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TITLE: Growth and/or Recruitment of a Novel Cell Population with Neural Crest Origin in Lung Fibrosis

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14. ABSTRACT

Pulmonary fibrosis is a debilitating disease characterized by progressive scarring of the lung, which destroys normal lung structure and leads to respiratory failure and death Abnormally increased fibroblasts/myofibroblasts is one of the key pathological changes in lung fibrosis. The origins of these abnormal lung fibroblasts/myofibroblasts are highly heterogeneous. In this project, we plan to determine whether abnormal growth and/or recruitment of a neural crest derived mesenchymal cell population contribute to lung fibrosis. In the past year, we have generated a transgenic reporter mouse line in which neural crest-derived cells were genetically labeled. In normal situation, neural crest-derived cells were detected as nerve fibers adjacent to airway smooth muscles. In contrast, in some bleomycin-induced fibrosis lungs, clusters of neural crest derived cells were detected. This abnormal cellular phenotype varied, and the potential factors affecting this change are currently under investigation. In addition, circulating neural crest-derived mesenchymal progenitor cells were detected in one of five fibrosis mice by peripheral blood mononuclear cell isolation and selective culture. These cultured cells, which were negative for epithelium-marker, were MSC-like progenitors and able to differentiate to other type cells.

15. SUBJECT TERMS
Lung fibrosis; Neural crest cells; Lung myofibroblasts; Lung mesenchymal cells; Bleomycin; Peripheral blood mononuclear cells

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1. INTRODUCTION:

Pulmonary fibrosis is a debilitating disease characterized by progressive scarring of the lung, which destroys normal lung structure and leads to respiratory failure and death. In particular, idiopathic pulmonary fibrosis (IPF) is a severe form with median survival ranging from 2.5 to 3.5 years from diagnosis. Thus, there is a critical need to fully understand the cellular and molecular mechanisms underlying lung fibrosis in order to develop new and effective therapies and reduce mortality. Abnormally increased fibroblasts/myofibroblasts and excessive production of extracellular matrix by these cells are key pathological changes in lung fibrosis. The origins of these abnormal lung fibroblasts/myofibroblasts are highly heterogeneous, possibly utilizing different mechanisms for these cell growth and accumulation, which may lead to different responses to therapeutic interventions. Our preliminary study suggests that there may be a new population of mesenchymal cells with neural crest origin specifically detected in fibrosis lung. Therefore, we plan to determine and characterize a new mesenchymal cell population of neural crest origin specifically in fibrosis lungs of bleomycin-treatment mice. In addition, we will also determine changes in circulating neural crest descendants in response to pulmonary fibrogenic injury.

2. KEYWORDS

Lung fibrosis
Neural crest cells
Lung myofibroblasts
Lung mesenchymal cells
Bleomycin
Peripheral blood mononuclear cells

3. ACCOMPLISHMENTS

What were the major goals of this project?

(1) To determine and characterize a new mesenchymal cell population of neural crest origin specifically in fibrosis lung.

(2) To determine changes in circulating neural crest descendants in response to pulmonary fibrogenic injury.

What was accomplished under these goals?

Major Activity 1 (Major Task 1 in SOW):
To generate lung fibrosis models in mice, in which neural crest cells and their descendants are permanently marked.

1) Specific objective: To expand mouse colonies and generate neural crest-specific reporter mice.

2) Key outcome: We have generated more neural crest reporter mice by crossing Wnt1-Cre and mT-mG reporter mice, which have been used for generating the lung fibrosis model. To exclude the possibility that the GFP-positive cells may result from late activation of Wnt1-Cre during lung fibrotic repair instead of neural crest origin, we also obtained an inducible Wnt1-rtTA/TetO-Cre driver line. By crossing Wnt1-rtTA/TetO-Cre and mT-mG mice, only neural crest cells are marked when inducing agent doxycycline is given during gestation. Unfortunately, the fibrosis study in these mice was aborted due to closure of the lab during COVID-19 pandemic. In addition, we have also optimized the dose for a new lot of bleomycin that induces significant pulmonary fibrosis but no lethality in mice.

Major Activity 2 (Major Task 2 in SOW):
To determine neural crest derived cells in fibrosis lungs and characterize these cells for their mesenchymal properties

1) Specific objective: To determine whether there are neural crest-derived mesenchymal cells in fibrosis lungs and what subtypes of mesenchymal cells they are.

2) Key outcome: We have examined the fibrosis lung sections in which Wnt-Cre-driven GFP-positive cells were detected. By co-immunofluorescence staining, we confirmed that all GFP^+ cells are negative for epithelial marker Cdh1, and found that some of the GFP^+-cells (neural crest origin) express Cspg4 (pericyte marker, Fig.1). We also tried immunostaining for several other markers including Tubb3, Calca, Pdgfra, and Pdgfrb, and did not obtain specific fluorescence signals due to high autofluorescence background caused by infiltrated inflammatory cells. We are in the process to optimize the staining protocol and purchase additional antibodies.

