NOV 30, 2023

DOI:
dx.doi.org/10.17504/protocols.io.5qpvo3wn9v4o/v1

Protocol Citation: Hussain Zubair 2023. Principles, Techniques, and Precautions in Transwell Migration and Invasion Assays for Cancer Cell Metastasis Research. **protocols.io** https://dx.doi.org/10.17504/protocols.io.5qpvo3wn9v4o/v1

Protocol status: Working
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Created: Nov 30, 2023

Principles, Techniques, and Precautions in Transwell Migration and Invasion Assays for Cancer Cell Metastasis Research

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ABSTRACT

This article outlines a comprehensive procedure for conducting Transwell assays, a critical technique in cancer biology for assessing cell migration and invasion - key factors in cancer metastasis. The aim is to evaluate the migratory and invasive capabilities of cells, and the effectiveness of drugs influencing these processes. The experiment employs Human Umbilical Vein Endothelial Cells (HUVEC) and utilizes a range of tools and reagents including cell culture media, FBS, PBS, DMSO, P/S, trypsin, and matrix gel. The assay involves coating Transwell chambers with matrix gel for invasion assays, cell starvation in serum-free medium, and subsequent seeding in chambers for migration or invasion analysis. The detailed method emphasizes maintaining specific conditions like temperature and concentration, particularly when handling the matrix gel. Post-experiment, cells are fixed, stained, and analyzed qualitatively and quantitatively using microscopy and ImageJ software. This protocol provides a robust framework for studying the mechanisms of cancer cell metastasis, contributing to the broader understanding of tumour progression and potential therapeutic strategies.

Last Modified: Nov 30, 2023

PROTOCOL integer ID: 91616

I. Experimental Objective

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1. To determine the migratory ability of cells.
2. To determine the invasive ability of cells.
3. To evaluate the efficacy of drugs that affect cell migration and invasion.

II. Background Introduction

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Tumor metastasis is a complex and critical process in the progression of cancer. In this process, cancer cells spread from the primary tumour to other parts of the body, forming secondary tumours or metastases. This series of processes is collectively known as the invasion-metastasis cascade. The process of metastasis from the primary tumour can be divided into several key steps as shown in **Figure 1**:

