Video Article

Ultrasound Imaging-guided Intracardiac Injection to Develop a Mouse Model of Breast Cancer Brain Metastases Followed by Longitudinal MRI

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Abstract

Breast cancer brain metastasis, occurring in 30% of breast cancer patients at stage IV, is associated with high mortality. The median survival is only 6 months. It is critical to have suitable animal models to mimic the hemodynamic spread of the metastatic cells in the clinical scenario. Here, we are introducing the use of small animal ultrasound imaging to guide an accurate injection of brain tropical breast cancer cells into the left ventricle of athymic nude mice. Longitudinal MRI is used to assessing intracranial initiation and growth of brain metastases. Ultrasound-guided intracardiac injection ensures not only an accurate injection and hereby a higher successful rate but also significantly decreased mortality rate, as compared to our previous manual procedure. *In vivo* high resolution MRI allows the visualization of hyperintense multifocal lesions, as small as 310 μm in diameter on T_2-weighted images at 3 weeks post injection. Follow-up MRI reveals intracranial tumor growth and increased number of metastases that distribute throughout the whole brain.

Video Link

The video component of this article can be found at https://www.jove.com/video/51146/

Introduction

Brain metastasis is the most common intracranial malignancy in adults. The prognosis is extremely poor, with a median survival of 4-6 months even with aggressive treatment. Breast cancer is one of the three major primary cancers with a high morbidity of brain metastasis[1-3]. Several brain-tropic breast cancer lines are capable of developing brain metastases after intracardiac or intracarotid injection[4]. Direct injection of tumor cells into the left ventricle can bypass the lung capillary bed and thus increase the incidence of forming brain metastases while minimizing visceral metastases. The MDA-MB231Br line is one of the most widely used human breast cancer lines to develop brain metastasis in rodent models[5,6].

Like many other studies[4,7], we have performed a manual procedure of intracardiac injection in our previous studies. However, only 50% successful rate was obtained with the manual injection and a fraction of mice died from the repeated invasive procedures if prior trials failed. Here, we are introducing the use of an imaging-guided procedure to secure the injection of brain-seeking breast cancer cells into the left ventricle of athymic mice. Longitudinal high resolution MRI is applied to follow intracranial development of brain metastases.

Protocol

All animal procedures were approved by the Institutional Animal Care and Use Committee of University of Texas Southwestern Medical Center.

1. Preparation of the MDA-MB231/Br-GFP Cells

1. Retrieve and culture the MDA-MB231/Br-GFP cells (kindly provided by Drs. Palmieri and Steeg, NCI) in DMEM medium containing 10% FBS, 1% glutamine and 1% penicillin/streptomycin.
2. Observe the condition of the cells and color of medium, and replace medium every 2-3 days.
3. Trypsinize and collect the cells when 80% confluence is reached as outlined in steps 1.3.1-1.3.5:
    1. Remove the old medium completely and add 5 ml PBS (1x) to wash the cells gently. Remove the PBS from the dish.
    2. Add 1.5 ml Trypsin in the dish; tilt the dish gently to ensure all the cells are covered by Trypsin. Put the dish back to the cell incubator and keep it at 37 °C for 1 min.
    3. Take the dish out of incubator and observe the cells under optical microscope to ensure the cells detach from the dish.
    4. Add 3 ml medium to stop the effect of trypsin. Collect the cell mixture in a centrifuge tube. Centrifuge the mixture at 2,000 rpm for 5 min.
    5. Remove the medium carefully and resuspend the cells in 5 ml serum free medium until homogenous.

4. Count appropriate number of cells and resuspend them in serum free DMEM medium with a final concentration of 1.75 x 10^5 cells in 100 µl volume.
    1. Take 50 µl cell mixture and add 50 µl Trypan Blue. After mixing, take 10 µl mixture and carefully add to the cell counting slide. Use multiple samples to ensure accurate cell number estimation.
    2. Stick in the cell counting slide, one end at a time and the cell counter starts counting automatically. Take average of all the counting results and calculate the total cell number.
    3. Centrifuge the cells again and resuspend them in the appropriate volume of serum free medium to result in the final concentration of 1.75 x 10^5 cells/100 µl volume.
5. Place cells on ice prior to intracardiac injection.

