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14. ABSTRACT
Aim 1 and 2: Using mouse models of lung injury, fibrosis and epithelial dysfunction, we have demonstrated that microenvironment is a key factor driving phenotype of profibrotic alveolar macrophages. We have shown that epithelial dysfunction promotes recruitment of monocyte-derived alveolar macrophages and leads to exaggerated pulmonary fibrosis.
Aim 3. Pulmonary fibrosis is a heterogeneous syndrome in which fibrotic scar replaces normal lung tissue. We performed massively parallel single-cell RNA-Seq on lung tissue from eight lung transplant donors and eight patients with pulmonary fibrosis. Combined with in situ RNA hybridization, with amplification, these data provide a molecular atlas of disease pathobiology. We identified a distinct, novel population of profibrotic alveolar macrophages exclusively in patients with fibrosis. Within epithelial cells, the expression of genes involved in Wnt secretion and response was restricted to non-overlapping cells. We identified rare cell populations including airway stem cells and senescent cells emerging during pulmonary fibrosis. Analysis of a cryobiopsy specimen from a patient with early disease supports the clinical application of single-cell RNA-Seq to develop personalized approaches to therapy.

15. SUBJECT TERMS
alveolar macrophages, pulmonary fibrosis

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Introduction

Purpose: To test the hypothesis that the replacement of tissue-resident alveolar macrophages with monocyte-derived macrophages in response to repeated injury over the lifespan explains the delayed onset of lung fibrosis until the later decades of life. We will use our experimental findings in mice to guide an unbiased assessment of macrophage heterogeneity in normal human lungs and lungs from patients with pulmonary fibrosis. The overarching goal of these studies is to identify clinically applicable biomarkers that can guide therapy and factors released from tissue-resident macrophages or bone marrow-derived macrophages that prevent or promote fibrosis, respectively so they can be targeted for prevention or therapy. This hypothesis will be tested in the following aims: Aim 1: To determine whether replacement of tissue-resident alveolar macrophages by monocyte-derived alveolar macrophages during aging contributes to the enhanced susceptibility to lung fibrosis in aged mice. Aim 2: To determine whether tissue-resident alveolar macrophages or monocyte-derived alveolar macrophages differentially respond to epithelial injury in a murine model of accelerated pulmonary fibrosis. Aim 3: To identify novel biomarkers expressed by human tissue-resident and monocyte-derived alveolar macrophages based on single cell molecular classification in patients with pulmonary fibrosis.

Keywords

Alveolar macrophages, pulmonary fibrosis, idiopathic pulmonary fibrosis

Accomplishments

Major Goal: Specific Aim 1: To determine whether replacement of tissue-resident alveolar macrophages by monocyte-derived alveolar macrophages during aging contributes to the enhanced susceptibility to lung fibrosis in aged mice.

Major Task 1: Are tissue-resident alveolar macrophages replaced by monocyte-derived alveolar macrophages during normal aging?
Subtask 1: Generate cohorts of shielded bone marrow chimeric mice. Dr. Misharin will perform the procedure, Drs. Chen and Soberanes will monitor animals, Dr. Budinger will oversee the project.
Subtask 2: Aging of shielded bone marrow chimeric mice. Monitor shielded bone marrow chimeric mice during aging. Track mortality (Drs. Misharin, Soberanes, Chen). Harvest lungs from shielded chimeric at designated time points (6 weeks, 6, 12, 18 and 24 months), FACSort alveolar macrophages, isolate RNA (Drs. Misharin, Soberanes and Chen). Prepare libraries for RNA-seq, perform sequencing and analyze the data (Drs. Budinger, Misharin, Soberanes, Bartom and Chen).

Accomplishments: We have completed experiment related to this subtask. We have found that "healthy" aging, i.e. aging mice without lung injury, does not lead to the accelerated replacement of tissue-resident alveolar macrophages with monocyte-derived alveolar macrophages. Moreover, using RNA-seq on FACSorted macrophages, we found a highly reproducible group of genes, differentially expressed between tissue-resident alveolar macrophages (originating from fetal monocytes) and monocyte-derived alveolar macrophages (originating from adult circulating monocytes) irrespective of age. This cluster of gene contained several transcription factors and pathogen recognition receptors, such as *Marco*. Both tissue-resident and monocyte-derived alveolar macrophages exhibited similar changes during the normal aging, characterized by the decreased expression of the cell cycle genes and increased expression of the components of immune-inflammasome. These findings informed our studies in Major Task 2.

