Original Article

Lobar lung transplantation in the mouse

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Contributions: (I) Conception and design: J He, X Xu, J Shi; (II) Administrative support: None; (III) Provision of study materials or patients: J Shi, G Peng, C Yang, M Liu, Y Xu, X Cheng; (IV) Collection and assembly of data: J Shi, G Peng, C Yang, M Liu, Y Xu, X Cheng; (V) Data analysis and interpretation: J Shi, C Luo, J He, X Xu; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

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Background: As an important supplementary approach to clinical in orthotopic lung transplantation (LTx), lobar LTx currently lacks a stable animal model and in the orthotopic left LTx model, the right lung of the donor mouse is completely removed and discarded. We introduce a novel mouse lobar LTx model that potentially provides a mouse model for clinical lobar LTx and increase the utilization rate of the experimental donor.

Methods: Lobar and orthotopic left LTx were performed in syngeneic strain combinations. We performed micro-computed tomography and tested arterial blood gases to assess the graft function 28 days after transplantation. Hematoxylin-eosin and Masson's trichrome staining were used to evaluate pathological changes.

Results: We performed ten lobar LTx with an operation success rate of 90%, accompanied by ten orthotopic left LTx from the same donors with an operation success rate of 100%. The graft preparation for lobar LTx was longer than that of the orthotopic left LTx (42.11±3.79 *vs.* 30.10±3.14 minutes, P<0.001). The recipient procedure for lobar LTx was nearly equivalent to the orthotopic left LTx. The graft function and histopathological changes for lobar LTx were comparable to those of orthotopic left LTx 28 days after transplantation.

Conclusions: We describe a lobar LTx model in the mouse, which potentially provides a model for clinical lobar LTx and effectively addresses the issue of resource wastage in the orthotopic left LTx model.

Keywords: Mouse; lobar lung transplantation (lobar LTx); model

Submitted Jan 13, 2024. Accepted for publication Apr 26, 2024. Published online Jun 25, 2024.
doi: 10.21037/jtd-24-79
View this article at: https://dx.doi.org/10.21037/jtd-24-79

Introduction

Lung transplantation (LTx) has been known as the only effective treatment for patients with end-stage pulmonary diseases. The operative choices of LTx mainly include bilateral LTx, single LTx, and lobar LTx. According to the report from International Society for Heart and Lung Transplantation (ISHLT) in 2021, more than 5,000 LTx have been performed worldwide and an increasing trend is maintained annually (1). However, the shortage of donors has always been the main problem, and numerous patients have not been able to wait for a suitable donor before they die. Lobar LTx can improve donor lungs' utilization rate and source, which is an effective method for donor shortage (2).

Lobar LTx was first described by Bisson and colleagues in 1992 and has been widely used in pediatric

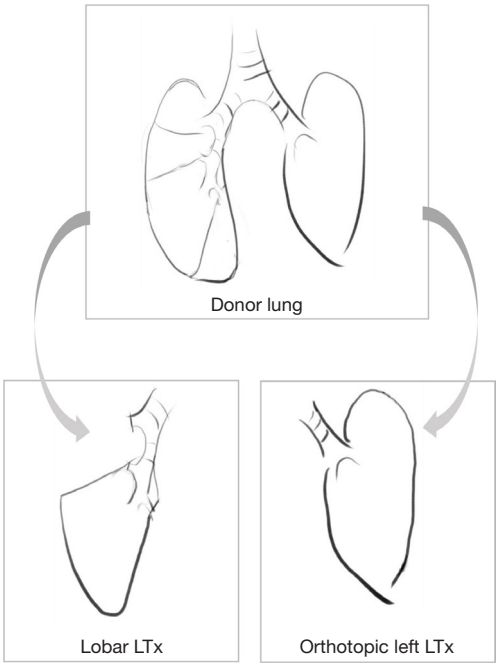


Figure 1 Patterns of lobar LTx in the mouse. The cardiac lobe of the right lung was used for lobar LTx, and the left lung was used for orthotopic left LTx. LTx, lung transplantation.

transplantation and small-sized adult recipients (3,4). It can be divided into living-donor lobar lung transplantation (LDLLT) and deceased-donor-lobar lung transplantation (ddLLTx) according to different donor sources. Various studies have shown that lobar LTx can effectively reduce the risk of death for waiting candidates (5,6). And its long-term outcomes are comparable to those of standard LTx (7). However, lobar LTx still faces some challenges that may limit the widespread application of the technique, such as an increased risk of primary graft dysfunction (PGD), early anastomotic complication, and reduced respiratory function in the long term (8).