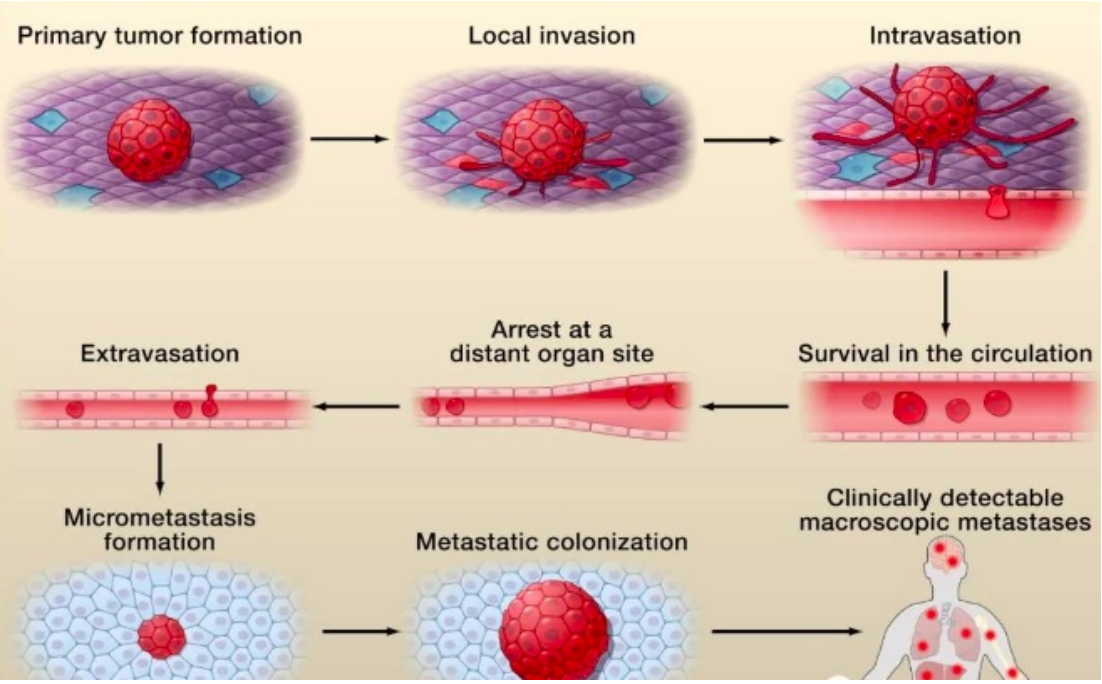

Figure 1: The Process of Tumor Cell Metastasis in the Body

1. Local Invasion: Tumor cells invade the extracellular matrix (ECM) and the matrix cell layer of nearby cells. The first step of metastasis is the invasion of the surrounding tissues by tumour cells. The secretion of extracellular proteases, such as matrix metalloproteinases (MMPs) by primary tumour

cells, can degrade the extracellular matrix (ECM), allowing tumour cells to penetrate surrounding tissues and enter the circulatory system.

2. Intravasation: Entering the lumens of lymphatic vessels or blood vessels. After the local invasion, some cancer cells enter nearby blood vessels or lymphatic vessels. This process is known as intravasation. These vessels provide a pathway for cancer cells to spread to distant parts of the body.

3. Survival in the Circulation: Cancer cells that enter the blood or lymphatic system face significant challenges, including shear stress, immune surveillance, and anoikis (a type of cell death that occurs when cells detach from the ECM). To survive in the circulatory system, cancer cells may form clusters or masses and evade immune detection, these cells are known as circulating tumour cells (CTCs).

4. Arrival at Anatomically Distant Organ Tissues: Once tumour cells enter the circulation, they are carried throughout the body. Eventually, they may become lodged in the capillary beds, where smaller vessels limit their movement. This stasis is due to interactions between adhesion molecules on cancer cells and endothelial cells of vessels at the secondary site.

5. Extravasation: Entering normal organ tissues at distant sites. To form secondary tumours, cancer cells must exit the bloodstream or lymphatic vessels. The adhesive properties of cancer cells change, and they penetrate the endothelial cells and pericytes that separate the vascular lumen from the interstitial microenvironment, a process known as extravasation.

6. Initial Survival and Micrometastasis in the New Environment: Once cancer cells successfully extravasate, they face the challenge of surviving in their new microenvironment at distant tissues. These include evading immune responses, adapting to the new microenvironment, and proliferating to form secondary tumours. The microenvironment at the secondary site may differ from that of the primary tumour, including types of stromal cells, ECM components, available growth factors and cytokines, and even the microarchitecture of the tissue itself. Cancer cells need to adapt to these changes to survive and grow.

7. Metastatic Colonization: Re-initiating the proliferation program and forming metastatic foci. After undergoing these seven steps, the primary tumour can produce macroscopic, clinically detectable tumour growth, often referred to as metastatic colonization. To promote their growth, metastatic cancer cells often induce the formation of new blood vessels through a process known as angiogenesis. This ensures a continuous supply of nutrients and oxygen to support the development of the secondary tumour. Secondary tumours can continue to invade surrounding tissues and may further spread through the bloodstream or lymphatic system. This process leads to the formation of multiple secondary tumours in different parts of the body [1, 2, 3]

III. Introduction to the Principle of Transwell Assay

3 The Transwell experiment, also known as Transwell migration or invasion assay, is a laboratory technique in cell biology and molecular biology used to study the movement of cells through a porous membrane. It is commonly used to study cell migration, chemotaxis, and invasion in response to

various stimuli such as growth factors, chemokines, or components of the extracellular matrix [4]. The Transwell experiment is particularly useful for assessing the ability of cells to migrate or invade through a porous barrier, where the porous barrier simulates the physiological conditions cells must traverse in the body's tissues. The Transwell experiment involves Transwell chambers (inserts) and well plates, which are devices consisting of a permeable membrane separating two compartments. Typically, the top chamber is filled with cells, while the bottom chamber contains chemical attractants or substances that induce cell migration. The porous membrane allows cells to migrate from the top chamber to the bottom chamber through small pores in the membrane.

