

Isolation of Primary Breast Cancer Cells from HER2 Transgenic Mice

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[Abstract] HER2 is a tyrosine kinase receptor, which is overexpressed in about 30% of breast cancer patients. Its overexpression leads to mammary tumorigenesis and increased invasion and metastasis (Slamon *et al.*, 1987). HER2 transgenic mouse (FVB/N-MMTVneu mouse) is a well-established model of mammary tumor in human (Fantozzi and Christofori, 2006). Although *in vivo* models are excellent for assessing the influence of various factors, especially microenvironment, on development of breast cancer, a convenient and less costly way to study the underlying molecular events is utilizing cells derived from the model under evaluation. In order to explore the molecular mechanism by which HOXB7 inhibits initiation, but promotes metastasis of breast tumors, we generated mouse breast cancer cell line from HER2 transgenic mouse (Liu *et al.*, 2015). This protocol may be useful for the generation of breast cancer cell line from mice with other genetic backgrounds.

Materials and Reagents

1. Cell strainer, sterile (100 μ m) (Corning, Falcon®, catalog number: 352360)
2. 10 cm standard tissue culture dish (Corning, catalog number: 430293)
3. 50 ml conical tubes, sterile (Corning, Falcon®, catalog number: 352070)
4. Disposable scalpels, forceps and scissors (VWR International)
5. HER2 transgenic mice (THE JACKSON LABORATORY, catalog number: 002376)
6. 0.25% trypsin-EDTA (1x) (Thermo Fisher Scientific, Gibco™, catalog number: 25200-056)
7. Phosphate-buffered saline (PBS), sterile (self-preparation)
8. HEPES (Sigma-Aldrich, catalog number: H3375)
9. DMEM/F12-with L-glutamine (Thermo Fisher Scientific, Gibco™, catalog number: 11875-093)
10. Bovine serum albumin solution (BSA) (Sigma-Aldrich, catalog number: A9576)
11. Hydrocortisone (Sigma-Aldrich, catalog number: H0888)
12. Type IV collagenase (Sigma-Aldrich, catalog number: C5138)
13. Hyaluronidase (Sigma-Aldrich, catalog number: H3884)
14. Pen-Strep (GE Healthcare, HyClone™, catalog number: SV30010)
15. Ammonium chloride (NH₄Cl) (Sigma-Aldrich, catalog number: A9434)
16. Tris (Sigma-Aldrich, catalog number: 93362)
17. Fetal bovine serum (FBS), heat inactivated (GE Healthcare, HyClone™, catalog number: SH30071.03)

18. Insulin (with transferrin/selenium) (Thermo Fisher Scientific, Gibco™, catalog number: 51500-056)
19. Digestion buffer (see Recipes)
20. Tris-buffered ammonium chloride (TAC buffer) (see Recipes)
21. Complete media (see Recipes)

Equipment

1. Shaking incubator (Thermo Fisher Scientific, Thermo Scientific, model: SHKA5000)
2. 37 °C, 5% CO₂ cell culture incubator (VWR, symphony™, model: 5.3A)
3. Pipette
4. Refrigerated centrifuge (Eppendorf, model: 5418R)
5. Water bath
6. Hemocytometer
7. Inverted microscope (Olympus Corporation, model: CKX41)
8. Tissue culture hood equipped with UV light source (Labconco, model: Purifier Logic+ Class II, Type A2 Biosafety Cabinet)

Procedure

A. Processing solid tumor tissue into single cells

1. Take out tumor from mouse mammary gland using forceps and scissors (Figure 1), and place it in a 10 cm Petri dish, pouring sterile DMEM/F12 medium on the tissue to keep it moist.



Figure 1. Spontaneous breast tumor in MMTV-HER2 mice

2. Cut the tumor into pieces using a scalpel, removing necrotic tissue if present. Necrotic tissue is normally darker and softer compared to the surrounding tumor.
3. Mince the tissue using disposable scalpels until finely chopped (Figure 2A and 2B), and transfer the minced tissue to a 50 ml conical vial.

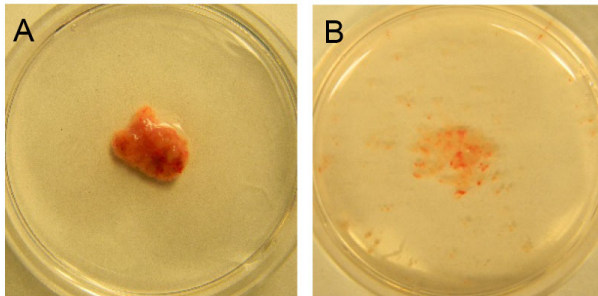


Figure 2. Tumor tissue from spontaneous tumor. A. Before mincing; B. After mincing.