For the mouse LTx model, the orthotopic left LTx has been widely applied in many basic studies, although the orthotopic right LTx was also introduced in 2010 (9). The complex operation blocks the widespread application of the technique. Therefore, the donor's right lung is heavily underused in the orthotopic left LTx model. In this study, we first describe a lobar LTx model in the mouse, aiming to provide a mouse model for clinical lobar LTx and increase the utilization rate of the experimental donor. We present this article in accordance with the ARRIVE reporting checklist (available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-79/rc).

Highlight box

Key findings

- The graft function and histopathological changes for lobar lung transplantation (LTx) are comparable to those of orthotopic left LTx.

What is known and what is new?

- Both in orthotopic left LTx and right LTx have been reported in mice.
- We introduce a novel mouse lobar LTx model that potentially provides a model for clinical lobar LTx and effectively addresses the issue of resource wastage in the orthotopic left LTx model.

What is the implication, and what should change now?

- A lobar LTx model in the mouse can increase the utilization rate of the donor's lung and may be a potential model to clinical lobar LTx. And we will apply the lobar LTx in allogeneic transplantation to explore the relative immunological issues in further study.

Methods

Animals

Inbred male Balb/c mice of age 6–8 weeks (weighing 20–25 grams) were purchased from GemPharmatech Co. (Jiangsu, China), and used as donors and recipients. Experiments were performed under a project license (No. 2021035) granted by the Experimental Animal Ethics Committee of Guangzhou Medical University, in compliance with Chinese guidelines for the care and use of animals.

Donor procedure

The donor heart-lung block was acquired as described in previous studies (10). In brief, the lung was exposed through a median sternotomy after heparinization with 100 units. The left atrium and right atrium were cut to vent the heart. Then lungs were flushed with 3 mL cold (4 ℃) low-potassium dextran glucose Perfadex solution and 26 cmH_2O constant infusion pressure from the pulmonary artery (PA). The heart-lung block was harvested at end-tidal volume with room air. The left lung was used for orthotopic left LTx, and the cardiac lobe of the right lung was used for lobar LTx as shown in *Figure 1*. The donor's left lung was prepared with cuffs as described in the previous study (11).

The donor right lung consisted of four lobes: the diaphragmatic lobe, cardiac lobe, azygous lobe, and apical

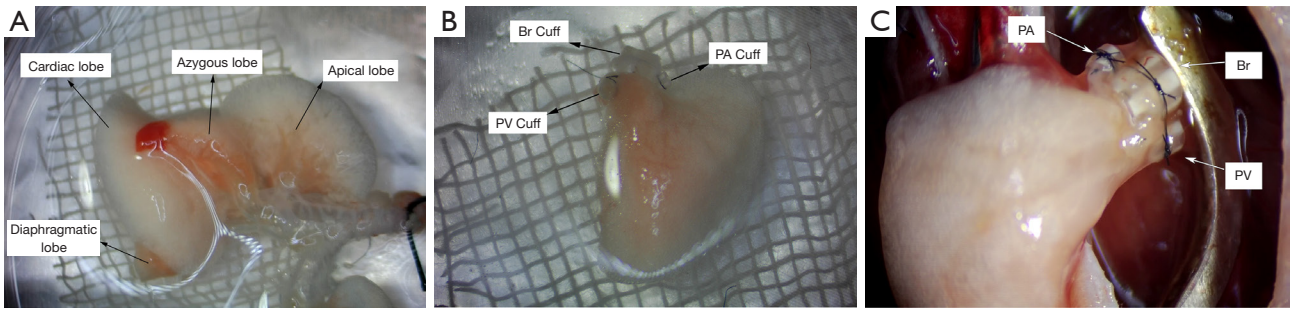
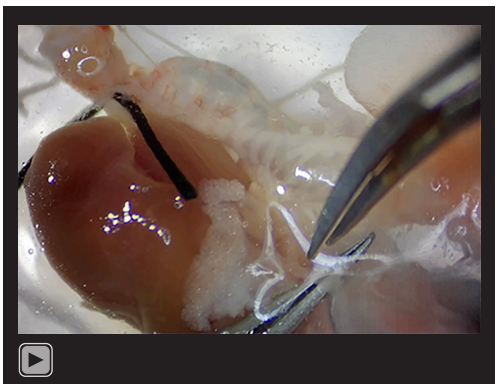
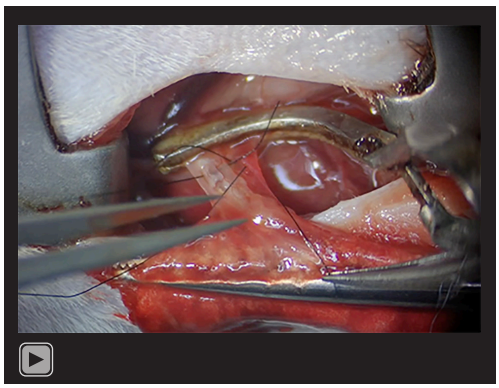