Major Task 2: Do insults associated with normal aging accelerate the replacement of tissue-resident alveolar macrophages with monocyte-derived alveolar macrophages?
Subtask 1: Generate cohorts of shielded bone marrow chimeric mice. Dr. Misharin will perform the procedure, Drs. Soberanes and Chen will assist and monitor animals, Dr. Budinger will oversee the project.
Subtask 2: Infect shielded chimeric mice with three strains of influenza A (A/WSN/33 and A/PR8/34 and A/Udorn/72) at 2, 4 and 6 months of age. Dr. Soberanes will perform the infections, Drs. Soberanes and Chen will assist and monitor animals. Dr. Budinger will oversee the project. Harvest lungs from shielded chimeric at designated time points (6 weeks, 6, 12, 18 and 24 months), FACSort alveolar macrophages, isolate RNA (Drs. Misharin, Soberanes and Chen). Prepare libraries for RNA-seq, perform sequencing and analyze the data (Drs. Budinger, Misharin, Bartom, Soberanes and Chen).
Accomplishments: As reported previously, generation of the shielded bone marrow chimeras is complete (100%). We have finished harvesting tissues from all experimental animals. Flow cytometric analysis demonstrates accelerated replacement of tissue-resident alveolar macrophages with monocyte-derived alveolar macrophages. Moreover transcriptomic profiling of FACSorted tissue-resident and monocyte-derived alveolar macrophages via RNA-seq at multiple time points demonstrated that lung injury can reprogram tissue-resident alveolar macrophages.

Major Task 3: Does replacement of tissue-resident alveolar macrophages by monocyte-derived alveolar macrophages lead to exaggerated fibrosis in mice?

Subtask 1: Generate cohorts of shielded bone marrow chimeric mice on using CD11c-DTR mice as a host. Dr. Misharin will perform the procedure, Drs. Soberanes and Chen will assist and monitor animals, Dr. Budinger will oversee the project.
Subtask 2: Initiate bleomycin-induced lung fibrosis in shielded chimeric mice and harvest macrophage populations. Dr. Soberanes will instill mice with bleomycin, Drs. Chen and Soberanes will assist and monitor animals. Dr. Budinger will oversee the project. Harvest lungs, FACSort alveolar macrophages, isolate RNA (Drs. Misharin, Soberanes and Chen). Prepare libraries for RNA-seq, perform sequencing and analyze the data (Drs. Budinger, Misharin, Bartom, Soberanes and Chen).
Accomplishments: As reported during the last year, during the initial phase experiments, we found out that CD11c-DTR mice develop severe lung injury upon depletion of CD11c-DTR-positive alveolar macrophages. These findings were recently confirmed and reported by another group (Mann et al., Eur J Immunol, 2016). To overcome this issue, we have developed an alternative system for depletion of tissue-resident alveolar macrophages using intratracheal instillation of clodronate-loaded liposomes. We found that intratracheal instillation of 50 ul of clodronate-loaded liposomes efficiently depletes tissue-resident alveolar macrophages and leads to the repopulation of the niche by monocyte-derived alveolar macrophages. Thus, we were able to overcome the technical issues and use regular shielded bone marrow chimeras for this subtask. We have generated sufficient number of shielded bone marrow chimeras and treated them with clodronate-loaded liposomes to deplete tissue-resident alveolar macrophages and induce repopulation with monocyte-derived alveolar macrophages.
Using transcriptomic profiling of the FACSorted alveolar macrophages we have demonstrated that local environment (normal vs inflammatory milieu) during the niche repopulation plays a crucial role in programming monocyte-derived alveolar macrophages. While monocyte-derived alveolar macrophages recruited into non-inflammatory environment after sterile depletion with clodronate-loaded liposomes did not exhibited pro-fibrotic activity and behaved similarly to tissue-resident alveolar macrophages, monocyte-derived alveolar macrophages recruited during bleomycin-induced or influenza A-induced lung injury exhibited marked profibrotic activity, resulting in marked transcriptional changes (as determined by RNA-seq) as well as changes in lung function (as determined by direct measurement of lung mechanics with Flexivent).