4. Add 10 ml digestion buffer (about 0.5 cm³ in 10 ml digestion buffer).
5. Close the tube with a cap and wrap the cap with Parafilm. Place the tube in a 37 °C shaker at low to moderate speed (e.g., 100-200 rpm) for 1-2 h.
6. Pipette up and down the digested tissue and centrifuge at 530 x g at room temperature for 5 min to pellet the cells.
7. Aspirate the supernatant, which contains fat. If the pellet contains red blood cells (observed as a red layer on top of the pellet), resuspend the pellet in 5-10 ml of TAC buffer and incubate 3-10 min in a 37 °C water bath. Centrifuge at 530 x g at room temperature for 5 min. Repeat this step until the red blood cells are no longer visible.
8. Resuspend the pellet in 10 ml of DMEM/F12 medium (room temperature, without supplements). Filter the digested tissue using cell strainer.
Note: It is not necessary. Normally, I do it because I need to use it for FACS or sorting.
9. Centrifuge at 530 x g at room temperature for 1 min. Resuspend the pellet in 10 ml of DMEM/F12 medium (room temperature, without supplements).
10. Perform a trypan blue exclusion test using a hemocytometer to determine the number of viable cells per ml.
11. Seed 4-6 x 10⁶ viable cells into 10 cm dish with complete media. Add 1x Pen-Strp.

B. Generating tumor cell line

1. Split cells once it is confluent. Normally the cells grow very fast in the first 3 passages.
2. Purify tumor cells via removing fibroblast. Fibroblasts are often more sensitive to trypsin and can therefore be removed from the plate while the tumor cells adhere for a longer time. So treating the cells with trypsin (normally 2-3 min at 37 °C), and constantly monitor it under a microscope. Once the fibroblast (blue arrowhead) detached from the plate, while the tumor cells (red arrowhead) still adhere on the plate (Figure 3A and 3B), gently suck trypsin and wash with

PBS. Then trypsinize and transfer the tumor cells to a new plate. After repeating this step for several passages, the majority of cells are tumor cells.

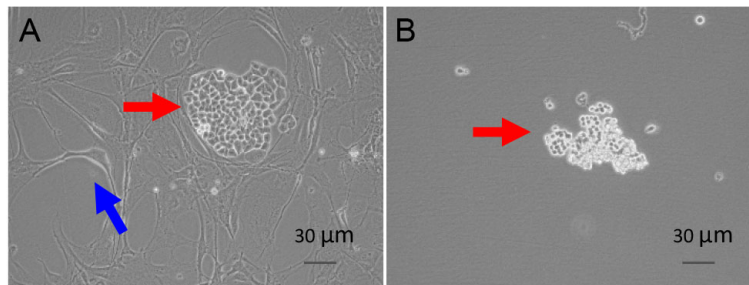


Figure 3. Purification of tumor cells. A. Before trypsinization; B. After trypsinization. Blue arrowhead: fibroblast; red arrowhead: tumor cells.

3. Passaging cells every 5-7 days after 3-4 passages. In the course of passage, the cells grow slowly or even no growth. Over 90% of cells die.
4. After passaging 8-9 times, the cells are immortalized, and grow faster and faster. Figure 4 shows the morphology of tumor cells at different passage.
5. The morphology of generated tumor cell line is shown in Figure 5. And the expression of HER2 is examined by IHC (Figure 6A) and Western (Figure 6B).
6. The tumorigenicity of generated tumor cell line is tested by inoculating cells into the mammary gland fat pad of HER2 transgenic mice (Figure 7).

