Tumor Cell Invasion Assay

Yanling Chen*

Department of Immunology, The Scripps Research Institute, La Jolla, CA 92037, USA
*For correspondence: ylchen@scripps.edu

[Abstract] Cell invasion assays have been used to study the interactions between tumor cells and the extracellular matrix (ECM), which not only provides a structural scaffold for the cell, but also contains various biological factors for the survival and growth of the cell. ECM gel contains the basic components of the ECM that provides a structural support for the cell to grow and move. Cells can secrete enzymes that degrade certain components of the ECM to move towards chemoattractants, or to simply establish niches for growth. Metastatic tumor cells often show more invasiveness to the ECM gel due to their higher motility and/or enzymatic activity for degrading ECM components. This protocol describes a tumor cell invasion assay to study the interactions between tumor cells and the ECM.

Materials and Reagents

1. ECM gel (Sigma-Aldrich, catalog number: E1270)
2. Human MDA-MB-231 cell line (ATCC, catalog number: HTB-26™)
3. Dulbecco's modified eagle medium (DMEM) (Life Technologies, Invitrogen™, catalog number: 10313-021)
4. Fetal bovine serum (FBS) (ATCC, catalog number: 30-2020™)
5. Trypsin-EDTA (Life Technologies, Invitrogen™, catalog number: 25200-056)
6. Phosphate buffered saline (PBS) (Life Technologies, Invitrogen™, catalog number: 14190-144)
7. Glutaraldehyde (Sigma-Aldrich, catalog number: G6257)
8. Ethanol (Sigma-Aldrich, catalog number: 459836)
9. Crystal violet (Sigma-Aldrich, catalog number: C3886)

Equipment

1. Cell culture incubator: 37 °C and 5% CO_2
2. Millicell cell culture inserts of 12 mm diameter 8 μm pores (Merck KGaA, catalog number: PI8P01250)
3. Cotton swabs

Copyright © 2011 The Authors; exclusive licensee Bio-protocol LLC.

Procedure

1. Grow cells in DMEM supplemented with 10% FBS.
2. Thaw ECM gel overnight at 4 °C and keep on ice.
3. Chill Millicell insert and plate to 4 °C, keep on ice.
4. Dilute ECM gel in ice-cold, serum-free DMEM to a final concentration of 2 mg/ml.
   Note: ECM gel final concentration may vary, depending on the cell type studied.
5. Add 100 µl ECM gel from step 4 into the upper compartment of the Millicell insert.
6. Immediately incubate the plate, with insert and ECM gel inside, at 37 °C for 2 h. This allows the liquid ECM gel to solidify.
7. Wash cells with 1x PBS and trypsinized the cells. Wash cells 2 times with serum-free DMEM and resuspend in DMEM at 5 x 10^5 cell/ml.
8. To the well of the plate (lower compartment), add 1.5 ml of DMEM supplemented with 10% FBS as attractant. Position the insert into the well, with the bottom of the insert merged in medium.
9. Gently add 1 x 10^5 cells from step 7 to the upper compartment of the insert.
10. Incubate the plate at 37 °C for 24 h.
11. After the incubation period, take the insert out carefully. Cells and the gel in the upper compartment of the insert need to be gently removed by gently wiping the upper side of the membrane with a cotton swab. We recommend to use each clean cotton swab for one wipe only, in one direction and do not swipe in back-and-forth movement. The cotton swab can be slightly moisturized with ddH_2O as needed but be sure to remove any excess water. Several wipes may be needed to completely remove any cell debris on the membrane.
12. Fix the cells on the lower side of the insert membrane with 5% glutaraldehyde for 10 min, followed by staining with 1% crystal violet in 2% ethanol for additional 20 min.
13. Quickly merge the insert in ddH_2O for 3-4 sec to remove excess dye, and immediately drain excess water using a cotton swab. Dry the insert completely.
14. Count the number of cells on the lower side of the filter under a microscope. Randomly choose different views and take average counting.

Acknowledgments

This protocol was developed in the Department of Immunology, Scripps Research Institute, La Jolla, CA, USA and adapted from Repesh (1989) and Valster *et al.* (2005). The work was funded by NIH grants CA079871 and CA114059, and Tobacco-Related Disease, Research Program of the University of California, 15RT-0104 to Dr. Jiing-Dwan Lee (Chen *et al.,* 2009).

Copyright © 2011 The Authors; exclusive licensee Bio-protocol LLC.

References

1. Chen, Y., Lu, B., Yang, Q., Fearns, C., Yates, J. R., 3rd and Lee, J. D. (2009). Combined integrin phosphoproteomic analyses and small interfering RNA--based functional screening identify key regulators for cancer cell adhesion and migration. *Cancer Res* 69(8): 3713-3720.
2. Hauc, C. R., Hsia, D. A., Puente, X. S., Cheresh, D. A. and Schlaepfer, D. D. (2002). FRNK blocks v-Src-stimulated invasion and experimental metastases without effects on cell motility or growth. *EMBO J* 21(23): 6289-302.
3. Repesh, L. A. (1989). A new *in vitro* assay for quantitating tumor cell invasion. *Invasion and Metastasis* 9(3): 192-208.
4. Valster, A., Tran, N. L., Nakada, M., Berens, M. E., Chan, A. Y. and Symons, M. (2005). Cell migration and invasion assays. *Methods* 37(2): 208-215.

Copyright © 2011 The Authors; exclusive licensee Bio-protocol LLC.