Major Task 4: Can adoptive transfer of tissue-resident or monocyte-derived alveolar macrophages rescue or exacerbate, respectively, the severity of experimental lung fibrosis?
Subtask 1: Sort alveolar macrophages, generate bone marrow derived macrophages, perform adoptive transfer and induce lung fibrosis. Dr. Misharin will perform sorting for alveolar macrophages and generate bone marrow derived macrophages in vitro. Dr. Soberanes will perform adoptive transfer of alveolar and bone marrow-derived macrophages and instill mice with bleomycin. Drs. Chen and Soberanes will assist and monitor animals. Harvest lungs, FACSort alveolar macrophages, isolate RNA (Drs. Misharin, Soberanes and Chen). Prepare libraries for RNA-seq, perform sequencing and analyze the data (Drs. Budinger, Perlman, Misharin, Bartom, Soberanes and Chen).
Accomplishments: We have established protocol for adoptive transfer of alveolar macrophages and performed initial experiments. Our data suggest that adoptive transfer of tissue-resident alveolar macrophages does not rescue experimental lung fibrosis. The experiments with adoptively-transferred monocyte-derived alveolar macrophages are currently ongoing. We expect to complete them in a timely manner during the third year of the project and include in our second publication.
We have recently **published** summary of our work for the Aim 1 of the current project in the Journal of Experimental Medicine (Misharin et al., JEM, 2017). We also presented our work at American Thoracic Society meeting in Washington, DC and 2017 MHSRS meeting in Florida. Importantly, our findings were independently confirmed by Dr. Janssen's group (McCubbrey et

al., AJRCMB, 2017), which speaks of high reproducibility of our data. Another manuscript, summarizing the data on the role of local environment in programming tissue-resident and monocyte-derived alveolar macrophages in currently in preparation.

Specific Aim 2: To determine whether tissue-resident alveolar macrophages or monocyte-derived alveolar macrophages differentially respond to epithelial injury in a murine model of the accelerated pulmonary fibrosis.

Major Task 1: Do macrophages from young and aged mice differentially regulate chronic stress in the lung epithelium?
Subtask 1: Generate cohorts of shielded bone marrow chimeric mice with tissue-resident or monocyte-derived alveolar macrophages using young and aged CD11c-DTR mice. Dr. Misharin will perform the procedure, Drs. Chen and Soberanes will monitor animals, Dr. Budinger will oversee the project.
Subtask 2: Sort tissue-resident alveolar macrophages, perform adoptive transfer and induce lung fibrosis. Dr. Misharin will perform sorting for alveolar macrophages. Dr. Soberanes will perform adoptive transfer of alveolar and instill mice with bleomycin. Dr. Soberanes will assist and monitor animals. Harvest lungs, FACSort alveolar macrophages, isolate RNA (Drs. Misharin, Soberanes and Chen). Prepare libraries for RNA-seq, perform sequencing and analyze the data (Drs. Budinger, Misharin, Bartom, Soberanes and Chen).
Subtask 3: Bleomycin-induced lung fibrosis in bone marrow chimeric mice. Monitor shielded bone marrow chimeric mice during aging. Track mortality (Drs. Misharin, Soberanes, Chen). Harvest lungs from shielded chimeric at designated time points, FACSort alveolar macrophages, isolate RNA (Drs. Misharin, Soberanes and Chen). Prepare libraries for RNA-seq, perform sequencing and analyze the data (Drs. Budinger, Perlman, Misharin, Soberanes and Chen).
Accomplishments: See above, Aim 1, Major task 4. During the initial phase experiments, we found out that CD11c-DTR mice develop severe lung injury upon depletion of CD11c-DTR-positive alveolar macrophages. These findings were recently confirmed and reported by another group (Mann et al., Eur J Immunol, 2016). To overcome this issue, we have developed an alternative system for depletion of tissue-resident alveolar macrophages using intratracheal instillation of clodronate-loaded liposomes. We found that intratracheal instillation of 50 ul of clodronate-loaded liposomes efficiently depletes tissue-resident alveolar macrophages and leads to the repopulation of the niche by monocyte-derived alveolar macrophages. Thus, we were able to overcome the technical issues and use regular shielded bone marrow chimeras for this subtask. We have generated sufficient number of shielded bone marrow chimeras and treated them with clodronate-loaded liposomes to deplete tissue-resident alveolar macrophages and induce repopulation with monocyte-derived alveolar macrophages.

