REPORT

Report of the Fellow who availed Fellowship / Training under Human Resource Development for Health Research.

1. Name and designation of Fellow : G. Bhanuprakash Reddy, Scientist-E

2. Address : National Institute of Nutrition
Jamai-Osmania, Hyderabad – 500007

3. Type of Fellowship and period : Short-term Fellowship for two months

4. Duration of fellowship : October 8-December 5, 2014
(Date of rejoin: December 8, 2014)

5. Frontline area of research in which Training /research was carried out : Disease Modelling and Genomics
(Tissue Remodeling Chronic Diseases)

6. Name & address of mentor and host institute: Dr. Satish K. Madala
Assistant Professor
Division of Pulmonary Medicine
Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229, USA
Tel: 513-636-9852
Email: satish.madala@cchmc.org

7. Highlights of work conducted :

i) Technique/expertise acquired (Give in about 150 words):

- Used the following cell-specific inducible transgenic mouse models for the isolation of mesenchymal cells to study the mechanisms involved in chronic tissue remodeling diseases.
  - Single transgenic Clara Cell Specific Protein-rtTA+/- mice (CCSP/-) and
  - Bitransgenic CCSP-rtTA+/-/(TetO)7-cmv TGFα+/- mice (CCSP/TGFα)
- Used of advanced cell isolation methods flow cytometry methods and antigen-coated metal bead technology
- Used RNA sequencing methods to identify cell-specific transcriptional factors responsible for signaling pathways in fibrosis
- Used of Ingenuity pathway (IPA) analysis to determine disease-specific transcriptional networks involved in cellular functions such as cell growth, proliferation and cell cycle.
- Using the cells isolated from the above cell-specific inducible models, preliminary studies were carried to investigate the signaling mechanism involved in tissue remodeling diseases due advanced glycation endproducts (AGE)
- Activated the cells with the AGE-modified proteins and studied the cell-proliferation, migration/ invasion mechanisms using state of art technology.

ii) Research results, including any papers, prepared/submitted for publication (Give in about 300 words)

Studies from the host laboratory have identified a specific, endogenous mechanism of pronounced and progressive fibrosis in mice caused by increased pulmonary levels of transforming growth factor-α (TGFα) signaling through the epidermal growth factor receptor (EGFR). The lab generated transgenic mice conditionally expressing TGFα in the lung epithelium under control of the CCSP promoter driving rtTA expression (CCSP/TGFα). In this model, TGFα is only expressed in airway and alveolar epithelial cells and only when mice are administered doxycycline (Dox). Overexpression of TGFα in adult CCSP/TGFα mice caused specific phenotypes that are observed in human fibrotic disease including fibroblast and epithelial cell proliferation, extracellular matrix deposition with myofibroblast transformation, severe restrictive changes in lung mechanics and secondary pulmonary hypertension. However, lung cell-specific epigenetic changes involved in age-related pulmonary fibrotic disease phenotypes have remained unknown. Importantly, the TGFα transgenic mouse is a powerful model for identifying epigenetic mechanisms of pulmonary fibrosis.

During my stay in the lab, I was involved in an ongoing study the lab whose goal was to understand the mechanisms of **fibrocyte-driven accumulation of Wilms Tumor 1-positive lung-resident stromal cells in severe fibrotic lung disease.** Because, the progressive accumulation of heterogeneous stromal cell populations, including fibrocytes and lung-resident mesenchymal cells have been shown to contribute to the formation of fibrotic lesions during idiopathic pulmonary fibrosis (IPF). However, the mechanisms involved in the expansion of fibrotic lung lesions and severe lung disease in IPF patients are unknown. In this study, we hypothesized that Wilms tumor 1-positive lung-resident stromal cells (WTRS) and fibrocytes accumulate in the pleural and sub-pleural fibrotic lesions of IPF and contribute to severe lung disease in a mouse model of TGFα-induced pulmonary fibrosis. To determine the effect of fibrocytes on WTRS accumulation and severity of lung disease, adoptive transfer studies were performed. Fibrocytes ($CD45^+$ collagen 1^+) were infused into control and TGFα mice on Dox for 14 days. On Day 21 after infusion, the lungs were assessed for changes in the number of WTRS, lung function, histology and hydroxyproline levels. RT-PCR was performed to assess changes in the expression of genes related to fibrosis. Immunohistochemical staining demonstrated the accumulation of WTRS in the pleural and sub-pleural fibrotic lesions of human IPF and a mouse model of TGFα-induced fibrosis. Adoptive fibrocyte transfer studies demonstrate increased accumulation of WTRS and severe lung disease, shown by increased airway resistance and hydroxyproline levels. In addition, the infusion of fibrocytes resulted in a significant increase in the expression of fibrosis associated genes, including collagen 1α and fibronectin1. These results demonstrate that WTRS and fibrocytes are the major lung stromal cells involved in the thickening of pleural and sub-pleural fibrotic lesions in IPF. We have demonstrated, for the first time, a heterogenous stromal cell interaction in which fibrocytes augment the accumulation of WTRS and induce severe lung disease in a mouse model of TGFα-induced fibrosis.

