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Mouse Models for Tumor Metastasis

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Abstract

Tumor metastasis is the main cause of death of cancer patients. Here we describe two mouse models for investigating tumor metastasis. In the first spontaneous metastasis mouse model, 4T1 mouse breast tumor cells are injected into the mammary gland of host mice and the metastasis of 4T1 tumor cells into the lung are examined with a colonogenic assay. In the second experimental metastasis mouse model, luciferase-labeled MDA-MB-231 human breast tumor cells are injected into the tail vein of NOD-SCID immunodeficient mice and the colonization of MDA-MB-231 tumor cells in the lung are monitored using noninvasive bioluminescence imaging.

Keywords

Tumwor metastasis; 4T1 mouse breast tumor cells; MDA-MB-231 human breast tumor cells; Allograft; Xenograft

1. Introduction

Metastasis is the multistep process wherein a primary tumor spreads from its initial site to secondary tissues/organs (1, 2). Despite the significant improvement in both diagnostic and therapeutic modalities for the treatment of cancer patients, tumor metastasis is still the major cause of mortality being responsible for ~90% of all cancer deaths (3, 4). Therefore, development of therapeutic agents that prevent tumor metastasis is very desirable.

To metastasize, cancer cells have to succeed in invasion, intravasation, survival in the circulation, extravasation, and proliferation within the distant parenchyma (5–7). In our laboratory, we have used two mouse models for tumor metastasis (8–10). The first model is an orthotopic allograft model (also called the spontaneous tumor metastasis model). In this model, 4T1 mouse mammary tumor cells are injected into the second mammary gland of syngenetic Balb/c mice and the spontaneous metastasis to the lung will be analyzed with a colonogenic assay (11, 12). The second model is an experimental metastasis model. In this model, luciferase-labeled MDA-MB-231 human breast tumor cells are injected into the tail vein of NOD-SCID immunodeficient mice and the presence of tumor cells in mice will be detected through the noninvasive bioluminescence imaging (13, 14).

2. Materials

1. 4T1 growth medium: RPMI 1640 medium supplemented with 10% FBS and 1% antibiotic-antimyotic.
2. 100× Antibiotic-antimyotic.
3. 0.25% Trypsin/1 mM EDTA.
4. Phosphate buffered saline.

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5. Collagenase solution: 2 mg/ml collagenase, Type IV, 0.01% DNase I in PBS. Filter the solution through a 0.2 μm filter to sterilize.
6. 6-Thioguanine: Dissolve 40 mg 6-thioguanine with 4 ml 0.1 N NaOH and filter the solution with 0.2 μm filter to sterilize.
7. Selection Medium: 4T1 growth medium supplemented with 10μg/ml 6-thioguanine.
8. Crystal violet staining solution.
9. 70-μm Cell strainer.
10. 60-cm Tissue culture dishes.
11. Dissecting scissors, curved scissors, and dissecting tissue forceps.
12. 4T1 mouse breast tumor cells and MDA-MB-231 human breast tumor cells (ATCC).
13. 6–8-weeks-old female BALB/c mice (Charles River) and 4–6 weeks old female NOD-SCID mice.
14. D-Luciferin: 10 mg/ml, dissolved in PBS and filter to sterilize.
15. Isoflurane, isoflurane vaporizer, induction chamber, oxygen tank, and gas regulator.
16. PEI stock solution: Dissolve polyethylenimine powder to a concentration of 1 mg/ml in water and adjust pH to 7.0 with 5 M HCl. Filter sterilizes and freeze aliquots at −80°C. If PEI precipitates after freeze and thaw just resuspend well before used for transfection. The precipitation does not affect the transfection efficiency.
17. DMEM with or without 10% FBS.
18. Polybrene: 10 mg/ml, dissolved in water; filter to sterilize.

3. Methods

3.1. 4T1 Cell Allograft Metastasis Mouse Model

3.1.1. 4T1 Cell Culture and Harvest

1. Culture 4T1 mouse mammary tumor cells in growth medium in a 37°C, 5% CO_2 tissue culture incubator. 4T1 cells double about every 12 h and should be split 1:5 or 1:10 every 2–3 days (see Note 1).
2. Discard culture medium and wash 4T1 cells in a 10 cm tissue culture plate with 5 ml PBS. Add 2 ml 1× Trypsin/EDTA solution to the plate and swirl to cover the entire plate. Incubate at room temperature for about 2 min and tap the side of the plate occasionally (see Note 2).
3. When the cells detach from the plate, stop the trypsinization by adding 2 ml growth medium to the plate and suspend the cells by pipetting up and down a couple times.
4. Spin down the cells at 500 rpm, room temperature in a bench-top centrifuge. Wash the cells once with 5 ml RPMI starvation medium and resuspend the cells in 1 ml

[1]Overgrowth reduces the viability of 4T1 cells and MDA-MB-231 cells. Therefore, precaution should be taken to avoid overgrowth. This is especially important for 4T1 cells, which is a rapidly proliferating cell line that doubles about every 12 h.