Major Task 2: Does chronic stress in the epithelium accelerate the replacement of tissue-resident alveolar macrophages with monocyte-derived alveolar macrophages during aging?
Subtask 1: Generate cohorts of shielded bone marrow chimeric mice using HPS-/- mice. Dr. Misharin will perform the procedure, Drs. Chen and Soberanes will assist and monitor animals, Dr. Budinger will oversee the project.
Accomplishments: See above, Major Task 2. While HPS-/- mice provided a valuable insight during the preliminary work, their utility was undermined because HPS deficiency was global and affecting not only epithelial cells, but all other cell types as well (including alveolar macrophages). Fortunately, soon after the work on the grant was initiated, a new strain of mice specifically causing stress in the lung epithelium by targeting telomerase (*Trf1*) in alveolar type 2 epithelial cells using highly specific *Cre*^{Sftpc} driver became available (Povedano et al., 2015, Cell Rep). These mice develop spontaneous age-dependent lung fibrosis. We have initiated collaboration with Dr. Mary Blasco and obtained *Trf1*-floxed mice for our colony. We have now finished crossing

these mice to *Cre*^{Sftpc} mice and initiated the proposed experiments. While this change of tools delayed the work on this subtask, it did not change the scope of our work. Moreover, this approach is much more specific and will allow us to reduce the number of required animals.
We have discussed our approach with Dr. Mary Armanios, one of the world's experts in telomerase deficiency-associated pulmonary fibrosis. This consultation has confirmed our current direction.
Experiments, conducted during the last quarter, demonstrated that young mice with deficiency of *Trf1* in alveolar epithelial cells exhibit enhanced response to profibrotic stimulus (bleomycin) and delayed recovery in response to influenza A infection. We are now aging a cohort of these animals to investigate the role of monocyte-derived alveolar macrophages in response to lung injury.
Subtask 2: Harvest and analyze the lungs. Harvest lungs from shielded chimeric at designated time points (6 weeks, 4, 12 and 18 months), FACSort alveolar macrophages, isolate RNA (Drs. Misharin, Soberanes and Chen). Prepare libraries for RNA-seq, perform sequencing and analyze the data (Drs. Budinger, Perlman, Misharin, Bartom, Soberanes and Chen).
Accomplishments: The work on this subtask was delayed because of the change in the mouse strain (see above). We have started our experiments using mice with *Trf1*-deficiency in alveolar type 2 cells. The proposed experiments involving second hit (influenza A and bleomycin) are currently under way, however, we already have data from single hit experiments, demonstrating that these animals develop more severe fibrosis and delayed recovery after influenza A lung injury. We have isolated macrophages from 4 months old mice and isolated RNA. RNA-seq on this first cohort will be performed during the next quarter.

Major Task 3: Can the adoptive transfer of tissue-resident alveolar macrophages improve chronic stress in the lung epithelium?
Subtask 1: Generate cohorts of shielded bone marrow chimeric mice using HPS-/- mice. Dr. Misharin will perform the procedure, Drs. Chen and Soberanes will assist and monitor animals, Dr. Budinger will oversee the project.
Accomplishments: See above, Major Task 2. While HPS-/- mice provided a valuable insight during the preliminary work, their utility was undermined because HPS deficiency was global and affecting not only epithelial cells, but all other cell types as well (including alveolar macrophages). Fortunately, soon after the work on the grant was initiated, a new strain of mice specifically causing stress in the lung epithelium by targeting telomerase (*Trf1*) in alveolar type 2 epithelial cells using highly specific *Cre*^{Sftpc} driver became available (Povedano et al., 2015, Cell Rep). These mice develop spontaneous age-dependent lung fibrosis. We have initiated collaboration with Dr. Mary Blasco and obtained *Trf1*-floxed mice for our colony. We have now finished crossing these mice to *Sftpc*^{Cre} and *Sftpc*^{ERCre} mice and initiated the proposed experiments. While this change of tools delayed the work on this subtask, it did not change the scope of our work. Moreover, this approach is much more specific and will allow us to reduce the number of required animals.
Subtask 2: Sort alveolar macrophages, perform adoptive transfer and induce lung fibrosis. Dr. Misharin will perform sorting for alveolar macrophages. Dr. Soberanes will perform adoptive transfer of alveolar and instill mice with bleomycin. Ms. Saber and Dr. Soberanes will assist and monitor animals. Harvest lungs, FACSort alveolar macrophages, isolate RNA (Drs. Misharin, Soberanes and Chen). Prepare libraries for RNA-seq, perform sequencing and analyze the data (Drs. Budinger, Perlman, Misharin, Soberanes and Chen).
Accomplishments: The work on this subtask was delayed because of the change in the mouse strain (see above). Once the experiments in Major Task 2 are complete, we will perform experiment in this Subtask.