The long term goal of these studies is to identify therapeutic targets for lung fibrosis in mouse studies which can be translated into clinical studies on IPF.

Based on these results an abstract was submitted for the forthcoming Annual meeting of American Thoracic Society to be held at Denver (USA) during May 15-20, 2015 (**Annexure I**)

iii) Proposed utilization of the experience in the Parent Institute. (Please specify the project developed whether originally proposed/ new project) :

Using the inducible transgenic mouse models, reagents and expertise gained in the host laboratory, I conducted some preliminary studies to investigate the effect of advanced glycation endproducts (AGE) that are accumulated during aging and in particular under chronic diabetic conditions, on tissue remodeling diseases of diabetic complications such as diabetic nephropathy and diabetic retinopathy. We have treated the mesenchymal cells obtained from transgenic animal models with the AGE-modified proteins and studied the cell-proliferation, migration/ invasion mechanisms using state of art technology. We have also investigated the expression of genes involved in cell survival and apoptotic mechanisms.

Based on the expertise gained and encouraging preliminary data, I would like propose a **NEW PROJECT** at the Parent Institute to investigate the "Role of advanced glycation endproducts (AGE) on tissue remodeling mechanisms in diabetic complications: effect of dietary agents and nutrition factors".

Diabetes mellitus is a global health problem and it covertly affects multiple organ systems that go undiagnosed long after the onset. A number of complications are associated with poorly controlled hyperglycemia such as diabetic nephropathy and retinopathy. The ongoing research in our laboratory at the National Institute of Nutrition (NIN) is focused on role of nutritional factors in diabetic complications. Research points towards a multifactorial etiology and complex interplay of several pathogenic pathways that can contribute to the declining function of various organs in diabetes. It is critical to inhibit the occurrence and development of fibrosis in order to preserve the function of critical organs such as retina and kidney in diabetic patients. It is very relevant for the NIN to conduct studies to understand the causative and protective dietary factors of fibrosis. The proposed **NEW PROJECT**, in collaboration with Dr. Madala's laboratory at the Cincinnati Children's Hospital Medical Center, could be a step forward in this direction.

Signature of Fellow

CLAIM SHEET

(To be filled in by the Fellow)

1. Name, Designation and Address: G. Bhanuprakash Reddy, Scientist-E

   National Institute of Nutrition
   Jamai-Osmania, Hyderabad – 500007

2. ICMR Sanction letter No. and date: DHR/HRD Fellowship/II(III)/ 13-14

3. Broad area of research: **Disease Modelling and Genomics**

4. Chosen area of training: **Chronic tissue remodeling diseases**

5. Name, designation & address

   of Professor/Mentor of host institute: Dr. Satish K. Madala
   Assistant Professor
   Division of Pulmonary Medicine
   Cincinnati Children's Hospital Medical Center, Cincinnati, OH 45229, USA
   Tel: 513-636-9852
   Email: satish.madala@cchmc.org

6. Duration of the training: October 8-December 5, 2014
   (Date of rejoin: December 8, 2014)

7. Details of expenditure on the training

A TRAVEL:

i) Place of work in India: Hyderabad

ii) Port of embarkation with date of departure: Hyderabad & October 8, 2014

iii) Port of disembarkation with date of arrival: Chicago & October 9, 2014

iv) Venue of training if in a city different from iii) above : Cincinnati, Ohio

v) Air fare for onward travel to venue by shortest route: Rs.

vi) Air fare for return travel : Rs.

vii) Total expenditure on Air travel (v + vi) : **PROVIDED BY DHR/ICMR**

B. Fellowship US $ 3000 x 2months: US $ 6000

6000 x Rs…….as per foreign ex. rate: Rs.

C. Contingency: Rs.

i) Visa charges: Nil (Already had a valid visa)

ii) Mediclaim insurance charges: **Rs. 3,046/-**

iii) Taxi charges from place of duty to airport and back: Nil (picked-up & dropped by the host)

D. Total (A to C) : Rs

Certificate:

Certified that I have participated in the above training/research programme and the particulars furnished above are correct. I also certify that I have not received any financial assistance from any other source.