Figure 2 Surgical procedure of lobar lung transplantation. (A) Right lung anatomy, including the diaphragmatic lobe, cardiac lobe, azygous lobe, and apical lobe. (B) The cardiac lobe after attachment of cuffs. (C) The cardiac lobe graft is implanted into the recipient's left chest cavity. PA, pulmonary artery; PV, pulmonary vein; Br, bronchus.



Video 1 Donor procedure of lobar lung transplantation in the mouse.



Video 2 Recipient procedure of lobar lung transplantation in the mouse.

lobe (*Figure 2A*). The cardiac lobe was chosen for lobar LTx. The cardiac lobe was prepared for the LTx also by the attachment of cuffs (*Figure 2B* and *Video 1*). Therefore, the bronchus, vein, and artery of the cardiac lobe were dissected and then prepared for placing cuffs as shown in the donor video. The PA, pulmonary vein (PV), and bronchus were pulled through 24G, 22G, and 18G angio-catheter cuffs without tail, respectively, and fixed to the cuff with an 11-0 nylon circumferential ligature. The main bronchus of the cardiac lobe was blocked by an 8-0 slipknot to prevent the perfusion solution from entering the trachea. The graft was covered with wet gauze in low-potassium dextran glucose solution at 4 ℃ until implantation.

Recipient procedure

Recipients were anesthetized with an intraperitoneal injection of 50 mg/kg sodium pentobarbital and local nerve block of compound lidocaine cream, orotracheal intubated with a 20G angio-catheter. A left thoracotomy was performed through the fourth intercostal space, and then a chest retractor was placed to expose the left lung. The original left lung was pulled out of the chest through a hemostat with gentle traction. We blocked the left hilar proximal to the heart by a micro-vessel clamp when the left PV and PA were isolated from the bronchus. The excess lung tissues near the hilum were cut off to expose the branches of the PA and PV. And the "V" shaped incisions were made for PA and PV. We then insert the donor PA, bronchus, and PV cuffs with a 10-0 nylon circumferential ligature. For lobar LTx, the cardiac lobe graft needed to inverse to match the left lung structure (*Figure 2C* and *Video 2*).

Micro-computed tomography (micro-CT)

Recipient mice were anesthetized by isoflurane. Micro-CT was performed on postoperative 28 days to evaluate

Table 1 Relevant times to orthotopic left LTx and lobar LTx

Procedure	Orthotopic LTx	Lobar LTx	P value
Donor procedure (min)	24.20±3.22	24.20±3.22	>0.99
Graft preparation (min)	30.10±3.14	42.11±3.79	<0.001
Recipient procedure (min)	28.70±2.45	29.56±2.46	0.46
Total procedure (min)	88.50±3.47	101.10±3.95	<0.001

Data are presented as mean ± standard deviation. LTx, lung transplantation.

the lobar LTx.

Blood gases analysis and histology

The lung grafts were harvested 28 days after implantation. The recipient mice were anesthetized and ventilated with FiO_2 of 1.0, at the rate of 140 breaths per minute and 15 cmH_2O respiratory pressure. About 100 µL of arterial blood was collected directly from the left ventricle after clamping the right hilum for 5 minutes. Blood gas was tested on an i-STAT of Care analyzer (Abbott and CG4+ cartridge, Chicago, USA). Then the recipient was euthanized, and the grafts were harvested and fixed with paraformaldehyde after the reperfusion procedure and then embedded in paraffin. Sections were stained with hematoxylin-eosin (HE) and Masson's trichrome staining as the standard protocol.