Specific Aim 3: To identify novel biomarkers expressed by human tissue-resident and monocyte-derived alveolar macrophages based on single cell molecular classification in patients with IPF.

Major Task 1: Collect samples, perform RNA-seq on populations of lung macrophages, perform single cell RNA-seq and analyze the data.
Subtask 1: Submit documents for local IRB review. Organize workflow, establish standard operating procedures, meeting with all co-investigators.
Accomplishments: Our study protocol was approved by the local IRB on 2016/06/05. This protocol was reviewed by the US Army Medical Research and Materiel Command (USAMRMC), Office of Research Protections (ORP), Human Research Protection Office (HRPO) and found to comply with applicable DOD, US Army, and USAMRMC human subjects protection requirements. We received an initial note of approval on 2015/12/01 and HRPO Approval Memorandum on 2016/1/9, HRPO Log Number A-18899.b.
Subtask 2: Begin study. Recruit first patients to the study (Drs. Mutlu, Bhorade, Budinger). Perform pilot RNA-seq on populations of pulmonary macrophages, establish optimal workflow and data analysis pipelines (Drs. Misharin, Budinger, Bartom, Berdnikovs, Perlman).
Accomplishments: We have achieved a significant progress on this subtask. Since approval of the protocol by IRB and HRPO over 70 lung samples were processed, including donor lungs and lungs from patients with various forms of lung fibrosis (SSc-ILD, IPF, ILD, MCTD, hypersensitivity pneumonitis, pneumoconiosis, CPFE). As proposed, we have FACSorted alveolar macrophages and alveolar type 2 cells from these samples and extracted RNA for gene expression profiling using RNA-seq. In addition, to emphasize the advantages of the focusing on specific cellular populations we analyzed gene expression profiles from the whole lung tissue (traditional approach implemented by other groups). All samples have yielded high-quality (RIN over 7) RNA. Libraries for RNA-seq were prepared using a high-throughput automated robotic platform (Agilent Bravo) to minimize a batch effect, all libraries have passed the QC. Libraries from were sequenced on Illumina NextSeq 500 instrument. Data was processed using an established bioinformatic pipeline on high-performance computational cluster QUEST (Dr. Bartom). Integrative analysis of the whole lung tissue, alveolar macrophages and alveolar type 2 cells demonstrated that alveolar macrophages and alveolar type 2 cells carry transcriptional signature of pulmonary fibrosis and can be used for the diagnostics. These findings were reported in our manuscript, which is now available as a preprint: Single-Cell Transcriptomic Analysis of Human Lung Reveals Complex Multicellular Changes During Pulmonary Fibrosis (https://doi.org/10.1101/296608).

Subtask 3: Single cell RNA-seq. Recruit patients to the study (Drs. Mutlu, Bhorade, Budinger). Process samples, generate single-cell RNA-seq libraries (Drs. Misharin, Chen, Soberanes). Perform RNA-seq, perform data analysis (Drs. Misharin, Budinger, Bartom, Berdnikovs, Perlman).
Accomplishments: We have recruited 17 subjects into the study: 9 subjects with various forms of pulmonary fibrosis, including 4 subjects with IPF, and 8 control subjects. Accordingly, we have performed single cell RNA-seq on these samples using 10x Genomics 3' V2 chemistry reagents. We have completed sequencing and analysis of all samples. We have identified several robust markers of profibrotic macrophages, including *SPP1, CHI3L1, MMP9* and others. We have validated these markers using fluorescent *in situ* RNA hybridization and immunohistochemistry. We have prepared manuscript and submitted it to the peer-reviewed journal. While the manuscript is under review, we have made efforts to disseminate our data via preprint server bioRxiv: Single-Cell Transcriptomic Analysis of Human Lung Reveals Complex Multicellular Changes During Pulmonary Fibrosis (https://doi.org/10.1101/296608). This manuscript received a lot of attention (viewed over 3800 times and pdf downloaded over 2300 times) and lots of positive feedback and requests for access to the data. To fulfill the community request we have developed an intuitive web-tool for the dataset exploration, which is available at http://lungatlas.s3-website-us-east-1.amazonaws.com Upon the publication this data will be publicly available via lungatlas.org/data/reyfman2018.

