AWARD NUMBER: W81XWH-18-1-0185

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

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REPORT DATE: JUNE 2019

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
MARCH 2019	Annual	1JUN2018 - 31MAY2019

4. TITLE AND SUBTITLE
Growth and/or Recruitment of a Novel Cell Population with Neural Crest Origin in Lung Fibrosis

5a. CONTRACT NUMBER
W81XWH-18-1-0185

5b. GRANT NUMBER
PR171133

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

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

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	16	19b. TELEPHONE NUMBER *(include area code)*

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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. This goal will be achieved in 18 months in the approved SOW.

(2) To determine changes in circulating neural crest descendants in response to pulmonary fibrogenic injury. This will be achieved in 18 months in the approved SOW.

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 obtain approval for animal protocol used in this project and start to generate the reporter mice, in which neural crest cells and their descendants are genetically labeled.

2) Key outcome: We have worked closely with the IACUC of Children's Hospital Los Angeles and ACURO, and obtained approval for our animal protocol from both IACUC and ACURO. We have crossed neural crest driver line Wnt1-Cre with fluorescence reporter mice mT-mG to generate Wnt1-Cre/mT-mG reporter mice. By examining the normal lungs from these reporter mice, a few GFP-positive cells were detected around airway smooth muscle cells, appearing as neural crest derived peripheral nerves (Fig.1). In addition, we have generated the fibrosis model in these reporter mice by intra-tracheal bleomycin administration. Due to decreased profibrotic activity of our previously frozen bleomycin aliquots, we had to purchase a new vial of bleomycin and optimized the appropriate dose of newly obtained bleomycin.

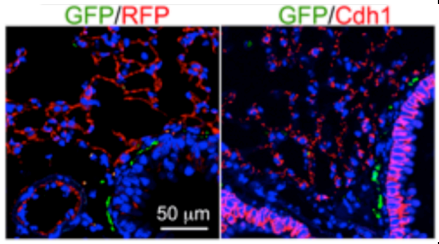

Fig.1. Wnt1-Cre/mT-mG mouse normal lung sections were stained with the indicated antibodies.

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: Among 5 fibrosis mice (three weeks after intra-tracheal bleomycin treatment), 2 of them had clusters of GFP-positive cells (neural crest derived) in the fibrosis lungs (Fig.2), while the other three did not, suggesting high variation in the cellular phenotypes. We are now focusing on the following issues in order to

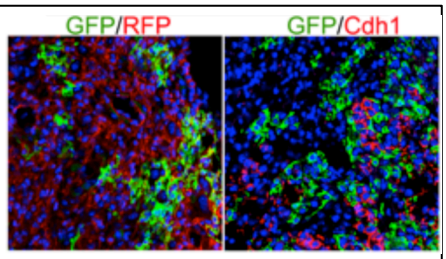

Fig.2. Wnt-Cre/mT-mG mouse lung fibrosis was induced by bleomycin. Lung sections (3 weeks after bleomycin injury) were stained with the indicated antibodies.

address the potential causes for such variation: (a) lung fibrosis severity, (b) mouse genetic background, (c) ages of experimental mice.

Major Activity 3 (Major Task 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 established the method to isolate and culture circulating monocytoid progenitor cells. Briefly, mouse peripheral blood was collected in a BD Microtainer tube with lithium heparin, and diluted with PBS (1:1), which was then layered onto Histopaque-1077. Following centrifugation at 400 g for 30 min, the mononuclear cell layer was transferred to a fresh tube and washed in PBS. The cell pellet was then resuspended in MSC culture medium and seeded onto a 100 mm culture dish. We have tried 5 samples (2 normal and 3 fibrosis mice), and only obtained MSC-like cell colonies from one fibrosis mouse sample. The cultured cells were able to grow more than 20 passages and became stable in culture, which had multipotent differentiation capacity. By immunostaining, these cells are GFP-positive and Tomato-negative (Fig.3), suggesting that they are neural crest-derived circulating mesenchymal progenitor cells. However, it seems that the number of these cells in circulation is extremely low. Whether such cells increase in response to bleomycin-induced fibrosis will be the focus of our studies in next 6 months.