Statistical analysis

Data analysis was performed with SPSS software (SPSS 20, SPSS Inc.). Data were expressed as mean ± standard deviation. The Student's *t*-test was used for statistical analysis. P values less than 0.05 were considered significant.

Results

General outcomes of lobar LTx

A total of ten donor lungs were used for orthotopic left LTx and lobar LTx. The operation success rate was 100% and 90% for orthotopic left LTx and lobar LTx, respectively. One lobar LTx failed to perform due to a bronchus tear for producing cardiac lobe cuff. The time of graft preparation was 42.11±3.79 minutes for lobar LTx, which was more than orthotopic LTx 30.10±3.14 minutes (P<0.001). Meanwhile, the whole time for lobar LTx was also longer than orthotopic LTx (101.10±3.95 *vs.* 88.50±3.47 minutes, P<0.001). There was no significant difference in the time of recipient procedure between orthotopic LTx and lobar LTx (28.70±2.45 and 29.56±2.46 minutes, P=0.46, *Table 1*).

Functional assessment of lobar LTx

We performed micro-CT and tested arterial blood gas to evaluate the post-transplanted function of lobar LTx. Although the mixed arterial blood from the left and right lungs was used to test blood gas, we considered it to be a reasonable representation of the graft function (12). There were no significant differences in arterial oxygen and carbon dioxide levels between the orthotopic LTx and lobar LTx (373.00±24.06 *vs.* 346.70±35.00 mmHg, P=0.29, and 32.50±2.84 *vs.* 33.56±3.78 mmHg, P=0.41, respectively) (*Figure 3*).

To evaluate the size match for lobar LTx, micro-CT scans were performed on recipient mice at postoperative 28 days. In orthotopic LTx matched well as expected (*Figure 4A-4C*). There was an excellent size match between the cardiac lobe and the left pleural space without obvious mediastinal shift for lobar LTx (*Figure 4D-4F*). Micro-CT at 28 days showed that the levels of the left diaphragm in lobar LTx almost even neared the orthotopic LTx, and the lobar LTx also recovered a dome-shaped diaphragm (*Figure 4*).

Histology of lobar LTx

The histologic presentation of LTx is demonstrated in *Figure 5*. The gross appearance of lung grafts for orthotopic LTx and lobar LTx is shown in *Figure 5A*,*5D*, respectively. Histological examinations of lobar LTx had no evidence of inflammation and were comparable to orthotopic LTx (*Figure 5B*,*5E*). The Masson's trichrome stain was used to evaluate the fibrosis for lobar LTx, and there was no significant difference in the degree of fibrosis between orthotopic LTx and lobar LTx (*Figure 5C*,*5F*).

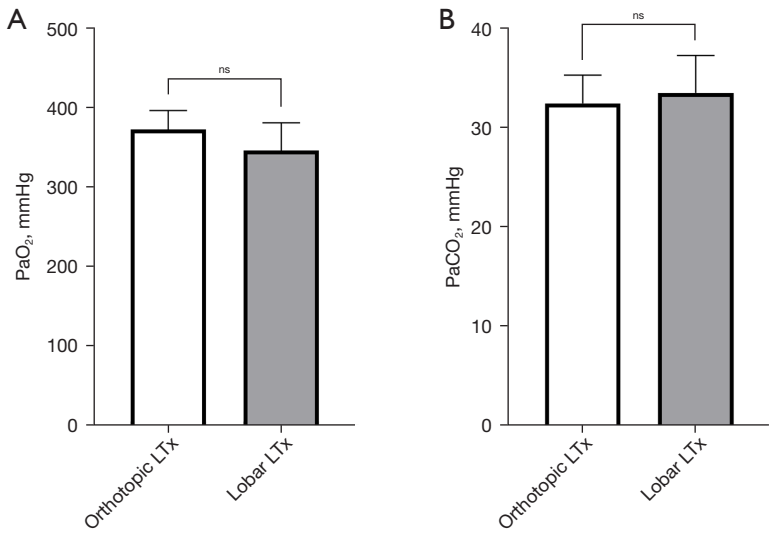


Figure 3 Arterial blood gas analysis. (A,B) There were no significant differences in the levels of arterial PaO_2 and $PaCO_2$ between lobar LTx and orthotopic left LTx. PaO_2, arterial partial pressure of oxygen; $PaCO_2$, arterial partial pressure of carbon dioxide; LTx, lung transplantation; ns, not statistically significant.

