Author’s response to reviews

Title: The role of HIF-1α-VEGF pathway in bronchiolitis obliterans after lung transplantation

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Reviewer reports:

Associate Editor: The manuscript used a meaningful methodology and nice results were obtained. The novelty is limited and the reviewers found important limitation. Please revise the manuscript according to the reviewers comments and please very explicitly explain why you think this study adds novel information (see comment #2 of reviewer 1). If you cannot give a good explanation for novelty of the work, the likelyhood of acceptance is low. I would prefer using box and whisker plots for the graphs which are easier to grasp.

Answer: Thank you for your affirmation. The rat left lung orthotopic transplantation is the most difficult organ transplantation, and only a few institutions in China can successfully make the animal model. As a Key Laboratory of Multiorgan Transplantation, our center has been active in the field of basic research in organ transplantation. Although Lung transplantation in rats is a well-established research methodology, there are few articles about long-term survival of rat model of left lung orthotopic transplantation has been published so far.

There are indeed many studies on HIF-1a and hypoxia, and the five references reviewer 1 mentioned are all very authoritative articles, however, these studies found that the effects of HIF-1a on chronic rejection after lung transplantation are not completely consistent, and our experimental design and results were not repeated with previous articles.
1. (Cell 2012;148:399-408, Journal of Clinical Investigation 2011;121:2336-49, Journal of Investigative Medicine 2016;64:361-3) Jiang et al, use an orthotopic tracheal transplantation model, found that HIF-1-dependent recruitment of recipient Tie2+ angiogenic cells and repair of airway microvasculature was a critical determinant of graft survival by mediating overexpression of HIF-1a; Mark Nicolls and his colleagues at Stanford performed orthotopic tracheal transplantation, found that HIF-1 α conditional knockout caused airway perfusion damage earlier and more seriously; Jiang et al found that increased airway perfusion on days 3 and 10 after transplantation, could protect early grafts (less than 1 month) from HIF-1a overexpression by maintaining the HIF-1a activity of donor trachea.

2. (Cellular Immunology 2012;273:59-66, Proc Am Thorac Soc 2009;6:108-121) On the contrary, Tiriveedhi et al, by using normal human bronchial epithelial (NHBE) cells in vitro, found that HIF-1α-mediated upregulation of fibrogenic growth factors induced by ligation of Kα1T Abs is critical for development of fibrosis leading to chronic rejection of lung allograft, considered that the pro-fibrotic role of HIF-1α is crucial to transplant rejection. Belperio et al also considered that local ischemia will stimulate hypoxia inducible factors (i.e., HIF-1α), which stimulates angiogenesis, a requirement to support chronic inflammation/fibroobliteration.

3. In the articles reviewer 1 mentioned and other tracheal or lung transplantation studies, we found that most of the subjects studied were cells in vitro or trachea transplants, and the research time for inducing chronic rejection was generally within 30 days. In our study, we used left lung orthotopic transplantation in rats. The survival time of recipients was 90 days after lung transplantation, which is more capable of simulating the actual situation. We found that at 90 days after lung transplantation, the pathological features of BOS were found in the allografts and the expression of HIF-1a, VEGF-A and VEGFR2 in the allograft tissue was up-regulated. It is consistent with point 2, but we get the data from animal models. In addition, clinically, lung biopsy is the gold standard for the detection of chronic rejection after lung transplantation. However, in China, many patients after lung transplantation are reluctant to receive a biopsy for graft tissue, and tend to be judged by chest CT examination. Therefore, in this paper, we performed stage chest CT scan on transplanted rats.

In summary, we believe that the main innovations of this study are as follows:

1. We adopted the model of left lung orthotopic transplantation in rats. The success rate of transplant surgery and the survival rate after lung transplantation were ensured by proficient surgical techniques. This model can better simulate the chronic rejection process after lung transplantation.
2. We performed chest CT scanning at multiple time points to observe the imaging changes of the transplanted lung more directly.

3. We found that the expression of HIF-1α, VEGF-A and VEGFR2 in the allograft tissue was up-regulated, consistent with histological findings by immunohistochemical staining and lung tissue score on 90th day after transplantation. We confirmed a link between the HIF-1α-VEGF-A pathway and BO, in a long-term survival model of orthotopic lung transplantation in rats for the first time.

Reviewer #1:

1. Lung transplantation in rats is a well-established research methodology with a history of more than 30 years, and numbers of simplified methods are reported. (Transplantation Proceedings 1989;21:2601-2, J Invest Surg 2008;21:33-7… etc.)

Answer: First of all, thank you for your affirmation. The rat left lung orthotopic transplantation is the most difficult organ transplantation, and only a few institutions in China can successfully make the animal model. As a Key Laboratory of Multiorgan Transplantation, our center has been active in the field of basic research in organ transplantation. Although Lung transplantation in rats is a well-established research methodology, there are few articles about long-term survival of rat model of left lung orthotopic transplantation has been published so far. In view of the comments of reviewer, we have added graft ischemic time and other data.