The Transwell experiment is divided into two types based on whether a matrix gel is added to the chamber **(Figure 2)**:

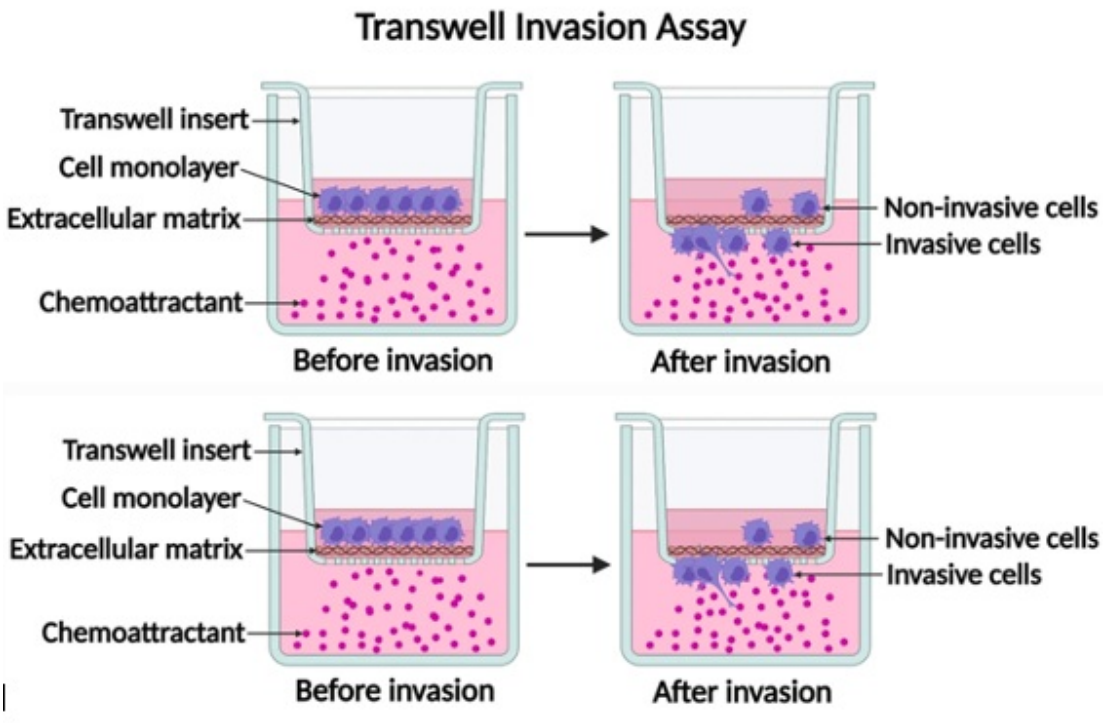

Figure 2: Transwell Assay Principle

1. Migration Assay (without matrix gel in the chamber): In this type of assay, cells migrate from the top chamber to the bottom chamber, but do not need to degrade or invade the membrane. This type of assay measures the ability of cells to move in response to a chemical attractant gradient.

2. Invasion Assay (with matrix gel in the chamber): In the invasion assay, cells not only migrate through the porous membrane but also need to degrade or invade the extracellular matrix (ECM) layer typically coated on top of the membrane. For example, invasion assays are often used to study the invasive behaviour of tumour cells.

The Transwell experiment is an important tool for in vitro study of cell behaviour, providing insights into various biological processes, including cancer metastasis, immune cell migration, and tissue development. Cell behaviour is quantified by counting the number of cells that migrate or invade to the bottom of the chamber or into the culture medium, or by measuring changes in various cell parameters such as cell motility and chemotactic responses.

IV. Instruments, Consumables, and Reagents

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- HUVEC cells
- Cell counter
- Benchtop centrifuge
- CO_2 incubator
- Inverted microscope
- Pipettes, pipette tips
- 4°C and -20°C refrigerators
- 24-well culture plates
- Transwell chambers
- Petri dishes
- Cotton balls or cotton swabs
- Crushed ice
- Cell culture medium
- FBS (Fetal Bovine Serum)
- PBS (Phosphate-Buffered Saline)
- DMSO (Dimethyl Sulfoxide)
- P/S (Penicillin/Streptomycin)
- Trypsin
- Matrix gel
- 4% Paraformaldehyde fixative
- Crystal violet staining solution