2. Ultrasound Imaging-guided Intracardiac Injection

1. Use female nude mice (BALB/c nu/nu) between 6-8 weeks old.
2. Log in the imaging system. Initialize the transducer 704 (40 MHz).
3. Start a new study and fill in the information.
4. Anesthetize (3% isoflurane/100% O_2 in an induction chamber) and maintain the animals with isoflurane (2%) in 100% O_2 (1 dm^3/min) during the whole procedure via a nose cone. Anesthesia is confirmed when no withdrawal reflex is observed with toe pinch.
5. Set the temperature of the imaging table to 37 °C. Tape the anesthetized mouse to the heated imaging table in supine position.
6. Keep the ultrasound gel at 37 °C prior to imaging. Apply the gel to the chest of the mouse.
7. Mount the transducer in the holder. Lower the transducer till the desired imaging depth is reached. Move the stage until the left ventricle is identified with the ascending aorta as the landmark (**Figure 1A**). Lock the stage when a clear view of left ventricle is visualized.
8. Draw 100 µl of cell mixture into a 1 ml syringe with a 22 G needle. Place and fix the syringe on the syringe mount.
9. Move the syringe forward towards the mouse chest and carefully move it side to side until the needle tip is in the imaging field of view (before entering the mouse).
10. Adjust the needle height and angle to aim down the left ventricle.
11. Penetrate the syringe needle into intercoastal space promptly through skin and muscle layers into the left ventricle under the guidance of ultrasound imaging.
    An indication of successful insertion of the needle into the left ventricle is the reflux of fresh arterial blood (pink color in contrast to dark red venous blood) into the syringe
12. Inject the cell mixture slowly (**Figure 1B**).
13. Upon completion, withdraw the needle, lift up the transducer, clean the ultrasound gel with dampened gauze and remove tape.
14. Prepare a clean cage with a preheated pad.
15. Place the animal on the pad and observe the animal till full recovery.
16. Monitor the behavior of the animal every 24 hr for two days.
17. General conditions and neurologic signs of complications in experimental mice are routinely monitored.

3. MRI Monitoring of Intracranial Tumor Development

1. Use a 9.4 Tesla magnet to monitor intracranial development of brain metastases.
2. Initiate MRI two weeks after tumor implantation and repeat once a week for up to three weeks.
3. Sedate the animals with 3% isoflurane and maintain them under general anesthesia (1.5% isoflurane).
4. Monitor and maintain the animal body temperature and respiration constant throughout the experiment.
5. High resolution multislice (14 slices with 1 mm thick, no gap) T_1- and T_2-weighted coronal images, covering the region from the frontal lobe to the posterior fossa, are acquired with the following parameters: T_1-weighted images: spin echo multiple slice (SEMS), TR/TE = 400 msec/20 msec, matrix: 256 x 256, FOV 20 x 20 mm, in plane resolution: 78 x 78 μm^2. T_2-weighted images: fast spin echo multiple slice (FSEMS) sequences, TR/TE = 2500 msec/48 msec, 8 echo trains, matrix: 256 x 256, FOV 20 x 20 mm, in plane resolution: 78 x 78 µm [28,9].
    Tumor lesions appear brighter than normal brain tissues on T_2-weighted images.
6. Tumor size is determined on T_2-weighted images by manually outlining the enhancing portion of the mass on each image by using MATLAB programs written by us[8].
    Given most of the tumor diameters are smaller than the slice thickness (1 mm), the tumor size is presented as in plane area rather than the volume.

