Experiment time: 2025-05-31, 18:00 - 2025-06-04, 21:00

Transwell Invasion Assay (I)

I. Objective of the experiment

This experiment aims to use Transwell invasion experiment to preliminarily detect the invasive ability of medium and high drug-resistant T24-RC48 cell lines, investigate the relationship between drug resistance and cell invasion behavior, establish optimal conditions for formal Transwell invasion experiment, and provide experimental basis for understanding the drug resistance mechanism of bladder cancer ADC.

II. Experimental content

2.1 Experimental design

Sample type: moderately resistant/highly resistant T24-RC48 cell line.

Cell density: 10,000 cells per well.

Number of replicates: 3 per group.

Matrix gel: Transwell chambers were pre-coated with Matrigel matrix gel (diluted 1:8)

2.2 Measurement principle

The Transwell invasion assay enhances the migration experiment by incorporating a Matrigel barrier. In this setup, the upper chamber of the Transwell microplate is pre-coated with Matrigel to simulate the extracellular matrix environment. Cells must secrete proteases to degrade the Matrigel before migrating through the permeable membrane into the lower chamber. The invasion capability of cells is assessed by quantifying the number of cells that penetrate the Matrigel barrier.

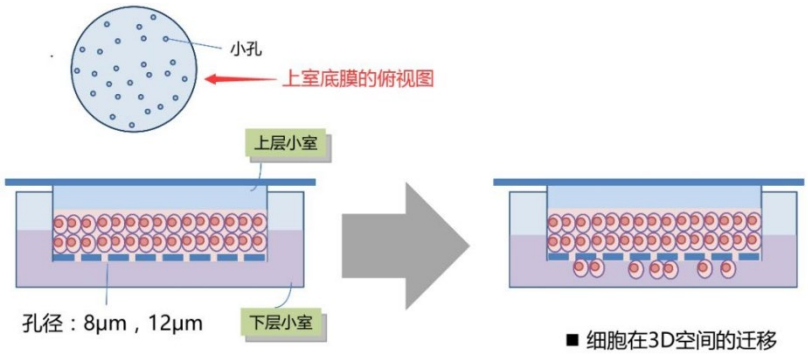

Figure 1 Transwell chamber principle

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)

Medium- and high-drug-resistant T24-RC48 cell lines

3.2 Reagents

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

Matrigel matrix gel was purchased from Corning, part number 354234

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 The medium and high resistant T24-RC48 cell lines were inoculated into the T25 cell culture bottle respectively.

5.2 After 24 hours, the complete medium of each cell bottle was replaced with McCoy's 5A medium containing 1% FBS for starvation treatment for 24 hours.

5.3 Under condition 4℃ (on ice), Matrigel was diluted with serum-free McCoy's 5A cell culture medium at 1:8.

5.4 Take 60μl and add it uniformly to the upper surface of the Transwell chamber. Incubate in the 37℃ incubator for 3h to make the matrix gel into a film.

5.5 Following polymerization, the excess liquid was carefully aspirated from the upper chamber, and 100μl of low serum medium was added to each chamber. The incubator was placed for 30min to carry out substrate membrane hydration.

Tips:

(1) The gun head is gently pushed along the wall of the chamber to push out Matrigel without bubbles, and avoid poking the filter membrane of the chamber.

(2) The volume of the added Matrigel glue should not be too large, and the polycarbonate film can be soaked.

(3) Matrigel is easy to solidify at too high or too low temperature, so the gun head and other equipment required for operation should be pre-cooled at 4℃.

(4) Ensure that the liquid surface is level when laying the glue, and the thickness of the glue is uniform. Do not produce bubbles.

5.6 Add 10% serum McCoy's 5A medium 600μl/cell, and then place the Transwell chamber into the 24-well plate with forceps.

5.7 Cells digested with trypsin were prepared into a single cell suspension and the cell concentration was adjusted to 1×10^5 cells/ml.

5.8 Take 100μl cell suspension and add it to the upper chamber of Transwell chamber, with three replicates per group, and continue to culture for 48h.