2. Also, the relationship between HIF-1a and hypoxic environment including chronic lung allograft rejection has been investigated for a long time. In these days, there are the trials to attenuate BO by targeting HIF-1a signaling. (Cell 2012;148:399-408, Proc Am Thorac Soc 2009;6:108-121, Cellular Immunology 2012;273:59-66, Journal of Clinical Investigation 2011;121:2336-49, Journal of Investigative Medicine 2016;64:361-3)

Answer: Thank you for your advice. There are indeed many studies on HIF-1a and hypoxia, and the five references you mentioned are all very authoritative articles, however, these studies found that the effects of HIF-1a on chronic rejection after lung transplantation are not completely consistent, and our experimental design and results were not repeated with previous articles.

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tracheal transplantation, found that HIF-1α conditional knockout caused airway perfusion damage earlier and more seriously; Jiang et al found that increased airway perfusion on days 3 and 10 after transplantation, could protect early grafts (less than 1 month) from HIF-1α overexpression by maintaining the HIF-1α activity of donor trachea.

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3. In the articles you mentioned and other tracheal or lung transplantation studies, we found that most of the subjects studied were cells in vitro or trachea transplants, and the research time for inducing chronic rejection was generally within 30 days. In our study, we used left lung orthotopic transplantation in rats. The survival time of recipients was 90 days after lung transplantation, which is more capable of simulating the actual situation. We found that at 90 days after lung transplantation, the pathological features of BOS were found in the allografts and the expression of HIF-1α, VEGF-A and VEGFR2 in the allograft tissue was upregulated. It is consistent with point 2, but we get the data from animal models. In addition, clinically, lung biopsy is the gold standard for the detection of chronic rejection after lung transplantation. However, in China, many patients after lung transplantation are reluctant to receive a biopsy for graft tissue, and tend to be judged by chest CT examination. Therefore, in this paper, we performed stage chest CT scan on transplanted rats.

3. So, I congratulate the authors for establishing the methodology of lung transplantation in rats in authors' lab, however, I can hardly agree with the opinion of authors that "this is the first report confirming the relationship between the HIF-1α and BO in vivo" as written in the last paragraph in discussion.

Answer: Thank you for your suggestion. We did not make these sentences clear. What we want to say is the association between the HIF-1α-VEGF-A pathway and BO in a long-term survival model of orthotopic lung transplantation in rats. We have made modifications according to your suggestion.
Reviewer #2:

1. I would suggest revising medical English, for example:
   - Page 2, line 21 "Graft function may be affected if the organ is exposed to hypoxia"
   - Page 6, line 86 "Recipients of all groups received"
   - Page 7, line 111 "Tests were performed with the Pearson's"
   - Page 8, line 127 "Lung fields appeared clearer"
   - Page 8, line 137-143: please reword the entire paragraph, it is very difficult to understand
   - Page 9, line 161-169: please reword the entire paragraph, it is very difficult to understand
   - Page 11, line 192-198: please reword the entire paragraph, it is very difficult to understand
   - Page 11, line 208-211: please reword the entire paragraph, it is very difficult to understand
   Answer: Thanks to your carefully review. In accordance with your suggestion, corresponding modifications have been made.

2. In the background section:
   - Please comment on the role of HIF-1, VEGF-A and VEGFR-2 as a target for potential treatment strategies.
   - Please clarify the role of HIF-1, VEGF-A and VEGFR-2 during hypoxemia and transplantation.
   Answer: Thank you for your suggestion. We have added the corresponding content.

3. In the methods section:
   - Please clarify some technical details such as graft ischemic time and type of donor graft perfusion.
   Answer: Thank you for your suggestion. We have added graft ischemic time and other technical details.
4. In the results section:

- Can you explain the meaning of a repeated CT on POD 27 and 30?

Answer: Thank you for your question. Since aseptic inflammation was induced by intratracheal injection of LPS on the 28th day after transplantation, we performed thoracic CT scans on the recipient rats on the 27th day after transplantation to determine whether the airway perfusion of the transplanted lung was good. A chest CT scan was performed on the 30th day to observe whether the model of LPS induced aseptic pneumonia was successfully established.

5. - Please add p-values when you report measurements from the different groups.

Answer: Thank you for your suggestion. We have added the corresponding content.

6. - Page 10, line 173: this sentence needs to be moved to the methods section.

Answer: Thank you for your suggestion. We have made modifications.

7. In the discussion section:

- I would expand on the role of HIF-1, VEGF-A and VEGFR-2 in acute and chronic lung injury; this is the key concept of the paper and would deserve a more detailed description.

- I would improve the background on the pathophysiology of HIF-1.

- I would improve the description of the relationship between HIF-1 and airway disease (large vs small).

Table 1: please expand the legend and clarify subgroups.

Answer: Thank you for your suggestion. We have added the corresponding content.