4. H&E Staining Confirming the Metastases

1. Sacrifice the mice immediately after the last MR scan, dissect the tumor-bearing brains and embed it in O.C.T medium and frozen in -80 °C.
2. Section a series of 10 µm-thick coronal brain specimens with cryostat.
3. Perform H&E staining on the brain sections.

Representative Results

With the high spatial resolution of MRI (78 µm in plane resolution), hyperintense lesions can be identified as small as 310 µm in diameter (**Figure 2**). Since the metastases in this study are very small and development of necrosis and edema is minimal, the hyperintense lesion on T_2-weighted images truly represented the tumor mass.

Longitudinal MRI studies allow *in vivo* noninvasive evaluation of tumor growth. As shown in **Figure 3**, the high resolution MRI was able to detect several small lesions 3 weeks after intracardiac injection (**Figure 3A**). On week 4, the lesions that were seen in the previous scan all became larger; more new lesions appeared on T_2-weighted images (**Figure 3B**).

H&E staining revealed either diffuse or cluster type metastatic lesions (**Figure 4**). Enlarged vessels were often seen around the tumor, indicating nonsprouting angiogenesis (**Figure 4D**).

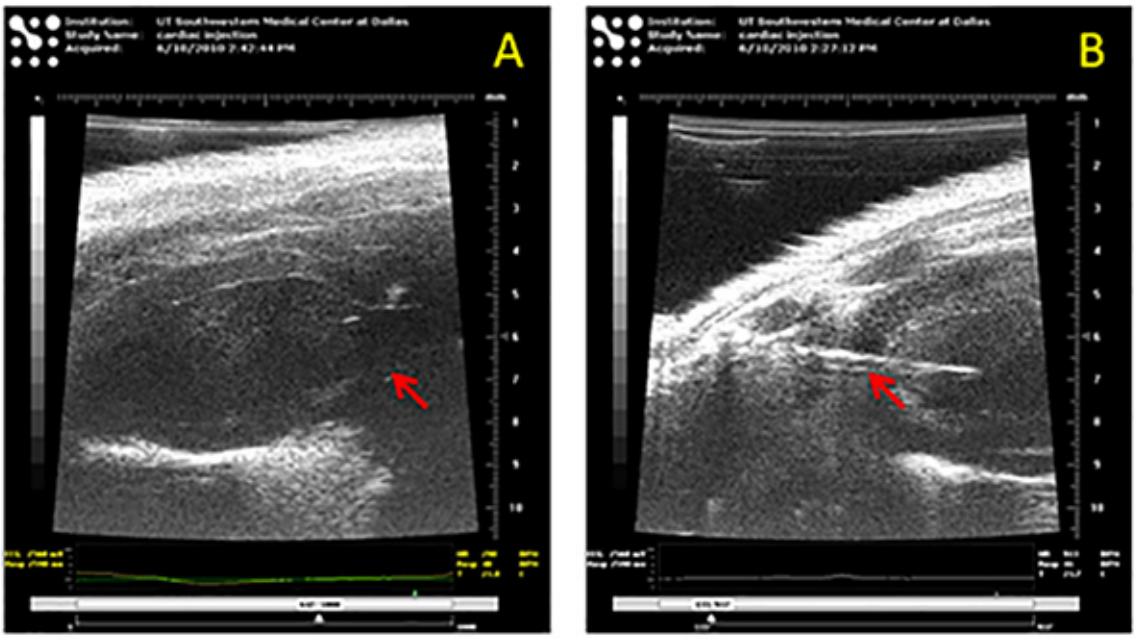

Figure 1. Ultrasound-guided intracardiac injection. (**A**) Identification of the ascending aorta (arrow) as the landmark of left ventricle of the mouse heart. (**B**) A needle (arrow) insertion into the left ventricle to inject the tumor cells. Click here to view larger image.