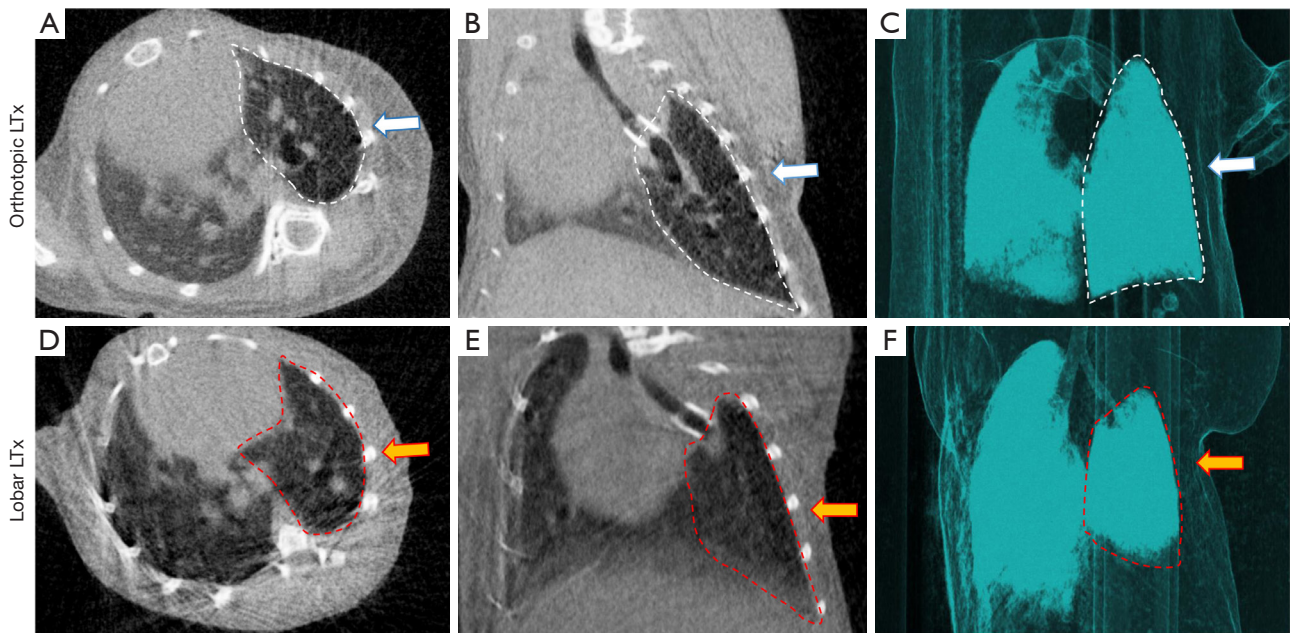


Figure 4 Micro-computed tomography was performed 28 days after transplantation to evaluate the size match for lobar LTx. (A-C) For orthotopic left LTx. (D-F) For lobar LTx. White and orange arrows represent transplanted lungs. LTx, lung transplantation.

Discussion

Since the technique for performing orthotopic left LTx in mice was introduced by Okazaki and his team in 2007 (10), an increasing number of centers have utilized this model to study common post-transplant complications such as PGD, acute rejection, and chronic lung allograft dysfunction (13-15). However, in this model, the right lung of the donor mouse is entirely removed and discarded, resulting in a