V. Experimental Steps

5 **DAY 1:** 2d

Pre-experimental preparations:

Observe the growth state of the cells, remove culture medium from cells in good condition, and starve in serum-free medium for 24 hours. 24:00:00

Observe the growth state of the cells, remove culture medium from cells in good condition, and starve in serum-free medium for 24 hours. 24:00:00

6 **DAY 2:** 2d 3h 30m

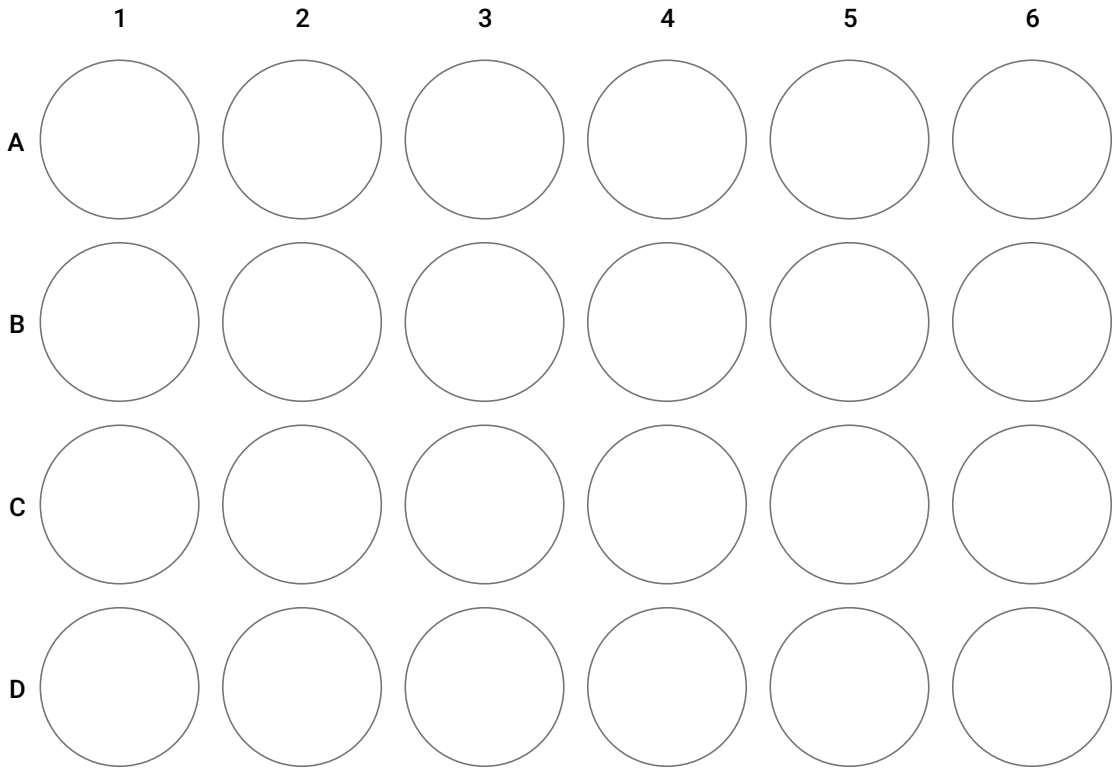
I. Coating matrix gel:

1. Mix matrix gel with pre-cooled pipette tips or pipette guns until uniform.
2. Dilute matrix gel with serum-free medium to 1 mg/mL on ice, and mix until uniform with pre-cooled pipette tips.

3. Add 60 µL of the above mixture vertically into the Transwell chamber to evenly spread it on the bottom, ensuring an even spread without creating bubbles. Then incubate at 37 °C for 1-3 hours. 03:00:00
4. Carefully aspirate unbound matrix gel.
5. Add 100 µL of serum-free culture medium to the chamber and incubate the culture plate at 37 °C for 00:30:00 min for hydration.
6. Remove liquid from the chamber, and check if any liquid has passed through the chamber to the lower compartment, if not, it is ready for cell seeding.

