Author's response to reviews

Title: Alveolar epithelial cells undergo epithelial-mesenchymal transition in acute interstitial pneumonia: a case report

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Author's response to reviews: see over
Reviewer's report

Title: Alveolar epithelial cells undergo epithelial-mesenchymal transition in acute interstitial pneumonia: a case report

Version: 1 Date: 7 December 2013

Reviewer: Hiroshi Iwasaki

Reviewer's report:

1. The authors reported that alveolar epithelial cells undergo epithelial-mesenchymal transition (EMT) in a case of acute interstitial pneumonia (AIP). They stated that this is the first report of alveolar epithelial cells transforming into myofibroblasts in the lung tissue with AIP. However, EMT in the interstitial pneumonia has been demonstrated by several investigators. By using a quantitative immunohistochemical analysis and double immunostaining, Harada et al (2010) demonstrated EMT of alveolar epithelial cells during pulmonary fibrogenesis.

Answers: Several articles reported that EMT may participate in various lung diseases, such as developmental disorders, fibrotic tissue remodeling, and lung cancer in humans. Harada et al (2010) demonstrated the presence of EMT in patients with UIP pattern and mentioned the EMT may not be specific for UIP and can be involved in other fibrotic processes in the lung. However, Yamada et al obtained conflicting results and did not detect double-positive cells for E-cadherin, ICAM-1, LEA CD44v9, SP-A, α-SMA, or vimentin
in lung tissues from patients with idiopathic pulmonary fibrosis and nonspecific interstitial pneumonia. Therefore, the evidence of myofibroblasts originating from epithelial cells through EMT in interstitial pneumonia remains controversial. Moreover, AIP is one of the six subtypes of major idiopathic interstitial pneumonia according to the American Thoracic Society/European Respiratory Society classification. To our knowledge, until now whether EMT exists in patients with AIP pattern is still unknown.

2. Page 7, line 2-3: … several filaments in the cytoplasm. What kind of filaments exist in the cytoplasm? The authors should specify the type of intracytoplasmic filaments: intermediate (10-nanometer) filaments, actin microfilaments, or both?

Answers: Several studies found that during EMT, cytoskeletal reprogramming establishes the presence of α-SMA stress fibers in epithelial cells. Therefore, the intracytoplasmic filaments are actin microfilaments.

Page 8, line 14: What means “systemic diseases”? The authors should explain more specific name or condition.

Answers: Systemic diseases could affect virtually any organ system, with principal manifestations including serositis, arthritis, renal, dermatologic, and pulmonary involvement. For example, Systemic lupus erythematosus and multiple myositis-dermatomyositis could
cause diffuse alveolar damage (DAD).

3. Page8, line21: What means NAIP? A full name should be described.

   Answers: We would like to apologize for writing “NSIP” instead of “NAIP.”

   The discussion (conclusion) is too long and redundant. Please highlight the essential point of this study.

   Answers: We have re-writing the conclusion according to your suggestions:

   AIP is one of the six subtypes of major idiopathic interstitial pneumonia according to the American Thoracic Society/European Respiratory Society classification. Given that many diseases could mimic AIP, multidisciplinary diagnosis, which requires a combination of clinical, radiological, and pathological findings, is needed. The case reported a patient with typical appearances of AIP. Apart from supplemental oxygen and mechanical ventilation, the patient received high-dose intravenous methylprednisolone for 5 days and normal dose of methylprednisolone for several days. However, the treatment could not contribute to the patient’s survival. The data from this case confirmed the occurrence of EMT in AIP and maybe beneficial for the treatment of this disease.

   The process of lung injury with subsequent development of scar tissue in idiopathic pulmonary fibrosis has been likened to an
abnormal wound healing model. A similar construct may be applied to AIP. In theory, intervention before the deposition of mature collagen should allow the restoration of normal lung architecture. The marked expansion of myofibroblast numbers within the alveolar septa respond for the subsequent collagen production in the proliferative and fibrotic AIP. Consistent with this conclusion, we also detected an amount of myofibroblast in the patient’s lung tissue. This myofibroblast may be related to the AIP process.

Previous observations revealed that injured epithelial cells could gradually lose their epithelial cell markers and polarity, thus expressing mesenchymal markers and acquiring single-cell motility, which was defined as EMT. During EMT, cytoskeletal reprogramming establishes the presence of α-SMA stress fibers in epithelial cells. Through this transition, alveolar epithelial cells serve as an importance source of myofibroblasts during tissue injury response. Kalluri et al. reported that under inflammatory stress, 30% of myofibroblasts can arise via EMT, whereas resident fibroblasts contribute only 23% in the kidney. Several articles reported that EMT may participate in various lung diseases, such as developmental disorders, fibrotic tissue remodeling, and lung cancer in humans. Harada et al. demonstrated the presence of EMT in patients with usual interstitial pneumonia pattern. However, Yamada
et al. obtained conflicting results and did not detect double-positive cells for E-cadherin, ICAM-1, LEA CD44v9, SP-A, α-SMA, or vimentin in lung tissues from patients with idiopathic pulmonary fibrosis and nonspecific interstitial pneumonia. Therefore, the evidence of myofibroblasts originating from epithelial cells through EMT in interstitial pneumonia remains controversial. Our ultrastructural data supported the existence of EMT in the lung tissue of patients with AIP. We also found that SPC and α-SMA, which are markers for alveolar epithelial cells and myofibroblasts, respectively, coexisted in the patient’s sections. The EMT program in epithelial cells is identified to be switched on by many transcription factors. For example, Snail, a major transcription factor governing EMT, could regulate the expression of genes related to epithelial and mesenchymal phenotype. We found that the expression of Snail in the lung tissue of the patient was upregulated in the proliferative phase of AIP compared with the exudative phase of AIP.