Major Task 2: Design diagnostic flow cytometry panel.

Subtask 1: Select prospective markers based on RNA-seq data (Drs. Misharin, Budinger, Berdnikovs, Perlman). Screen antibodies for panel design (Dr. Misharin). Design and validate the panel (Drs. Misharin, Budinger).

Accomplishments: We have performed validation of the markers of profibrotic macrophages via fluorescent *in situ* RNA hybridization and immunohistochemistry. We are now adapting these techniques to develop diagnostic flow cytometry panel in bronchoalveolar lavage fluid from patients with pulmonary fibrosis.

Opportunities for training and professional development:

Impact on society beyond science and technology: Nothing to report.

Impact

Impact on the development of the principal discipline(s) of the project: Nothing to report.

Impact on other disciplines: Nothing to report.

Impact on technology transfer: Nothing to report.

Impact on society beyond science and technology: Nothing to report.

Changes/Problems

Changes in approach:

No changes in approach since last progress report.

Actual or anticipated problems or delays and actions or plans to resolve them: Nothing to report.
Changes that had a significant impact on expenditures: Nothing to report.
Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents: Nothing to report.

Products

Journal publications:

1. Reyfman PA, Walter JM, Joshi N, Anekalla KR, McQuattie-Pimentel AC, Chiu S, Fernandez R, Akbarpour M, Chen C, Ren Z, Verma R, Abdala-Valencia H, Nam K, Chi M, Han S, Gonzalez-Gonzalez FJ, Soberanes S, Watanabe S, Williams KJN, Flozak AS, Nicholson TT, Morgan VK, Hrusch CL, Guzy RD, Bonham CA, Sperling AI, Bag R, Hamanaka RB, Mutlu GM, Yeldandi AV, Marshall SA, Shilatifard A, Amaral LAN, Perlman H, Sznajder JI, Winter DR, Hinchcliff M, Argento AC, Gillespie CT, Dematte JD, Jain M, Singer BD, Ridge KM, Gottardi CJ, Lam AP, Bharat A, Bhorade SM, Budinger GRS, Misharin AV. Single-Cell Transcriptomic Analysis of Human Lung Reveals Complex Multicellular Changes During Pulmonary Fibrosis. BioRxiv 296608, April 6 2018, available from: https://doi.org/10.1101/296608

2. Joshi N, Walter JM, Misharin AV. Alveolar Macrophages. Cell Immunol. 2018 Aug;330:86-90. doi: 10.1016/j.cellimm.2018.01.005. Epub 2018 Jan 20.

3. Soberanes S, Misharin AV, Jairaman A, Morales-Nebreda L, McQuattie-Pimentel AC, Cho T, Hamanaka RB, Meliton AY, Walter JM, Chen CI, Chi M, Chiu S, Gonzalez-Gonzalez FJ, Antalek M, Adbala-Valencia H, Chiarella SE, Sun KA, Woods PS, Ghio AJ, Jain M, Perlman H, Ridge KM, Morimoto RI, Sznajder JI, Balch WE, Bhorade SM, Bharat A, Prakriya M, Chandel NS, Mutlu GM, Budinger GRS. Metformin Targets Mitochondrial Electron Transport to Reduce Air-Pollution-Induced Thrombosis. Cell Metab. 2018 Oct 4. pii: S1550-4131(18)30586-2. doi: 10.1016/j.cmet.2018.09.019. PMID: 30318339

Presentations:

1. Alexander Misharin. Dissecting the Role of Alveolar Macrophages in Pulmonary Fibrosis: Insights from Mouse Models, Clinical Samples and Single Cell Transcriptomic. Division of Pulmonary and Critical Care, Duke University, Durham, NC. December 12, 2017.

2. GR Scott Budinger. Understanding molecular signatures of aging. NIA NIH. June 6, 2018. Washington, DC. Available from https://videocast.nih.gov/Summary.asp?File=23935&bhcp=1

Websites:

LungAtlas: http://lungatlas.s3-website-us-east-1.amazonaws.com This website contains the webtool for visualization and exploration of the single cell RNA-seq dataset from our recent preprint (Single-Cell Transcriptomic Analysis of Human Lung Reveals Complex Multicellular Changes During Pulmonary Fibrosis https://www.biorxiv.org/content/early/2018/04/06/296608). Upon the publication this dataset will be linked to the peer-reviewed publication.

