Video Article

An Orthotopic Mouse Model of Pleural Cancer

Elliot Servais[1], Christos Colovos[1], Valerie Rusch[1], Michel Sadelain[2], Prasad Adusumilli[1]

[1]Division of Thoracic Surgery, Memorial Sloan-Kettering Cancer Center

[2]Center for Cell Engineering, Memorial Sloan-Kettering Cancer Center

Correspondence to: Prasad Adusumilli at adusumip@mskcc.org

URL: http://www.jove.com/video/2172
DOI: doi:10.3791/2172

Keywords: Mesothelioma, Mouse model, Pleural, Orthotopic

Date Published: 6/15/2015

Citation: Servais, E., Colovos, C., Rusch, V., Sadelain, M., Adusumilli, P. An Orthotopic Mouse Model of Pleural Cancer. *J. Vis. Exp.* (), e2172, doi:10.3791/2172 (2015).

Abstract

The pleura is a thin serosal layer covering the lungs and chest wall and is a common location for solid tumor involvement with an estimated incidence of 250,000 patients annually in the United States. Primary pleural malignancies, such as malignant pleural mesothelioma (MPM), as well as metastatic pleural malignancies, commonly originating from lung and breast cancers, are characteristically resistant to standard therapies. Emerging novel therapies are currently investigated in preclinical flank and intraperitoneal tumor models that do not accurately recapitulate the pleural tumor microenvironment. A clinically relevant mouse model of pleural malignancy, in conjunction with the ability to non-invasively monitor tumor burden, will not only facilitate a better understanding of pleural tumor biology, but will also allow the investigation of novel therapies. Herein we describe a simple, reproducible technique for establishing a mouse model of MPM that recapitulates human disease and allows for the study and development of novel therapies for pleural-based malignancy.

Video Link

The video component of this article can be found at http://www.jove.com/video/2172/

Protocol

1. Cell preparation

a. Tumor cells are harvested at appropriate confluency based upon specifications of the cell line and experiment.
b. The cells are prepared either in serum-free media or PBS and placed on ice prior to mouse injection

2. Mouse preparation

The following mouse procedure is performed in accordance with the guidelines and regulations set forth by the Institutional Animal Care and Use Committee (IACUC) at Memorial Sloan-Kettering Cancer Center.

a. Adult mice are anesthetized using inhaled Isoflurane and supplemental oxygen.

3. Identification of landmarks, surgical prep, and draping

a. The mouse is transferred from the anesthesia chamber to the operating stage and the mouse's nose carefully placed into the nose cone to provide continuous inhaled anesthesia.
b. Appropriate depth of anesthesia is confirmed by observing and slowed, steady respiratory pattern and absence of withdrawal to toe or tail pinch.
c. The mouse is positioned in the left-side down position, and an axillary roll is placed underneath the mouse's left axilla, propping up the right chest. The incision will be placed approximately 1/3 the distance up from the inferior costal margin to the shoulder superiorly.
d. The operative site is prepared by shaving the right or left chest to cover at least 150% of the planned incision site using electronic clippers. It is important to note that in mice the right and left pleural cavities communicate and any cells injected into one side will spread throughout the chest.
e. The skin is prepped using a Povidone-Iodine swab stick covering the entire surgical area. This is followed by a second prep using a 70% Isopropyl alcohol prep pad.
f. A fenestrated drape is placed over the mouse so that only the prepped surgical site is exposed during the procedure.

4. Surgical Implantation

a. To begin the procedure, skin is grasped just superior to the planned incision using toothed forceps. The skin is tenting up in order to safely cut the skin and soft tissues without injuring underlying structures.
b. The incision should be carried down to the chest wall using a combination of sharp dissection with the dissecting scissors as well as blunt tissue spreading.
c. Through the exposed intercostal spaces, the lung superiorly and the liver inferiorly can be visualized. Appropriate level of anesthesia facilitates slowing of respirations which will aid in relatively easier intrapleural injection without injury to the lung.
    i. Note: There is a small vein that reliably runs cranial-caudally through the area of the incision, which should be identified and avoided. Injury to this vein can cause significant bleeding.

d. The location of the diaphragm at the junction of the lung and liver is identified and the 28-gauge insulin syringe with 200uL of the cell solution is brought into the field for the injection.
e. Using forceps to expose the area of injection the needle is placed through the intercostal space just inferior to the diaphragm. The needle is directed superiorly at an angle not more than 15-20 degrees in order to enter the pleural space.
    i. The needle should advance freely; resistance could indicate a subpleural or intraparenchymal injection in to the lung. Gentle advancement of the needle tip allows displacement of lung superiorly and avoids intrapulmonary injection.

f. Following the injection, use forceps to cover the needle track with tissue as the needle is withdrawn to avoid air entry into the pleural space which can precipitate a pneumothorax.
g. The soft tissues are repositioned covering the wound and the incision closed with a single steel 7 or 9mm wound clip.