Date: Signature
Name & Address of Fellow

Please attach the following documents:

1. Original receipts for items claimed against contingency grant (Item. C).
2. Original receipt of Hotel/Guest house accommodation charges to be submitted by Senior Fellows.

Details to be furnished by Fellow

1. Total grant received:

2. Sanction Letter no. & date: DHR/HRD Fellowship/II(III)/ 13-14

3. Total expenditure incurred on training

   of the Fellow :

4. Amount to be released to the concerned Fellow :

5. Balance (if available) returned/being returned to DHR/ICMR:

6. Utilization certificate in the proforma enclosed:

Certificate:

Certified that Fellow has been paid grant as sanctioned by ICMR column 2 above for training abroad and as per guidelines of the DHR Fellow of HRD scheme.

Date:

Signature
Name & Address of Fellow

UTILIZATION CERTIFICATE

1. Title: HRD Fellowship Sponsored by DHR
2. Address of the Institution: National Institute of Nutrition
   Jamai-Osmania, Hyderabad – 500007
3. Name & designation of Fellow: G. Bhanuprakash Reddy, Scientist-E
4. DHR/ICMR sanction letter no. & date: DHR/HRD Fellowship/II(III)/ 13-14
5. Amount that was available for expenditure :
6. Actual expenditure:
7. Unspent balance if any:
8. Balance to be reimbursed to the Fellow:

Certified that out of Rs……..of grant-in-aid sanctioned during the financial year…….in favour of Dr……...DHR Fellowship awardee under DHR/ICMR sanction letter No…….dated…….. . A sum of Rs……..has been utilized for the purpose of research/training abroad in respect of Dr……The fellow for which it was sanctioned and that the balance amount of Rs…….due to the awardee may please be reimbursed.

Date: Signature of Fellow

Annexure-I

Fibrocyte-Driven Accumulation of Wilms Tumor 1-Positive Lung-Resident Stromal Cells in Severe Fibrotic Lung Disease

V. Sontake[1,3], B. DiPasquale[2], S. K. Shanmukhappa[2], G. B. Reddy[3], E. S. White[4] and S. K. Madala[1]

[1]Divisions of Pulmonary Medicine and [2]Pathology, Cincinnati Children's Hospital Medical Center, Cincinnati, USA; [3]National Institute of Nutrition, Hyderabad, India; [4]Department of Internal Medicine, University of Michigan, USA
Corresponding author's email: satish.madala@cchmc.org

Rationale: The progressive accumulation of heterogeneous stromal cell populations, including fibrocytes and lung-resident mesenchymal cells have been shown to contribute to the formation of fibrotic lesions during idiopathic pulmonary fibrosis (IPF). However, the mechanisms involved in the expansion of fibrotic lung lesions and severe lung disease in IPF patients are unknown. In this study, we hypothesized that Wilms tumor 1-positive lung-resident stromal cells (WTRS) and fibrocytes accumulate in the pleural and sub-pleural fibrotic lesions of IPF and contribute to severe lung disease in a mouse model of TGFα-induced pulmonary fibrosis.
Methods: To determine whether WTRS accumulate in the pleural and sub-pleural fibrotic lesions of IPF patients, lung sections from human IPF were immunostained for WT1 and vimentin. To determine the effect of fibrocytes on WTRS accumulation and severity of lung disease, adoptive transfer studies were performed. Fibrocytes ($CD45^+$ collagen 1^+) were infused into control and TGFα mice on Dox for 14 days. On Day 21 after infusion, the lungs were assessed for changes in the number of WTRS, lung function, histology and hydroxyproline levels. RT-PCR was performed to assess changes in the expression of genes related to fibrosis.
Results: Immunohistochemical staining demonstrated the accumulation of WTRS in the pleural and sub-pleural fibrotic lesions of human IPF and a mouse model of TGFα-induced fibrosis. Adoptive fibrocyte transfer studies demonstrate increased accumulation of WTRS and severe lung disease, shown by increased airway resistance and hydroxyproline levels. In addition, the infusion of fibrocytes resulted in a significant increase in the expression of fibrosis associated genes, including collagen 1α and fibronectin1.
Summary: Our results demonstrate that WTRS and fibrocytes are the major lung stromal cells involved in the thickening of pleural and sub-pleural fibrotic lesions in IPF. We have demonstrated, for the first time, a heterogenous stromal cell interaction in which fibrocytes augment the accumulation of WTRS and induce severe lung disease in a mouse model of TGFα-induced fibrosis.

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Abstract submitted to Annual meeting of American Thoracic Society to be held at Denver (USA) during May 15-20, 2015