[2]When harvesting the tumor cells for xenograft/allograft, cells should not be overtrypsinized. Overtrypsinization reduces the invasiveness of tumor cells.

RPMI medium. Determine the cell number with a hemocytometer. Dilute the cells to 5×10^6/ml with RPMI starvation medium.

3.1.2. Orthotopic Allograft

1. Shave female Balb/c mice (4–6 weeks old) with a hair clipper around the second mammary gland.
2. Load a 1 ml tuberculin syringe with cells. Attach a 26 gauge ½ in. needle to the syringe; align the bevel of the needle toward the metric numbers on the syringe. Carefully eliminate air bubbles in the needle and syringe (see Note 3).
3. Restrain a 6–8 weeks old female Balb/c mouse with one hand and inject 100 μl 4T1 cell (5×10^5 cells) subcutaneously into the second mammary gland. Palpable primary tumors usually develop within 1 week (see Note 4).
4. Administer mice with antimetastasis agents 1 week after the tumor cell injection. Antimetastasis agents are typically diluted into 100 μl PBS and administered intraperitoneally on a daily basis, although other route of administration or doses could also be used. 100 μl PBS or other vector could be administered as control.

3.1.3. Colonogenic Assay

1. Euthanize the mice 4 weeks after the tumor cell injection or when they are moribund. Spray the mice with 70% ethanol so that the fur on the chest is wet. Open up the chest with dissecting scissors and forceps, and retrieve the lung.
2. Rinse the lung with 2.5 ml PBS and then transfer the lung to another 2.5 ml PBS in a 60-mm tissue culture plate. Mince the lung to pieces as small as possible with curved scissors. Transfer the lung tissue suspension to a 15-ml Falcon tube containing 2.5 ml of collagenase/DNase I solution using a 5 ml tissue culture pipette. Thoroughly minced lung tissue should not clog the pipette.
3. Incubate the mixture in a 37°C shaker and shake the tube at 250 rpm for 2 h.
4. Gently pipette the suspension a couple of times to break up the chunks and filter the solution through a 70 μm cell strainer.
5. Spin down the cells, wash the pellet with 10 ml PBS once and resuspend the cells in 10 ml Selection Medium. Make four 1/10 serial dilutions by removing 1 ml from higher concentration cell suspension and mixing with 9 ml Selection Medium. Culture the cells in a 7% CO_2 incubator at 37°C for 10–14 days to allow the formation of 4T1 cell colonies (see Note 5).
6. Discard the Selection Medium and fix the cells by adding 5 ml methanol to each plate and incubate at room temperature for 5 min. Rehydrate the cells by washing with 5 ml distilled water and stain the cells with 2 ml crystal violet staining solution. Swirl to cover the entire plate with staining solution. Wash the cells a couple of times with distilled water and count the number of colonies in the dilution with about 100 colonies.

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[3]It takes some practice to properly inject 4T1 cells subcutaneously. Proper injection is the key to minimize variation among individual animals. When properly injected, the tumor cell suspension looks like a "bubble" underneath the skin.

[4]To avoid contamination, mice need to be thoroughly sprayed with 70% ethanol before harvesting the lungs for colonogenic assays. The forceps and scissors need to be soaked in 70% ethanol for at least 30 min before starting the experiment, and washed with 70% ethanol before used on a different animal.

[5]Do not disturb the cell culture plates during the colonogenic assay. It would be best to culture the plates in a separate CO_2 incubator committed for this assay. Frequent opening and closing of incubator doors and moving the plates during the 10–14 day incubation may result in the formation of secondary colonies and artificially increase colony numbers.