5. Recovery

a. The mouse can be removed from the Isoflurane nose cone and allowed to recover.
b. The mouse should remain in the left-side down position during recovery and can be placed back into the cage with attention to make sure no bedding or other materials are blocking the mouse nose or mouth.
c. It is important to look for any evidence of bleeding at the surgical site during a post-operative check.

Representative Results

The resultant orthotopic mouse model develops pleural tumor which recapitulates human pleural disease in gross appearance, histopathologically, and biological phenotype. We performed necropsy studies and magnetic resonance imaging (MRI) to demonstrate the tumor growth pattern (Figure 1). As seen in the figure, tumor grows along visceral and parietal pleural surfaces, encasing mediastinal structures, and the diaphragm. The MRI appearance of pleural tumor spread is similar in the mouse model and human patients. Primary pleural tumors, such as pleural mesothelioma have high degree of lymphangiogenesis and secreted VEGF levels. As pleural tumors are known for extensive lymphangiogenesis compared to other malignancies, we evaluated neo-lymphangiogenesis in the orthotopic pleural model as compared to subcutaneous flank tumors by immunofluorescence staining for CD34 and LYVE-1, validated markers of angio- and lymphangiogenesis, respectively[1-2]. We found that the orthotopic pleural model demonstrated extensive lymphangiogenesis in the tumor nodules, which was in contrast to the flank tumors that had a relative lack of immunofluorescence staining (see figure 2). We compared survival of the orthotopic mouse model to flank and intraperitoneal tumor models following injection of equivalent number of pleural mesothelioma cells. We found that only the orthotopic pleural model recapitulated an aggressive disease with mice displaying symptoms similar to mesothelioma patients of tachypnea, weight loss, and early demise. In contrast mice in the flank and intraperitoneal groups did not display systemic symptoms and were sacrificed due to large flank tumors or large volume abdominal ascites, respectively (see figure 3). In addition, we have performed chemo- and radiotherapy studies in the orthotopic pleural mice by providing isolated thoracic radiation to the mouse chest; these studies could not be performed in the intraperitoneal model due to severe gastrointestinal related toxicities (see Table 1 for mouse model summary comparisons). The biological characteristics of MPM were reproduced in both immunocompetent (BALB/c) and immunodeficient (SCID-bg and nude athymic) mice.

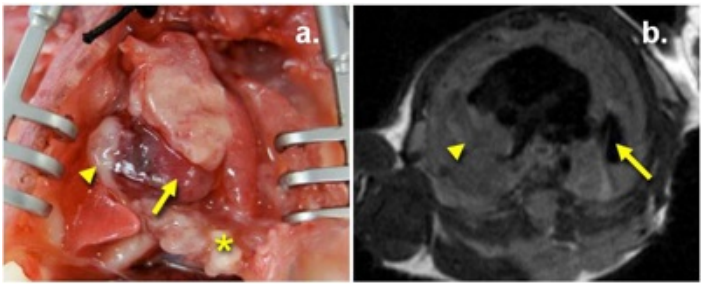

Figure 1. (a) Mouse necropsy showing pleural tumor growing along visceral and parietal pleural surfaces (arrowhead), diaphragm (asterisk), and encasing mediastinal structures such as the heart (arrow). (b) MRI clearly demonstrates MPM tumor (arrowhead) encasing lungs (arrow).

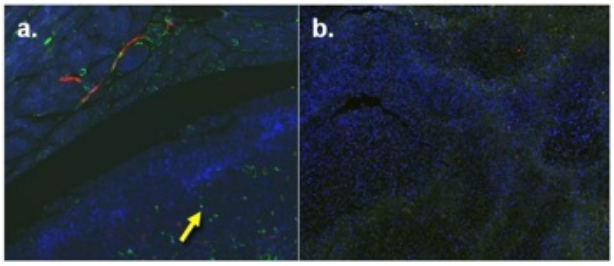

Figure 2. Immunofluorescence for angiogenesis (CD34, green) and lymphangiogenesis (LYVE1, red) shows extensive angio- and lymphangiogenesis in the orthotopic pleural tumor (a), and lack thereof in the flank tumor (b).

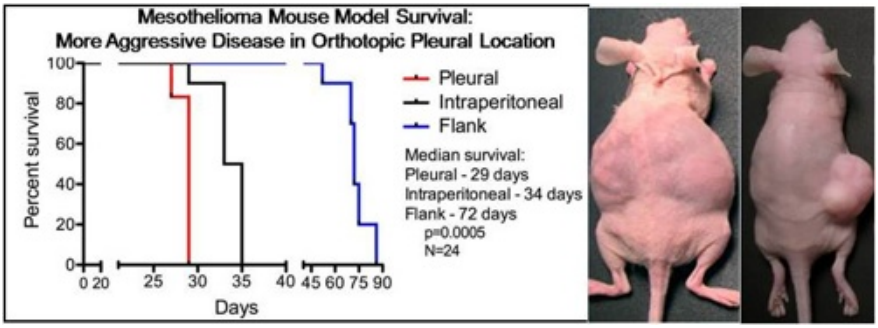

Figure 3. Survival comparison following equivalent tumor burden inoculation shows only the orthotopic pleural model recapitulates an aggressive human MPM. In contrast, mice with intraperitoneal and flank tumors are sacrificed due to abdominal ascites and large flank tumors, respectively, rather than systemic or respiratory symptoms.