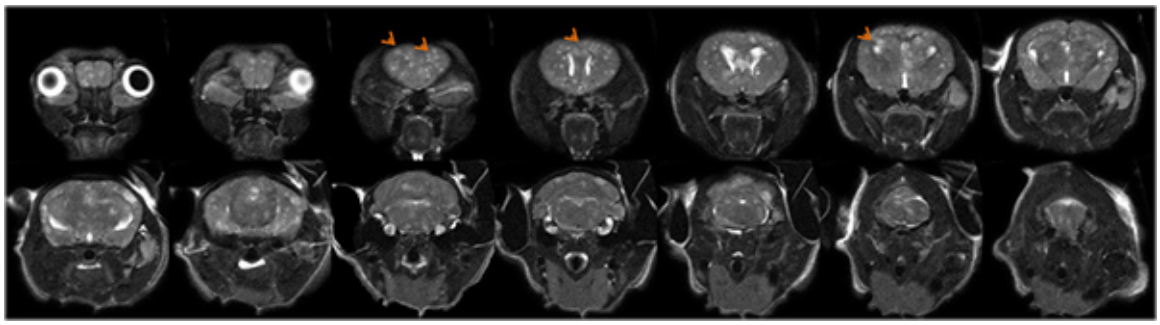

Figure 2. High resolution T_2-weighted images of breast cancer brain metastases. Fourteen consecutive MRI slices of a representative mouse brain, acquired four weeks after intracardiac injection, clearly revealed the multifocal metastases distributing through the whole mouse brain, from olfactory bulb to pontine and medulla. Click here to view larger image.

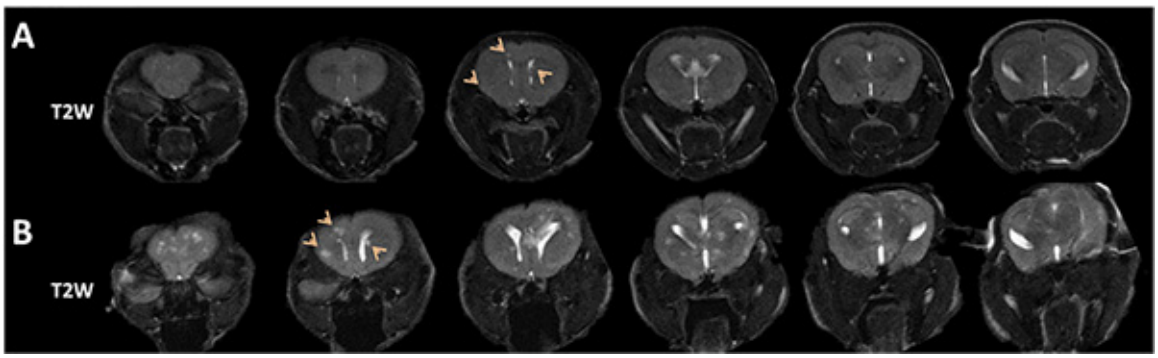

Figure 3. Longitudinal MRI of development of brain metastases. A. Six consecutive coronal MRI sections at week 3 identified multiple lesions with hyper-intensity on T_2-weighted images. **B**. An increased number of lesions appeared on the images at week 4, and those lesions seen on week 3 became larger. Click here to view larger image.