In conclusion, the results of this study confirmed that alveolar epithelial cells underwent EMT, which maybe an important origin of myofibroblasts in the progression of AIP. Although pathological manifestation may vary from one case of AIP to another, our finding partly indicated the possibility and importance of EMT in AIP and provided a potential therapeutic method of preventing EMT in AIP.
Quality of written English: Needs some language corrections before being published.

We have sent our manuscript to ShineWrite.com in order to perfect our writing.
Reviewer's report

Title: Alveolar epithelial cells undergo epithelial-mesenchymal transition in acute interstitial pneumonia: a case report

Version: 1 Date: 14January2014

Reviewer: shaoxi SC cai

Reviewer's report:

1, Actually, since it was reported in 1935 by Hamman and Rich, the clinical management of AIP has been well documented. In present manuscript, the emergence of EMT was evident via the upregulation of EMT related proteins including SPC, α-SMA and Snail through IHC and ultrastructural examinations. Although the finding is interesting, I have major problems with the references. In other words, how does the upregulation define? Immunostaining is not an appropriate technique for quantification and at least how does the quantification carry out need to be clearly described.

Answers: We then determined the changes in EMT-related proteins, such as surfactant associated protein (SPC), α-smooth muscle actin (α-SMA), and Snail. (Santa Cruz Biotechnology Inc. Dallas, Texas, USA). The results of the double immunostaining of the lungs showed mesenchymal specific protein α-SMA in the alveolar epithelial cells (SPC-positive cells). The double positive cells were evidently increased in the lung tissue from the 62 d autopsy
compared with that from the 5 d biopsy (Figure 5). The zinc finger transcription factor Snail was evidently expressed in the lung tissue, especially in the lung tissue from the 62 d autopsy (Figure 6).

Although immunostaining is not an appropriate technique for quantification, this technique is often used to detect the presence of EMT to co-immunostain markers of epithelial and fibroblast cell lineage. In this study, we counted the double positive cells and used average fluorescence intensity to quantify expressional changes of Snail. Data represent three independent experiments.

![Figure 5](image-url)
2. Without a reliable control, it is really difficult to reach the conclusion. Also, it is a single case report about the development of EMT in AIP patient and the individual variation owing to the small sample size make the conclusion less confident.

Answers:

Although no control was employed in our study, the expression of EMT-related genes was evidently different in the process of AIP in our patient; Moreover Jayachandran et al (2012) detected only weak Snail
expression in lungs specimens of transplant donors. Cytokeratins are the characteristic intermediate filaments in epithelial cells. During EMT cells are programmed to express vimentin and α-SMA instead. In the normal lung tissue, epithelial cells do not express α-SMA.

The results of this study confirmed that alveolar epithelial cells underwent EMT, which maybe an important origin of myofibroblasts in the progression of AIP. Although pathological manifestation may vary from one case of AIP to another, our finding partly indicated the possibility and importance of EMT in AIP and provided a potential therapeutic method of preventing EMT in AIP.

The conclusion of the existence of EMT in AIP is based on the data from the patient presented with typical appearances of AIP. Given that AIP is a rare interstitial lung disease, collecting many human lung tissues from patients with AIP is difficult. Nevertheless, in the future, we would further confirm the possibility and importance of EMT in AIP by using a large sample size.

3. Furthermore, EMT has been shown in other pulmonary diseases, such as asthma and COPD. In this case, the conclusion could only be made after careful exclusion of other potential reasons.

Answers: Though EMT has been shown in other pulmonary diseases, such as asthma and COPD, the conclusion of the existence of EMT in AIP is based on the data from the patient presented diagnosed typical AIP
by clinicians, radiologists and pathologists.

4. The authors need to put some more words in interpreting what is the possible involvements of EMT in AIP and how the finding maybe beneficial for the treatment of AIP in discussion?

Answers: We have given more interpretation in discussion according to your advices:

Our ultrastructural data supported the existence of EMT in the lung tissue of patients with AIP. We also found that SPC and α-SMA, which are markers for alveolar epithelial cells and myofibroblasts, respectively, coexisted in the patient’s sections. The EMT program in epithelial cells is identified to be switched on by many transcription factors. For example, Snail, a major transcription factor governing EMT, could regulate the expression of genes related to epithelial and mesenchymal phenotype. We found that the expression of Snail in the lung tissue of the patient was upregulated in the proliferative phase of AIP compared with the exudative phase of AIP.

In conclusion, the results of this study confirmed that alveolar epithelial cells underwent EMT, which maybe an important origin of myofibroblasts in the progression of AIP. Moreover, the marked expansion of myofibroblasts numbers within the alveolar septa responds for the subsequent collagen production in the proliferative
and fibrotic AIP. Although pathological manifestation may vary from one case of AIP to another, our finding partly indicated the possibility and importance of EMT in AIP and provided a potential therapeutic method of preventing EMT in AIP.

Minor

1. The arrangement of the figures is confused.

Answers: We have adjusted the arrangement of the figures:

Figure 1 Radiological findings of the patient
Figure 2 Hematoxylin and eosin (H&E) stain
Figure 3 Emergence of myofibroblasts in lung tissue
Figure 4 Ultrastructural findings of the lung tissue from the patient
Figure 5 Double positive cells for SPC and α-SMA existed in the human lung tissue
Figure 6 Detection of Snail expression in human lung tissue

2. page 8, last line “…relatively little collagen deposition compared to that in NAIP.” “NAIP” should be read “NSIP”.

Answers: We would like to apologize for writing “NSIP” instead of “NAIP.”