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3.2. Human Breast Tumor Cell Xenograft Mouse Model

3.2.1. Labeling Human Breast Tumor Cells with Luciferase Using Retroviruses

1. Culture 293T cells in DMEM medium supplemented with 10% FBS and antibiotic-antimyotic agents.
2. Split one confluent plate of 293T cells 1:3 12–16 h before transfection. Feed the cells with fresh growth medium 2–3 h prior to the transfection. The cells should be 60–70% confluent at the time of transfection.
3. We used a triple-fusion protein reporter retrovirus construct encoding herpes simplex virus thymidine kinase 1, green fluorescent protein (GFP) and firefly luciferase (TGL) (9, 13). However, any retroviral vector encoding fire fly luciferase can also be used. Mix luciferase retroviral construct with VSV-G (e.g., Addgene plasmid 12259) and gag-pol (e.g., Addgene plasmid 14487), 5 μg each and dilute the plasmid with 500 μl Opti-MEM medium. Dilute 45 μl PEI stock (1 mg/ml) with 500 μl Opti-MEM and incubate at room temperature for 5 min (see Note 6).
4. Mix the DNA solution and the PEI solution and vortex vigorously for 10 s. Incubate the mixture at room temperature for 15 min (see Note 7).
5. Add the mixture, drop by drop, to a 10-cm dish of 293T cells. Return the cell to the 37°C 5% CO_2 incubator.
6. 12–16 h later discard the medium and feed the cells with fresh growth medium.
7. Harvest retroviruses in the medium 48 h and 72 h after transfection.
8. Centrifuge the medium containing retroviruses at 4,000 × *g* for 30 min at 4°C to remove cell debris. Carefully transfer the supernatant to a transparent centrifuge tube.
9. Centrifuge the supernatant at 48,000 × *g* for 2 h at 4°C. The virus precipitate appears as a small white or yellow pellet on the wall of the centrifuge tube. Carefully remove the supernatant without disturbing the pellet. Resuspend the viruses in growth medium. Typically 1 ml growth medium is used for every 10 ml of supernatant. The retroviruses can be snap-frozen with liquid nitrogen and stored at −80°C or used immediately for infection (see Note 8).
10. To label MDA-MB-231 cells with luciferase, 1 ml concentrated viruses encoding luciferase are added to cells in a 10-cm culture dish (50–70% confluent). Add polybrene to the final concentration of 8 μg/ml.
11. The medium can be changed 12 h post-infection and the cells can be assayed for luciferase activity 72 h post-infection.

3.2.2. Tail Vein Injection of MDA-MB-231 Cells

1. Culture MDA-MB-231 human breast cancer cells stably expressing firefly luciferase in a 37°C, 5% CO_2 tissue culture incubator.

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[6]The VSV-G pseudotyped retrovirus could be prepared using packaging cell lines stably expressing VSV-G and gag-pol, or by co-transfection of 293T cells with plasmids encoding VSV-G and gag-pol. We find that retroviruses produced with co-transfection approach generally have much higher titers.

[7]PEI transfection is an affordable and high efficient substitute for other lipid based transfection reagents such as Lipofectamine 2000. PEI transfection routinely gives us high transfection efficiency close to 100%, and is much more consistent and reproducible than calcium phosphate method.

[8]Biosafety level 2 standard should be taken when handling VSV-G pseudotyped retroviruses. All the utensil and disposable plastics must be treated with concentrated bleach.

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2. Trypsinize to detach cells from the culture plate and determine cell number with a hemocytometer. Dilute the cells with DMEM medium (without FBS) to 5×10^6/ml.
3. Use a heating lamp to dilate the tail vein of female NOD-SCID mice (4–6 weeks old). More than one animal can be heated together in a plastic cage, with a heating lamp above them. Proper dilation of the tail vein is crucial to the success of tail vein injection (see Note 9).
4. Transfer a mouse to a restraining device; properly secure the mouse with the tail exposed.
5. Load a 1 ml tuberculin syringe with luciferase-labeled tumor cells. Attach a 26 gauge ½ in. needle to the syringe; align the bevel of the needle toward the metric numbers on the syringe. Carefully eliminate air bubbles in the needle and syringe. Inject 100 μl luciferase-labeled cells into one of the lateral tail veins.