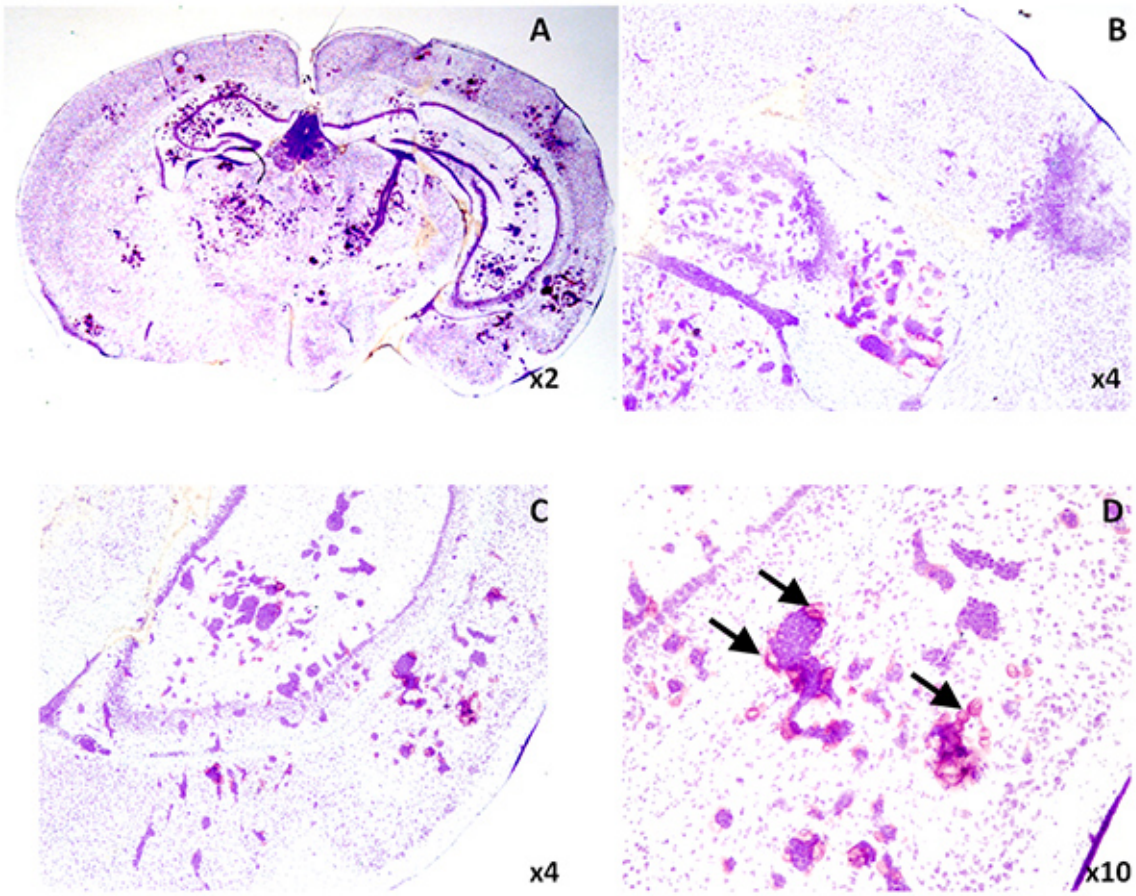

Figure 4. Microscopic lesions were observed on H&E staining. A. A whole mount coronal section depicted multiple lesions. **B-D**. Higher magnification images showed either diffuse or cluster type lesions (**B** and **C**). Enlarged vessels were seen around the tumor. Click here to view larger image.

Discussion

In the present study, we have demonstrated that ultrasound imaging-guided left ventricular injection ensures the accuracy so that every animal in this study developed brain metastases and no animal death was observed. The beauty of image-guided injection is that the path of needle penetration of skin and finally into the left ventricle can be monitored and adjusted under image, which is distinct from the manual procedure requiring strict anatomic landmarks to follow.

Current understandings of intracranial development of brain metastases are largely based on histological studies on animal models[4,10]. However, histological studies normally require a large number of mice that are killed at different time points after tumor implantation. More importantly, information about temporal development in individual lesions is lacking from histological studies.

In vivo imaging promises greater efficiency since each animal serves as its own control and multiple time points can be examined sequentially[7,11]. High resolution T_2-weighted images enable the detection of multifocal tumor initiation at very early stage (lesions with diameter as small as 310 μm are visible). Longitudinal MRI allows individual brain metastases to be examined over time. Moreover, *in vivo* noninvasive MRI will be particularly valuable in longitudinal study of therapeutic response, *e.g.* whole brain radiation.