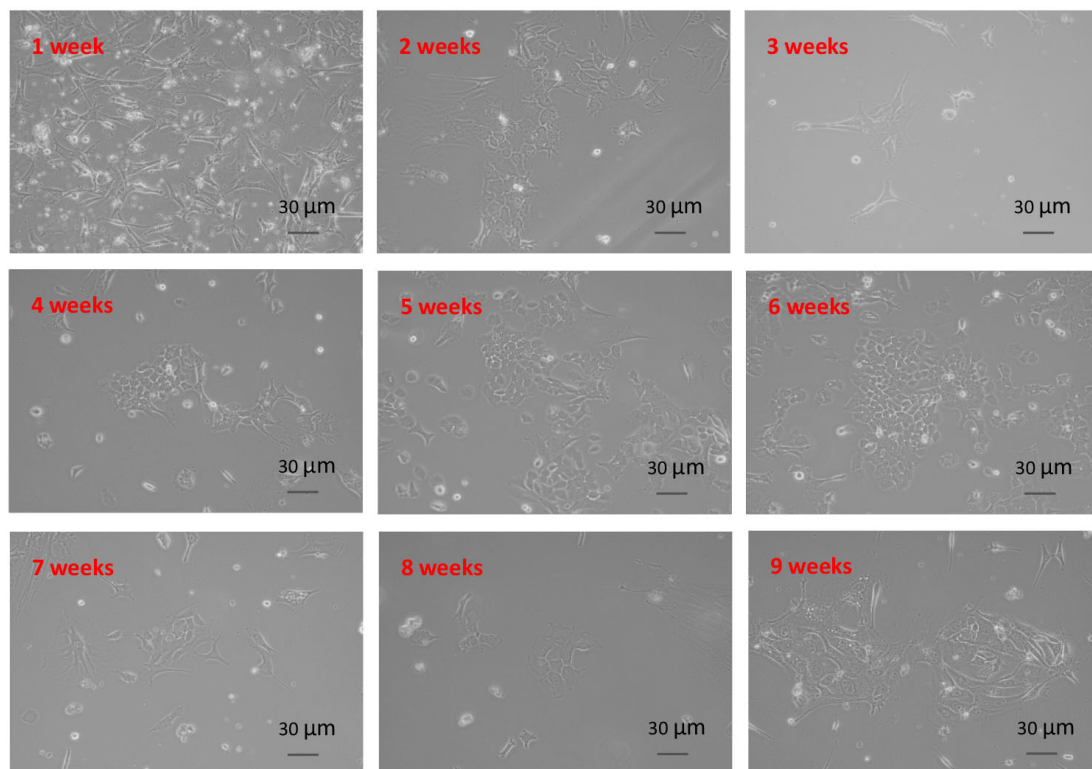


Figure 4. Morphology of tumor cells at different passages

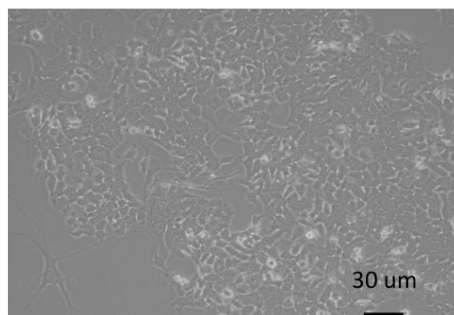


Figure 5. Representative tumor cell line

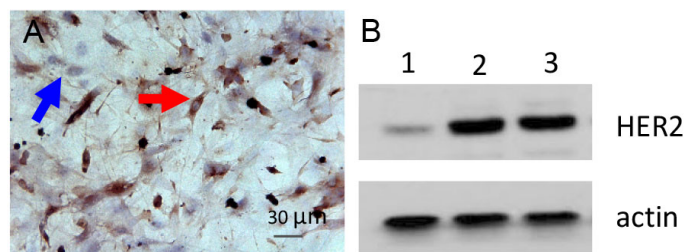


Figure 6. HER2 expression in tumor cells. A. IHC staining for HER2 in tumor cells (blue arrowhead: fibroblast; red arrowhead: tumor cells). B. HER2 expression in tumor cell line (1: MCF10A-Vector; 2: MCF10A-HER2; 3: Representative cell line).



Figure 7. Tumorigenicity of established tumor cell line. Tumors formed in HER2 transgenic mouse after inoculating representative cell line in 10^4 /site for 4 weeks.

Notes

1. Based on what I did, stable cell lines can be generated in about 30% of tumor tissues.
2. The cells start to proliferate after passaging 8-9 times or more. I think it is a sign for immortalization. However, I do not know the underlying mechanism.

Recipes

1. Digestion buffer
DMEM-F/12 supplemented with:
10 mM HEPES
2% BSA
0.5 μ g/ml hydrocortisone
1 mg/ml IV collagenase
0.1 mg/ml hyaluronidase
1x Pen-Strep
Sterile filter and store at 4 °C.
2. Tris-buffered ammonium chloride (TAC buffer)
0.16 M NH_4Cl
0.17 M Tris
Adjust pH to 7.65 with HCl
To make the working solution:
Mix 90 ml of 0.16 M NH_4Cl and 10 ml of 0.17 M Tris
Adjust to pH 7.2 with HCl
Sterile filter and store at 4 °C.
3. Complete media
DMEM-F/12 supplemented with 10% FBS and 10 μ g/ml insulin.

Acknowledgments

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References

1. Fantozzi, A. and Christofori, G. (2006). [Mouse models of breast cancer metastasis](#). *Breast Cancer Res* 8(4): 212.
2. Liu, S., Jin, K., Hui, Y., Fu, J., Jie, C., Feng, S., Reisman, D., Wang, Q., Fan, D., Sukumar, S. and Chen, H. (2015). [HOXB7 promotes malignant progression by activating the TGF \$\beta\$ signaling pathway](#). *Cancer Res* 75(4): 709-719.
3. Slamon, D. J., Clark, G. M., Wong, S. G., Levin, W. J., Ullrich, A. and McGuire, W. L. (1987). [Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene](#). *Science* 235(4785): 177-182.