3.2.3. Noninvasive Bioluminescence Imaging

1. Check the level of isofluorane in the vaporizer and gas level in the oxygen tank to ensure adequate amount of isofluorane and oxygen for the duration of the procedure. Make sure that the system is set to flow to the induction chamber and/or IVIS imaging system.
2. Turn on the supply gas and adjust the flow meter to 500–1,000 ml/min.
3. Place mice in the induction chamber and seal the chamber. Adjust the vaporizer to 2.5–3% and monitor the mice until recumbent. Lift one end of the induction chamber to roll the mice over to ensure that the animals are properly anesthetized.
4. Inject 100 μl d-Luciferin (15 mg/ml) via i.p. into each mouse. Transfer the mice to the IVIS imaging machine. Align the noses of the mice with the nosecones to ensure that the mice stay immobile during the imaging process.
5. Record the luminescence image with the IVIS imaging machine.
6. Image the mice on day 0 (within 2 h after the xenograft), on day 1, and on a weekly basis thereafter.
7. Properly label the mice so that each individual mouse can be identified in the imaging process. Measure the luminescence photon flux signal by selecting a ROI (region of interest) over the thoracic region of the mice. Calculate the normalized photon flux using the following formula:

$$P_{\mathrm{n}}=100 \times (P_{\mathrm{t}}/P_0),$$

where P_{n} is the normalized photon flux, P_{t} is the photon flux of a given date, and P_0 is the photon flux signal of day 0 (see Note 10).

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[9]The proper dilation of tail vein with heating lamp is the key to tail vein injection. When properly dilated, the veins look bulged and the tail feels warm.

[10]The mice need to be properly labeled before the first bioluminescence imaging. The photon flux derived from the first imaging (day 0) should be used to normalize the luminescence signal from subsequent imaging.

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References

1. Weiss L. Metastasis of cancer: a conceptual history from antiquity to the 1990s. Cancer Metastasis Rev. 2000; 19:I–XI. 193–383. [PubMed: 11394186]
2. Fidler IJ. The pathogenesis of cancer metastasis: the 'seed and soil' hypothesis revisited. Nat Rev Cancer. 2003; 3:453–458. [PubMed: 12778135]
3. Hanahan D, Weinberg RA. The hallmarks of cancer. Cell. 2000; 100:57–70. [PubMed: 10647931]
4. Christofori G. New signals from the invasive front. Nature. 2006; 441:444–450. [PubMed: 16724056]
5. Partin AW, Schoeniger JS, Mohler JL, Coffey DS. Fourier analysis of cell motility: correlation of motility with metastatic potential. Proc Natl Acad Sci USA. 1989; 86:1254–1258. [PubMed: 2919174]
6. Aznavoorian S, Murphy AN, Stetler-Stevenson WG, Liotta LA. Molecular aspects of tumor cell invasion and metastasis. Cancer. 1993; 71:1368–1383. [PubMed: 8435813]
7. Condeelis J, Singer RH, Segall JE. The great escape: when cancer cells hijack the genes for chemotaxis and motility. Annu Rev Cell Dev Biol. 2005; 21:695–718. [PubMed: 16212512]
8. Shan D, Chen L, Njardarson JT, Gaul C, Ma X, Danishefsky SJ, Huang XY. Synthetic analogues of migrastatin that inhibit mammary tumor metastasis in mice. Proc Natl Acad Sci USA. 2005; 102:3772–3776. [PubMed: 15728385]
9. Yang S, Zhang JJ, Huang XY. Orai1 and STIM1 are critical for breast tumor cell migration and metastasis. Cancer Cell. 2009; 15:124–134. [PubMed: 19185847]
10. Chen L, Yang S, Jakoncic J, Zhang JJ, Huang XY. Migrastatin analogues target fascin to block tumour metastasis. Nature. 2010; 464:1062–1066. [PubMed: 20393565]
11. Mitsuhashi M, Liu J, Cao S, Shi X, Ma X. Regulation of interleukin-12 gene expression and its anti-tumor activities by prostaglandin E2 derived from mammary carcinomas. J Leukoc Biol. 2004; 76:322–332. [PubMed: 15123779]
12. Pulaski BA, Ostrand-Rosenberg S. Reduction of established spontaneous mammary carcinoma metastases following immunotherapy with major histocompatibility complex class II and B7.1 cell-based tumor vaccines. Cancer Res. 1998; 58:1486–1493. [PubMed: 9537252]
13. Minn AJ, Kang Y, Serganova I, Gupta GP, Giri DD, Doubrovin M, Ponomarev V, Gerald WL, Blasberg R, Massague J. Distinct organ-specific metastatic potential of individual breast cancer cells and primary tumors. J Clin Invest. 2005; 115:44–55. [PubMed: 15630443]
14. Minn AJ, Gupta GP, Siegel PM, Bos PD, Shu W, Giri DD, Viale A, Olshen AB, Gerald WL, Massague J. Genes that mediate breast cancer metastasis to lung. Nature. 2005; 436:518–524. [PubMed: 16049480]