Participants and other collaborating organizations

Name: **GR Scott Budinger**
Project Role: Principal Investigator
Researcher Identifier: NA
Nearest person month worked: 1.2
Contribution to Project: Dr. Budinger has prepared IRB protocol and worked on its revisions. He supervised generation of the shielded bone marrow chimeras and development of the new fate-mapping mouse models. He performed acquisition of the human lung samples for FACSorting, interpretation of RNA-seq results and presented data at the conferences.

Name: **Raul Piseaux**
Project Role: Postdoctoral Researches
Researcher Identifier: NA
Nearest person month worked: 1.5
Contribution to Project: Dr. Piseaux monitored shielded bone marrow chimeras, managed mouse colony and worked on generating new fate-mapping mouse models.

Name: **Sergejs Berdnikovs**
Project Role: Co-Investigator
Researcher Identifier: NA
Nearest person month worked: 0.6
Contribution to Project: Dr. Berdnikovs provided his expertise in transcriptomic analysis of the population-based (mouse and human) and single cell RNA-seq (human) data.

Name: **Elizabeth Bartom**
Project Role: Co-Investigator
Researcher Identifier: NA
Nearest person month worked: 1.2
Contribution to Project: Dr. Bartom developed and continuing to provide support to bioinformatics pipelines for analysis of the population-based and single cell RNA-seq data. She plays crucial role in integrating our sequencing pipeline into current computational infrastructure at Northwestern (Hundred Genomic Nodes/QUEST project).

Name: **Sangeeta Bhorade**
Project Role: Co-Investigator
Researcher Identifier: NA
Nearest person month worked: 0.6
Contribution to Project: Dr. Bhorade coordinate recruitment of the human subjects into lung transplant program and provided us with invaluable human samples. She is one of the first co-authors our manuscript on flow cytometric analysis of macrophages in the human lung.

Name: **Runyan, Constance E**
Project Role: Res Lab Mgr 1
Researcher Identifier: NA
Nearest person month worked: 2.45
Contribution to Project: Ms. Rynyan processed human lung specimens for single cell RNA-seq and assisted with mouse experiments.

Name: **Gokhan Mutlu**
Project Role: Co-Investigator

Researcher Identifier: NA
Nearest person month worked: 1.2
Contribution to Project: Dr. Mutlu coordinated recruitment of the human subjects into lung transplant program (University of Chicago site) and provided us with invaluable human samples.

Special reporting requirements

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

Dr. Budinger:
New:

U19AI135964 (Wunderink) 01/17/2018-12/31/2022
0.6 calendar
NIH/NIAID

Successful Clinical Response in Pneumonia Therapy (SCRIPT) Systems Biology Center
This innovative integrated systems biology application seeks to delineate the complex host/pathogen interactions that lead to unsuccessful response to therapy in serious pneumonia.

1P01DK117824-01 (PI: John Erik Pandolfino) 07/15/18-06/30/23
0.24 calendar
NIH
Disordered Tissue Biomechanics as a Driver of Esophageal Disease
The goal is to develop a complex model that will incorporate physiologic biomarkers measures of mechanical properties of the esophageal wall with psychological mediators of symptom generation
Role: Co-I

1R01HL141459-01 (PI: Jing Liu) 08/19/2018-05/31/2022
0.36 calendar
NIH/ NHLBI
The transcription factor Miz1-mediated mechanisms of lung aging
The goal is to provide fundamental insights into the pathogenesis of COPD and thus provide therapeutic targets.
Role: Co-I

Berdnikovs, Sergejs
New:
1R01HL134800-01A1 (PI: Lam; Role: CO-I) 1.2 effort 12/22/17-11/31/2021
Wnt-beta-catenin cross interactions in alveolar macrophages and epithelial cells in persistence of SSc-ILD

Bhorade, Sangeeta Maruti
New:
1R56HL135124-01 (PI: Misharin; Role: CO-I) 0.36 effort 09/25/17-08/31/2019
Differential role of tissue-resident and monocyte-derived alveolar macrophages in the development of pulmonary fibrosis

Other organizations involved as partners:
Organization Name: University of Chicago, Section of Pulmonary and Critical Care Medicine, Dr. Gokhan Mutlu
Location of Organization: Chicago, IL
Partner's contribution to the project: Dr. Mutlu provided us with human lung specimens for to fulfill the research as described in Aim 3.
Financial support: not applicable

Appendices

Nothing to report.