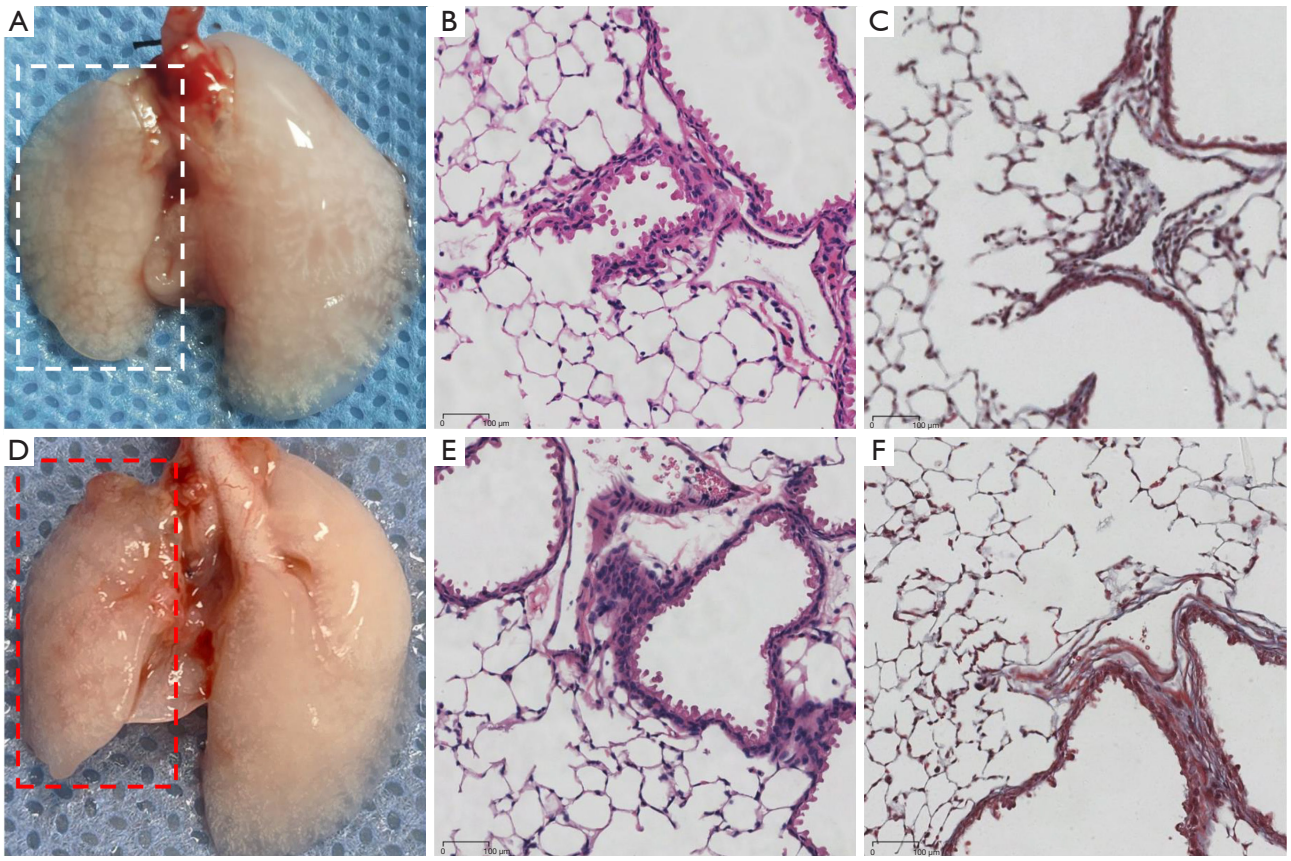


Figure 5 Gross appearance and histology. (A-C) The gross appearance and histology of orthotopic left LTx at 28 days after transplantation. (D-F) The gross appearance and histology lobar LTx at 28 days after transplantation. (B,E) Hematoxylin-eosin stain (200×). (C,F) Masson's trichrome staining (200×). The graft was marked with a rectangle in gross appearance. LTx, lung transplantation.

serious animal ethics problem of wasting the right lung of the donor mouse. Li and colleagues in 2010 first reported orthotopic right LTx in the mouse aiming to mimic a survival model for an experimental LTx (9). Due to the complexity of the anatomy, the orthotopic right lung transplant is technically challenging to most surgeons, which limits the widespread use of the model. However, there is currently a lack of documented reports on lobar LTx models in small animals internationally. Additionally, research on clinical lobar LTx, as a crucial therapeutic approach in an era marked by a shortage of LTx donors, remains relatively scarce, while encountering challenges such as an increased risk of PGD, early anastomotic complication, and reduced respiratory function in the long term. In this study, we introduce a novel mouse lobar LTx model that provides a mouse model for clinical lobar LTx and increase the utilization rate of the experimental donor.

The mouse's right lung is composed of four lobes, known as the diaphragmatic lobe, cardiac lobe, azygous lobe, and apical lobe (9). Our center also tried to perform the mouse orthotopic right LTx but failed due to the short and deep right PV. However, we found the mouse's right lung is suitable for a lobar LTx, as Voswinckel *et al.* described that the right lung in adult mice accounts for about 70% of the total lung function, and that the cardiac lobe compensates for the most significant increase in size after resection of the left lung, which accounts for about 40% of the function of the right lung, which is comparable to the percentage of the function of the left lung (16). In the study, the cardiac lobe of the right lung was implanted in the recipient's left chest. And the left lung from the same donor was used for orthotopic left LTx, which can improve the utilization rate of the donor organs.