II. Sample preparation and cell seeding:

1. Prepare samples with serum-free medium, it is recommended to set the working solution concentration to twice the final concentration. Then add to the chamber in a **sample: cell suspension ratio of 1:1**.
2. Add 500 µL of complete medium containing 10 % FBS to the lower compartment of a 24-well plate, and place the Transwell chamber in the 24-well plate with tweezers.
3. Digest starved cells, resuspend in serum-free medium, and adjust cell density according to the number of cells seeded in the upper chamber.
4. First, add 100 µL of sample working solution, then add 100 µL of cell suspension, thus the sample concentration is diluted by half to the final concentration.
5. Incubate the 24-well plate at 37 °C, 5 % CO_2, 90 % humidity for 24-48 hours. 48:00:00



7 **DAY 3/4:** 40m

Cell fixation and staining:

1. Remove the Transwell chamber, discard the culture medium, and gently wipe the matrix gel and cells inside the chamber with a PBS-wetted cotton swab or cotton ball.
2. Add 600 µL of 4 % paraformaldehyde fixative to a clean well of a 24-well plate, and place the chamber in it for 00:30:00 min for fixation.
3. Discard the fixative, and wash the inside and outside of the chamber once with PBS.
4. Add 600 µL of crystal violet staining solution to a clean well of a 24-well plate, and place the chamber in it for 00:10:00 min for staining.
5. Remove the chamber, and wash the inside and outside of the chamber three times with PBS.
6. After appropriate air drying, observe under a microscope for qualitative study; take photos of 3-5 fields of view, then use ImageJ for counting and take the average value for the quantitative study.

VI. Example

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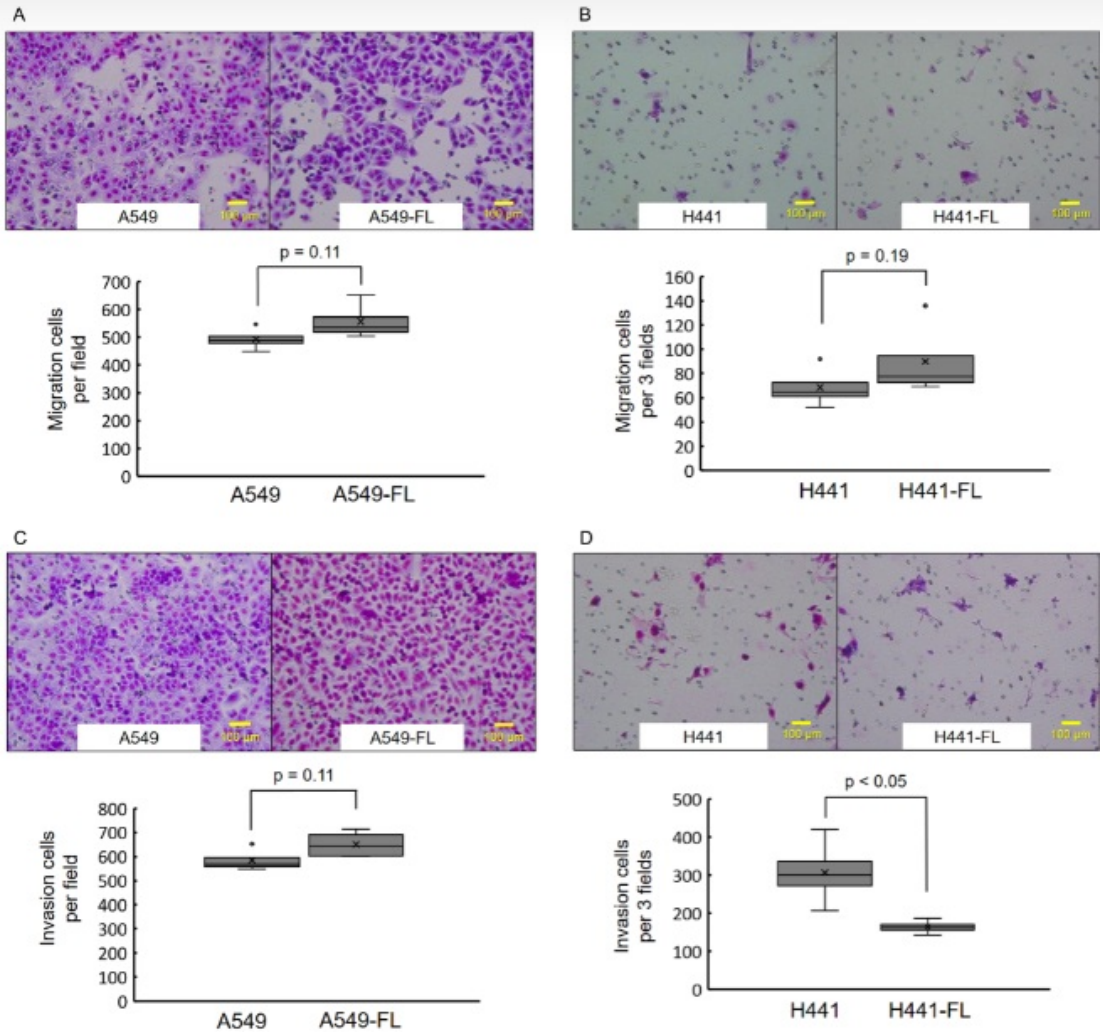