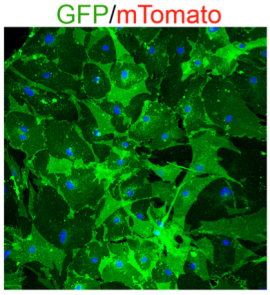

Fig.3. Established cell culture from PBMCs. The cells were stained by the indicated antibodies.

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. In addition to expanding Wnt1-Cre/mT-mG mice, we are generating an alternative reporter mouse line with genotypes of Sox10-Cre/mT-mG. These mice will be used for lineage tracing in experimental lung fibrosis.

(2) To compare neural crest-derived cells between fibrosis lungs and normal controls, and further characterize these cells. More fibrosis lungs at different fibrotic stages will be harvested and analyzed using the proposed quantitative and qualitative approaches.

(3) To measure circulating neural crest-derived monocytoid progenitor cells in lung fibrosis mice. PBMCs will be isolated from the Wnt1-Cre/mT-mG reporter mice with or without lung fibrosis. The cells with mGFP vs. the cells with mTomato will be directly quantified and compared between fibrosis mice and normal controls.

(4) To culture and isolate mouse monocytoid cells with neural crest origin and characterize their mesenchymal transdifferentiation capacity. We will continue isolation and culture of circulating monocytoid cells, and characterize the cells for their neural crest origin and progenitor properties.

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:	*1.8*
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:	*2*
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:	*6*
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:

1R01HL146541-01 (Shi & Henske) 04/01/19-03/31/23 3.50 calendar
NIH/NHLBI
“Pathogenic Mechanisms of Pulmonary Lymphangioleiomyomatosis”

This project is focused on the mechanisms underlying hyperactive mTORC1 in subsets of lung mesenchymal cells leads to estrogen-dependent proliferation of nodular-like lesions, associated with metabolic and mitochondrial dysfunction, recruitment of extrapulmonary cells and lung destruction with two specific aims (1) To dissect the molecular and cellular mechanisms of LAM using a novel mouse model with spontaneous LAM-like lung lesions. The role of hyperactivated mTORC1 in the pulmonary LAM-like nodules will be examined using both pharmacologic (reversible) and genetic (permanent) approaches. The impact of Tsc2 deficiency on cellular metabolism and mitochondrial function will be addressed for the first time in lung mesenchyme-derived cells as a potential mechanism for the abnormal cell growth. The origin of LAM cells and the mechanisms of recruitment of extrapulmonary mesenchymal cells to LAM-like nodules will be examined using both parabiosis and allograft models to elucidate the cellular heterogeneity of LAM nodules (2) To determine the specific role of gender in contributing to the formation and progression of LAM-like nodules in mice with lung mesenchymal *Tsc2* delet**ion.** The roles of estrogen in the pulmonary LAM-like lesions will be investigated *in vivo* by altering estrogen activity in lung mesenchyme-specific *Tsc2* knockout mice, or by simultaneous deletion of ERa/Tsc2 or ERb/Tsc2. The mechanisms will be further dissected *in vitro* by estrogen-mediated metabolic profiling, RNA-seq, ChIP-seq in *Tsc2*-null vs. wild-type cells. Although the lung mesenchyme-specific *Tsc2* knockout mouse model is used in this project, the major research questions and approaches proposed in this R01 are totally different. There is no significant scientific overlap between this new R01 project and our ongoing DoD project.
Role: PI

Dr. Wei Shi has one project closed this year.

1U01 HL122681-01 (Warburton) 06/14/14-04/30/19 1.80 calendar
NIH/NHLBI
“Molecular anatomy of human alveolar development”
The major goal of this grant is to generate a digital map of alveolar development in mouse and human lung, using various imaging approaches.
Role: Co-investigator

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.