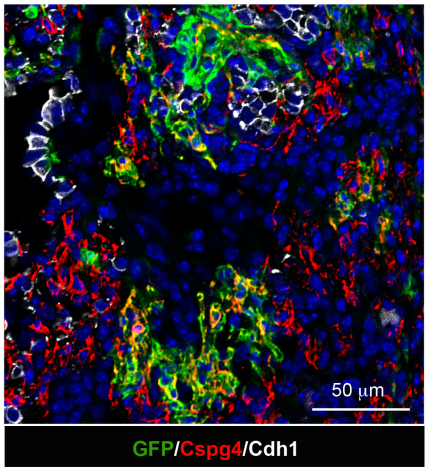

Fig.1. Co-immunostaining of GFP, Cspg4, and Cdh1 of the lung section from bleomycin-induced fibrosis mouse lung. Yellow signals result from overlapped red and green signals.

Major Activity 3 (Major Task 3 & 4 in SOW):
To isolate and culture mouse monocytoid cells with neural crest origin and characterize their mesenchymal transdifferentiation capacity.

1) Specific objective: To establish the isolation and culture methods, and to characterize neural crest derived circulating mononuclear cells in culture.

2) Key outcome: Following the established method as reported in the last annual report, we have repeated isolation and culture of circulating monocytoid progenitor cells from the blood of lung fibrosis mice. Of four samples, one gave MSC-like cell colonies. Their neural crest origin was confirmed by their GFP expression. The data support previous observation that the number of circulating neural crest-derived cells is extremely

low even in bleomycin-induced lung fibrosis model, which suggests that direct quantitative analysis of circulating monocystoid cells may not be feasible.

What opportunities for training and professional development has the project provided?

Nothing to report

How were the results disseminated to communities of interest?

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?

(1) To continually generate mice in which neural crest cells and their descendants are GFP labeled using Wnt1-Cre/mT-mG mice or Sox10-Cre/mT-mG mice. In particular, the experiments aborted due to the COVID-19 pandemic will be resumed.

(2) To compare neural crest-derived cells between fibrosis lungs and normal controls, and further characterize these cells. Immunofluorescence staining will be continued and alternative FACS analysis of single cell suspension from enzyme-dissociated lung tissues will be introduced.

(3) To continue isolate and culture circulating monocytoid cells, and determine their properties by measuring cellular markers including hematopoietic, neural, and mesenchymal markers.

(4) To measure circulating neural crest-derived monocytoid progenitor cells in lung fibrosis mice. Blood samples will be pooled from at least 5 mice per condition. PBMCs will be isolated, cultured, and quantitatively analyzed.

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?

(1) This project will reveal a new mechanism by which neural crest derived cells may be one of the important sources for abnormal lung fibroblasts and myofibroblasts during fibrosis progression.
(2) Dynamic measurement of the neural crest origin mesenchymal cells in lung fibrosis models will provide new knowledge regarding the heterogeneity of fibrotic fibroblasts and myofibroblasts.
(3) Determination of neural crest origin monocytoid progenitor cells in circulation and potential recruitment of these cells to fibrotic lung will provide novel targets for lung fibrosis treatment and prevention.
(4) Analyses of the multiple transdifferentiation capacity for these neural crest derived progenitor cells in both circulation and lung during fibrosis will be critical to understanding fibrosis mechanisms and other lung injury pathology.

What was the impact on other disciplines?

Nothing to report

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS

Nothing to report.

6. PRODUCTS

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Wei Shi
Project Role:	Project Director/Principal Investigator
Researcher Identifier (e.g. ORCID ID):	0000-0001-6499-2473
Nearest person month worked:	*0.6 Calendar*
Contribution to Project:	Dr. Shi is the PI on this project, and oversees the project, including data generation, analysis, and presentation. He will ensure that the project goals are accomplished in a scientifically rigorous and timely manner.
Funding Support:	*DoD, NIH*

Name:	Hui Chen
Project Role:	Research Specialist
Researcher Identifier (e.g. ORCID ID):	0000-0003-0346-1732
Nearest person month worked:	*0.6 Calendar*
Contribution to Project:	Hui performs day-to-day work as proposed in this project, including animal breeding, genotyping, tissue fixation and histology/morphometry, and immunohistochemistry.
Funding Support:	None

Name:	Yongfeng Luo
Project Role:	Postdoctoral Research Associate
Researcher Identifier (e.g. ORCID ID):	0000-0001-8765-0273
Nearest person month worked:	*0.6 Calendar*
Contribution to Project:	Dr. Luo is responsible for bleomycin-induced lung fibrosis mouse models, cell isolation and characterization, immunofluorescence staining, confocal imaging, and FACS analysis.
Funding Support:	None

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Dr. Wei Shi has one new active project:

T30IP1028 (Lien) 09/01/19-08/31/21 1.08 calendar
California Tobacco Related Disease Research Program $200,000
"Effects of tobacco and e-cigarettes on heart repair and regeneration"
This pilot grant is to test a hypothesis that tobacco (including both combustible tobacco cigarette and e-cigarette) not only increases the chances of a first-time heart attack but can also affect heart repair and regeneration after myocardial infarction by regulating the cardiac lymphatic system. We aim to determine (1) the effects of tobacco and e-cigarettes in zebrafish heart regeneration and cardiac lymphatic vessel functions; (2) the effects of tobacco

smoke on mouse heart repair, fibrotic scar formation, development of heart failure and lymphangiogenesis. There is no scientific overlap between this project and the current DoD project.
Role: Co-I

What other organizations were involved as partners?

None

8. SPECIAL REPORTING REQUIREMENTS

- **COLLABORATIVE AWARDS:** *Not applicable*
- **QUAD CHARTS:** .*Not Applicable*

9. APPENDICES

Nothing to report.