Animal Model	Flank	Intraperitoneal (Pseudo-orthotopic)	Pleural (Orthotopic)
Survival	✗	✗	✓
Growth Pattern	✗	✗	✓
IHC	✗	✗	✓
Angio- & Lymphangiogenesis	✗	✓	✓
SMRP	✗	✓	✓
Quantitative BLI	✗	✗	✓
Volumetric MRI	✓	✗	✓
Radiation Tx	✓	✗	✓

Table 1. Comparison of mesothelioma animal models.

Discussion

The pleural space constitutes the area between the linings of the lung (visceral pleura) and the chest wall (parietal pleura), which are normally in close approximation, separated by only a thin layer of serous fluid. The rich pleural lymphatic network of the pleura, however, provides an efficient route for metastases from distant tumors to the pleura and an egress for tumor cells from primary pleural or lung cancers to reach systemic circulation[3-6]. These characteristics of the pleural microenvironment account for the frequency at which pleural involvement occurs in cancer patients. Thus, translational research focusing on pleural malignancy is applicable to patients with primary pleural tumors and lung cancer as well as the larger subset of patients with distant primary tumors including those with a propensity to develop pleural implants such as breast cancer, ovarian cancer, and lymphoma[7-9]. Unfortunately, preclinical trials are limited by the lack of a validated, clinically-relevant animal model of pleural malignancy. The use of flank, intraperitoneal, or other substitute mouse models of malignancy do not recapitulate disease in humans, nor do they allow investigations of therapies targeted to the pleural tumor microenvironment. The orthotopic pleural tumor model described herein provides an appropriate platform for studying pleural tumor biology and evaluating novel therapies as demonstrated in our publications[10-11].

We have provided a protocol to facilitate the highly reproducible establishment of this orthotopic murine tumor model and demonstrated the superiority of this model to other heterotopic mouse models frequently used in cancer research.

Disclosures

No conflicts of interest declared.

Acknowledgements

The authors would like to acknowledge the gracious support of Desiree Ehleiter from the MSKCC Research Animal Resource Center (RARC). This work is partially supported by the following funding sources: AATS-Third Edward D. Churchill Research Scholarship, Mesothelioma Applied Research Foundation (MARF) in memory of Lance S. Ruble.

References

1. Fiedler, U. et al. The sialomucin CD34 is a marker of lymphatic endothelial cells in human tumors. Am J Pathol 168, 1045-1053 (2006).
2. Skobe, M. et al. Induction of tumor lymphangiogenesis by VEGF-C promotes breast cancer metastasis. Nat Med 7, 192-198 (2001).
3. Manac'h, D. et al. Visceral pleura invasion by non-small cell lung cancer: an underrated bad prognostic factor. Ann Thorac Surg 71, 1088-1093 (2001).
4. Osaki, T., Nagashima, A., Yoshimatsu, T., Yamada, S. & Yasumoto, K. Visceral pleural involvement in nonsmall cell lung cancer: prognostic significance. Ann Thorac Surg 77, 1769-1773; discussion 1773 (2004).
5. Shimizu, K. et al. Visceral pleural invasion is an invasive and aggressive indicator of non-small cell lung cancer. J Thorac Cardiovasc Surg 130, 160-165 (2005).
6. Zocchi, L. Physiology and pathophysiology of pleural fluid turnover. Eur Respir J 20, 1545-1558 (2002).
7. Alexandrakis, M. G., Passam, F. H., Kyriakou, D. S. & Bouros, D. Pleural effusions in hematologic malignancies. Chest 125, 1546-1555 (2004).
8. Rodriguez-Panadero, F., Borderas Naranjo, F. & Lopez Mejias, J. Pleural metastatic tumours and effusions. Frequency and pathogenic mechanisms in a post-mortem series. Eur Respir J 2, 366-369 (1989).
9. Sahn, S. A. Malignancy metastatic to the pleura. Clin Chest Med 19, 351-361 (1998).
10. Adusumilli, P. S. et al. Radiation-induced cellular DNA damage repair response enhances viral gene therapy efficacy in the treatment of malignant pleural mesothelioma. Ann Surg Oncol 14, 258-269 (2007).
11. Adusumilli, P. S. et al. Imaging and therapy of malignant pleural mesothelioma using replication-competent herpes simplex viruses. J Gene Med 8, 603-615 (2006).