Fig 3: Transwell migration and invasion assays (Example)

VII. Precautions

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1. Matrix gel solidifies in room temperature solvent, so the entire gel laying process needs to be performed on ice.
2. The concentration of the matrix gel is also a factor affecting cell migration and invasion. Therefore, the concentration and dilution ratio of the matrix gel should be adjusted according to the information provided by the supplier and the specific conditions of the experiment. If necessary, set up a preliminary experiment to explore the optimal concentration and dilution ratio. A commonly used concentration is 1 mg/mL.
3. When laying the gel, try to add it vertically to the centre of the bottom of the chamber to avoid the formation of bubbles.
4. When aspirating unbound matrix gel from the chamber, ensure that the tip of the pipette does not scratch the surface of the gel layer.
5. Different cells have different migration and invasion capabilities. A range of cell density gradients can be set to find the appropriate cell seeding density.
6. The process of placing the chamber often produces bubbles. Once bubbles are formed, the chemotactic effect of the lower culture medium is weakened or even disappears, so special attention is needed. If bubbles appear, lift the chamber or use a pipette tip to remove them, and then replace the chamber after removing the bubbles.
7. 1-2 hours after seeding the cells, inspect the culture plate to ensure no large bubbles have formed. Sometimes small bubbles may be observed, but these do not affect cell migration and invasion and can be removed by gently tapping the well plate.
8. 8. When washing the chamber with PBS, avoid physically touching the bottom of the chamber. Prepare several sterile beakers or Petri dishes, pour in an appropriate amount of PBS, and wash in sequence to improve efficiency.
9. Before taking photographs, allow the chamber to dry appropriately, as moisture can affect the microscopic field of view.

VIII. References

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1. Valastyan S, Weinberg RA. Tumor metastasis: molecular insights and evolving paradigms. Cell. 2011;147(2):275-292. doi:10.1016/j.cell.2011.09.024
2. Steeg PS. Tumor metastasis: mechanistic insights and clinical challenges. Nat Med. 2006;12(8):895-904. doi:10.1038/nm1469
3. Wan L, Pantel K, Kang Y. Tumor metastasis: moving new biological insights into the clinic. Nat Med. 2013;19(11):1450-1464. doi:10.1038/nm.3391
4. Justus CR, Marie MA, Sanderlin EJ, Yang LV. Transwell In Vitro Cell Migration and Invasion Assays. Methods Mol Biol. 2023;2644:349-359. doi:10.1007/978-1-0716-3052-5_22
5. Nakano T, Kanai Y, Amano Y, et al. Establishment of highly metastatic KRAS mutant lung cancer cell sublines in long-term three-dimensional low attachment cultures. PLoS One. 2017;12(8):e0181342. Published 2017 Aug 7. doi:10.1371/journal.pone.0181342
6. [6] Wang CG, Lou YT, Tong MJ, et al. Author Correction: Asperosaponin VI promotes angiogenesis and accelerates wound healing in rats via up-regulating HIF-1α/VEGF signaling. Acta Pharmacol Sin. 2022;43(6):1617-1618. doi:10.1038/s41401-021-00777-3

7. Li Q, Liu J, Jia Y, Li T, Zhang M. miR-623 suppresses cell proliferation, migration and invasion through direct inhibition of XRCC5 in breast cancer. Aging(Albany NY). 2020;12(11):10246-10258. doi:10.18632/aging.103182