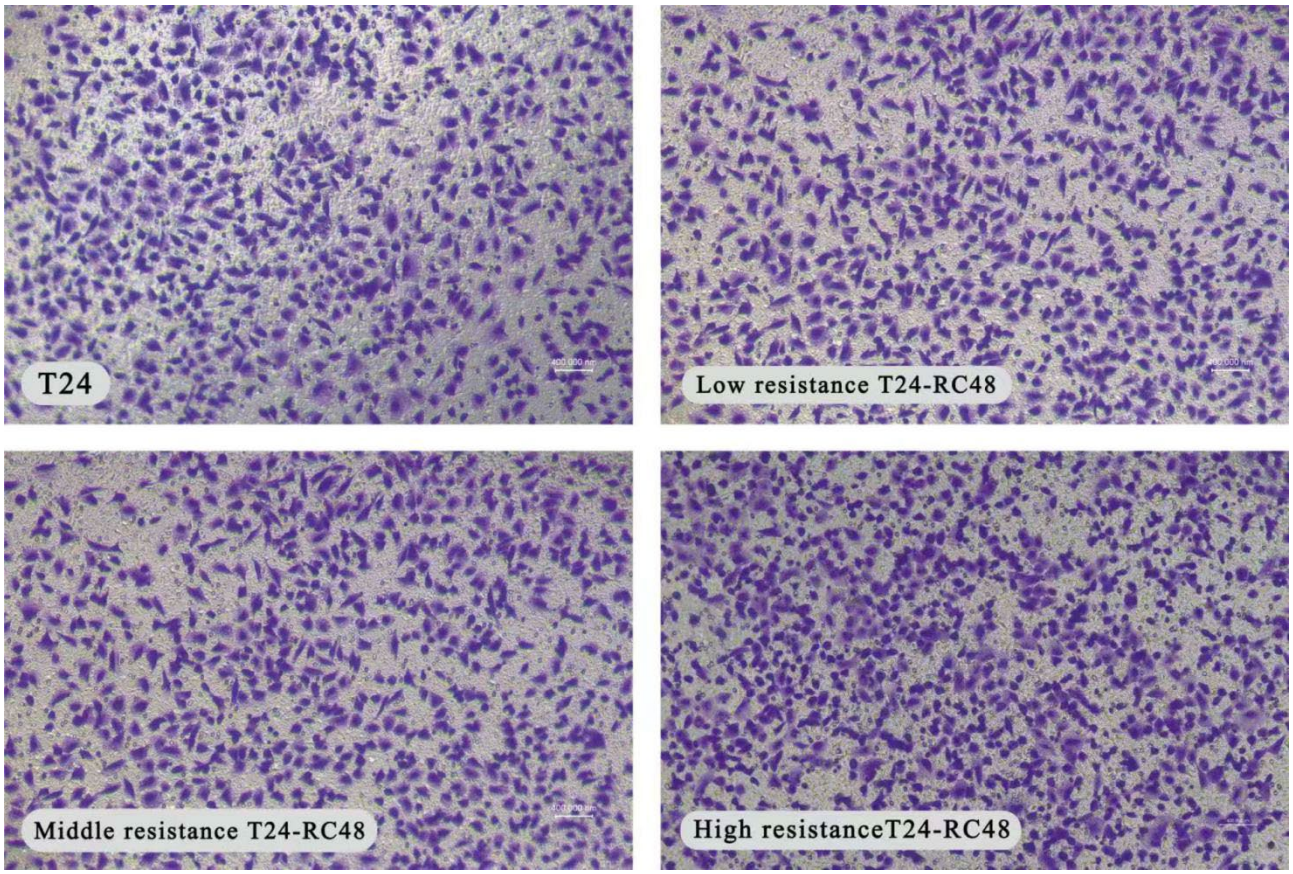

Figure 2 Transwell chambers stained with crystal violet under inverted phase contrast microscopy

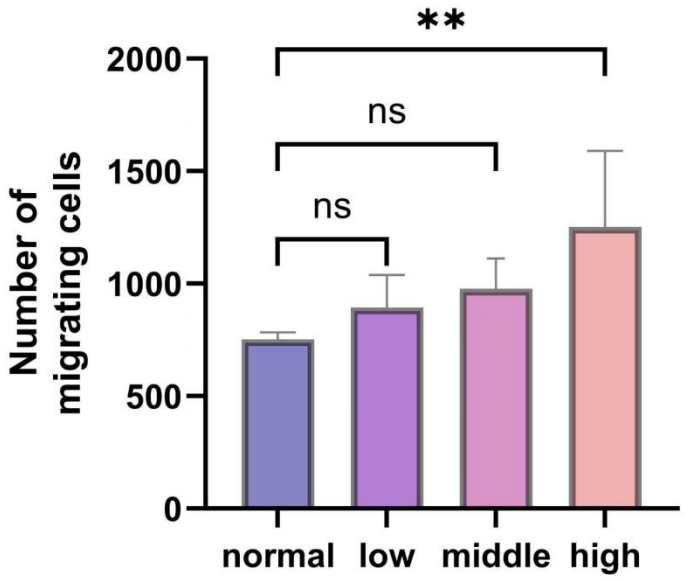

Figure 3 Cell migration of T24 wild type and low, medium and high drug resistant T24-RC48 cell lines

VII. Results analysis

By reducing cell density to 1×10^5 cells/ml, this experiment effectively prevented cell overlap

and significantly improved the accuracy of migrating cell counts. Results showed that T24-RC48 highly drug-resistant cells exhibited significantly enhanced migration capacity compared to wild-type cells ($p<0.01$), while low-and moderate-drug-resistant cells showed no statistically significant difference. This suggests that high drug resistance may promote migration through specific mechanisms.

Further analysis revealed that optimizing cell density was crucial for the experiment's success: In the initial trial, high-density culture caused cell aggregation that obscured the true migration capacity of highly drug-resistant cells. The adjusted protocol significantly improved data reliability. The difference between wild-type and highly drug-resistant groups may relate to their tolerance to chemotherapeutic agents like ADCs, while highly drug-resistant cells might activate migration-related signaling pathways to evade drug-induced cell death. Subsequent studies could combine molecular mechanisms experiments (e.g., Western blotting of migration markers) to further elucidate the correlation between drug resistance and migration capacity, providing new insights for understanding bladder cancer resistance mechanisms.