5.9 Add 800 μl PBS to the blank culture well, gently place the chamber into which the medium has been sucked out, and gently washed (care was taken throughout all washing steps to avoid scraping or dislodging the migrated cells on the lower surface of the membrane).

5.10 Gently wipe off the inner layer of cells in the chamber: use a cotton swab with a slightly loosened tip to ensure thorough cleaning, including the edges. After wiping, rinse the inner wall with PBS.

5.11 Transfer the chamber to a new well containing 800μL of 4% paraformaldehyde (PFA) per well and fix at room temperature for 15 min; then wash twice with PBS, 2 min each time (all washes were performed by gentle immersion without shaking or swishing).

5.12 Transfer the chamber to a blank culture hole with 600ul/ hole of crystal violet dye, and stain for 10min; after staining, wash off the floating color with clean water twice, each time for 2min.

5.13 Place the stained chamber on a clean glass slide, and pay attention to not completely dry it. Slightly moistening will make the cell morphology better; capture images according to a predefined pattern: first along the crosshairs, and then at each of the four corners.

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

VI. Experimental results

In this experiment, the invasive ability of each group of cells was low. Crystal violet staining showed that only a small number of cells were scattered on the surface of the lower chamber of Transwell (Figure 2).

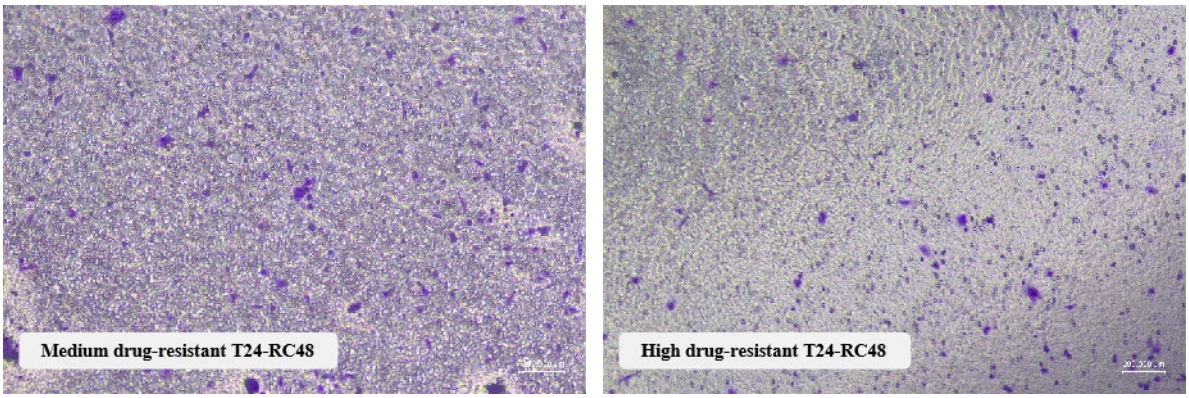

Figure 2 Transwell chambers stained with crystal violet under inverted phase contrast microscopy

VII. Results analysis

The results indicated that the number of invaded cells in all groups was significantly lower than anticipated. Possible reasons include: 1) The formation of effective migrating cell clusters was challenging with 10,000 cells per well in the 48-hour invasion assay, particularly under the matrix gel barrier where cell penetration efficiency decreased further; 2) The invasion assay may require longer time to complete matrix gel degradation and migration processes; 3) The pre-placed Matrigel dilution ratio or thickness might hinder cell invasion; 4) Insufficient serum concentration in the lower chamber failed to establish an effective concentration gradient with the upper chamber. Furthermore, the reuse of Transwell chambers led to high background staining after fixation, compromising the clarity of

observation.

In the next experiment, we will employ a new Transwell chamber with 50,000 cells per well to increase cell penetration. We will appropriately adjust the serum concentration in the lower chamber to establish an effective gradient. Incubation time may also be extended to ensure complete matrix gel degradation. Additionally, we will optimize matrix gel conditions by testing different Matrigel dilution ratios to evaluate their impact on invasion efficiency. These adjustments are expected to significantly enhance cell invasion numbers, providing reliable data for subsequent analysis of the correlation between drug resistance and invasive capacity.