Formation of multifocal brain metastases is the characteristics of the MDA-MB231Br model. Besides the MDA-MB231/Br-GFP cell line we showed in the present study, the parental clone MDA-MB231/Br and MDA-MB231/Br-luc also exhibit similar metastatic lesions. In contrast, several other brain-seeking lines such as MCF-7Br and 4T1 have been shown to develop a solitary metastasis after intracardiac injection[12]. Both of the models, mirroring clinical counterparts, are useful animal models of brain metastasis. However, in addition to brain metastases, visceral metastases, such as lung and bone metastases are often observed in this model. Establishment of animal models that develop brain metastases exclusively will be critical, in particular, for evaluation of treatment response.

In conclusion, we have demonstrated the usefulness of imaging-guided intracardiac injection to establish a brain metastasis mouse model and the high resolution MRI to assess intracranial development of multifocal metastases in a mouse model.

Disclosures

No conflicts of interest declared.

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References

1. Schouten, L. J. *et al.* Incidence of brain metastases in a cohort of patients with carcinoma of the breast, colon, kidney, and lung and melanoma. *Cancer.* **94**, 2698-2705 (2002).
2. Lin, N. U. *et al.* CNS metastases in breast cancer. *J. Clin. Oncol.* **22**, 3608-3617, doi:10.1200/JCO.2004.01.175 22/17/3608 [pii] (2004).
3. Eichler, A. F. *et al.* The biology of brain metastases-translation to new therapies. *Nat. Rev. Clin. Oncol.* doi:nrclinonc.2011.58 [pii] 10.1038/nrclinonc.2011.58 (2011).
4. Lockman, P. R. *et al.* Heterogeneous blood-tumor barrier permeability determines drug efficacy in experimental brain metastases of breast cancer. *Clin. Cancer Res.* **16**, 5664-5678, doi:1078-0432.CCR-10-1564 [pii] 10.1158/1078-0432.CCR-10-1564 (2010).
5. Yoneda, T. *et al.* A bone-seeking clone exhibits different biological properties from the MDA-MB-231 parental human breast cancer cells and a brain-seeking clone *in vivo* and *in vitro*. *J. Bone Miner. Res.* **16**, 1486-1495 (2001).
6. Palmieri, D. *et al.* Her-2 overexpression increases the metastatic outgrowth of breast cancer cells in the brain. *Cancer Res.* **67**, 4190-4198, doi:67/9/4190 [pii] 10.1158/0008-5472.CAN-06-3316 (2007).
7. Percy, D. B. *et al. In vivo* characterization of changing blood-tumor barrier permeability in a mouse model of breast cancer metastasis: a complementary magnetic resonance imaging approach. *Invest. Radiol.* **46**, 718-725, doi:10.1097/RLI.0b013e318226c427 (2011).
8. Zhou, H. *et al.* Longitudinal MRI evaluation of intracranial development and vascular characteristics of breast cancer brain metastases in a mouse model. *PLoS One.* **8** e62238, doi:10.1371/journal.pone.0062238 (2013).
9. Zhou, H. *et al.* Dynamic near-infrared optical imaging of 2-deoxyglucose uptake by intracranial glioma of athymic mice. *PLoS One.* **4**, e8051, doi:10.1371/journal.pone.0008051 (2009).
10. Zhang, R. D. *et al.* Differential permeability of the blood-brain barrier in experimental brain metastases produced by human neoplasms implanted into nude mice. *Am. J. Pathol.* **141**, 1115-1124 (1992).
11. Mason, R. P. *et al.* Tumor oximetry: comparison of 19F MR EPI and electrodes. *Adv. Exp. Med. Biol.* **530**, 19-27 (2003).
12. Gril, B. *et al.* Pazopanib reveals a role for tumor cell B-Raf in the prevention of HER2+ breast cancer brain metastasis. *Clin. Cancer Res.* **17**, 142-153, doi:1078-0432.CCR-10-1603 [pii] 10.1158/1078-0432.CCR-10-1603 (2010).

