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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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CONTRACTING ORGANIZATION: Children's Hospital Los Angeles

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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: As reported previously, our mouse experiment was suspended due to COVID-19 shutdown. We have resumed mouse colony expansion and cross-breeding since early January 2021. About 20 Wnt1-Cre/mT-mG neural crest reporter mice have been obtained for the lung fibrosis study.

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: As reported last year, presence of the Wnt1-Cre-driven positive cells (neural crest origin) has high variation in the fibrotic mouse lungs that is induced by one dose bleomycin. We now determine whether more consistent cellular phenotypes are presented in the mouse lungs with severe and irreversible fibrosis lesions. The Wnt1-Cre/mT-mG mice have been given relatively low dose of bleomycin (1.5 u/kg) every other week for 8 doses (~4 months) through non-invasive intra-tracheal aerosol spray. We expect to harvest and examine the lung tissues and circulating mononuclear cells (n>5 in both experimental and saline control groups) in the mid of July.

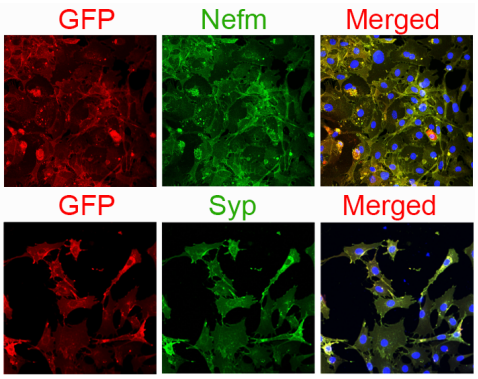

Fig.1. Immunofluorescence co-staining of the cultured cells with GFP and neural markers.

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: We have further characterized the cells in the previously established culture, which was originally isolated from peripheral blood mononuclear cells. By co-staining these cells with antibodies against GFP (neural crest reporter) and neural markers (Nefm and Syp) or mesenchymal cell marker (Vimentin), we found that most cells in culture are positive for the above markers (Fig.1 & 2), suggesting that these are neural crest-derived ectomesenchymal cells.

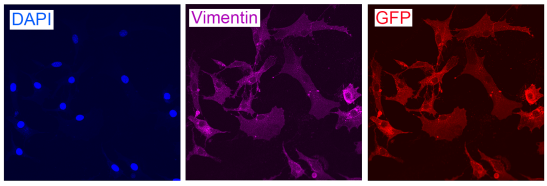

Fig.2. Immunofluorescence co-staining of the cultured cells with GFP and mesenchymal markers.

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 compare neural crest-derived cells between the irreversible fibrosis lungs and normal controls, and further characterize these cells.

(2) To analyze quantitatively and qualitatively circulating neural crest-derived monocytoid progenitor cells in the irreversible lung fibrosis mice.

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:	*0.3 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:	Yongfeng Luo
Project Role:	Postdoctoral Research Associate
Researcher Identifier (e.g. ORCID ID):	0000-0001-8765-0273
Nearest person month worked:	*0.3 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 two new active projects:

T31IP1685 Shi (PI) 07/01/20-06/30/22
California Tobacco Related Disease Research Program $200,000
"The deleterious effects of nicotine and e-cigarette flavorants on lung mesenchymal stem cells".
The pilot grant is to test a hypothesis that nicotine and/or e-cigarette flavorants induce lung damage through adversely affecting lung mesenchymal stem cell-mediated lung injury repair and homeostasis. We aim to determine (1) the direct toxic effects of nicotine and/or e-cigarette flavorants on lung mesenchymal stem cells in culture and the related cellular and molecular mechanisms. (2) the alterations of endogenous lung mesenchymal stem cells in mice that expose to e-cigarette vapor vs. conventional tobacco cigarette smoke. There is no scientific overlap between this project and the current DoD project.
Role: PI

W81XWH-21-1-0257 Shi (PI) 04/15/21-04/14/23
DoD/CDMRP $100,000
"Mesenchymal Folliculin defects as a novel pathogenic mechanism of polycystic kidney lesions"
The project is to test a hypothesis that *Flcn* deficiency in distinct subsets of renal mesenchymal cells leads to polycystic kidney lesions via Wnt and Tfe3-dependent signaling to renal epithelium. Two specific aims are: (1) To determine the key subsets of mesenchymal cells in which *Flcn* deletion results in polycystic kidney lesions. (2) To determine the mechanisms by which *Flcn* deletion in the defined mesenchymal cells causes renal cysts. There is no scientific overlap between this project and the current DoD project.
Role: PI

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.