Lobar LTx is a well-established technique in clinical transplantation (7,8), which includes LDLLT and ddLLTx. ddLLTx was first reported by Bisson and his colleagues (3).

Starnes and colleagues first introduced LDLLT in 1993 due to the cadaveric lung donor shortage (17). Lobar LTx is an effective method to reduce wait-list time and mortality, especially for patients with severely restricted chest cavities and small-sized recipients (8). Although numerous studies have reported that lobar LTx achieves postoperative survival outcomes comparable to those of standard LTx (18), there are still some potential drawbacks for lobar LTx, such as a supposed increased risk of PGD, early anastomotic complication, and reduced respiratory function in the long term. The mouse lobar LTx would be a potential model to investigate those drawbacks.

To our knowledge, we are the first to describe the mouse lobar LTx techniques in that the cardiac lobe from the donor's right lung was inversed into the recipient's left chest. For donor procedures, the cuffs of the cardiac lobe are less challenging compared to those of the right lung because of the cardiac lobe with enough long PV. We need to isolate the cardiac lobe from the right lung by separating, ligating, and cutting off the other lobes, leading to longer operative times for the donor procedure of lobar LTx than the left lung donor. For the recipient's procedure, there are nearly no differences between the lobar LTx and the orthotopic left LTx. Therefore, the operative time for graft implantation in lobar LTx should be less than the orthotopic right LTx (9), and nearly equal to the left LTx.

In this study, we aim to investigate the model feasibility of mouse lobar LTx merely. Therefore, we used syngeneic murine transplantation (Balb/c to Balb/c) for orthotopic left LTx and lobar LTx to avoid interference from immunological rejection. Clinical studies have reported that the long-term outcomes of lobar LTx are favorable and comparable to standard LTx (8,19). We performed a histological examination at postoperative 28 days, which showed similar changes between mouse lobar LTx and orthotopic left LTx. Considering the allograft's function cannot be reflected by the recipient's health status in the mouse orthotopic left LTx model (20), the micro-CT was applied to assess the lobar allograft function. We found that the cardiac lobe was a good size match to the left pleural space without obvious mediastinal shift at postoperative 28 days. The average arterial blood gas in the lobar LTx model was slightly lower than those in orthotopic LTx, though there was no statistical difference. We consider that the lobar allograft reduced some respiratory function compared with the left lung allograft at postoperative 28 days.

There were some limitations to the study. Due to longer operative time for donor preparation, the cold ischemia time were longer for lobar LTx than left LTx. However, the longest cold ischemic time for lobar LTx were less than 2 hours, which is acceptable for organ protection. On the other hand, we only performed syngeneic lobar LTx, which cannot assess the immunological reflection of lobar LTx. For technological purposes, syngeneic transplantation may exclude some interferences with the study. And we will apply the lobar LTx in allogeneic transplantation to explore the relative immunological issues in further study.

Conclusions

In conclusion, in this study, we propose a novel mouse lobar LTx model that provides a mouse model for clinical lobar LTx and increase the utilization rate of the experimental donor. Function assessments and histopathological changes in syngeneic transplantation for lobar LTx are comparable to those of orthotopic left LTx. The lobar LTx model may be a potentially valuable supplement to orthotopic left LTx, which can increase the utilization rate of the experimental donor, providing a foundational model for subsequent clinical studies on lobar LTx.

Acknowledgments

Funding: None.

Footnote

Reporting Checklist: The authors have completed the ARRIVE reporting checklist. Available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-79/rc

Data Sharing Statement: Available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-79/dss

Peer Review File: Available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-79/prf

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-79/coif). J.H. serves as Executive Editor-in-Chief of *Journal of Thoracic Disease*. W.L. serves as an unpaid editorial board member of *Journal of Thoracic Disease* from December 2022 to January 2025. The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all

aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Experiments were performed under a project license (No. 2021035) granted by the Experimental Animal Ethics Committee of Guangzhou Medical University, in compliance with Chinese guidelines for the care and use of animals.

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Cite this article as: Shi J, Luo C, Xu Y, Cheng X, Zhang J, Peng G, Yang C, Liu M, Liang W, Xu X, He J. Lobar lung transplantation in the mouse. J Thorac Dis 2024;16(6):3636-3643. doi: 10.21037/jtd-24-79