Spotlight

The Newsletter of the ENTELLIGENCE™
Young Investigators Award Program
http://entelligencemd.org

Spring 2013 Vol. 4 Issue 1

Chairman's Note

On behalf of the ENTELLIGENCE Steering Committee, I am pleased to announce the completion of the 8th review cycle for the Young Investigators Award (YIA) Program. Underwritten by generous support from Actelion, 42 young investigators from around the United States and Canada have now received grant awards for pulmonary vascular disease research since 2006.

The 2013 award recipients are:

Harry Karmouty-Quintana, PhD
University of Texas-Health Science Center
Mentor: Michael R. Blackburn, PhD
Title: *The Role of Hyaluronan in Pulmonary Hypertension Associated with Idiopathic Pulmonary Fibrosis*

Tien Peng, MD
University of Pennsylvania
Mentor: Edward Morrisey, PhD
Title: *The Role of Sonic Hedgehog (Shh) Signaling in Pulmonary Arterial Hypertension*

Michael L. O'Byrne, MD
Children's Hospital of Philadelphia
Co-Investigators: Brian D. Hanna, MD, PhD; Steven M. Kawut, MD, MS; Russell T. Shinohara, PhD
Mentor: Jonathan J. Rome, MD
Title: *Adverse Outcomes Associated with Cardiac Catheterization in Children with Pulmonary Arterial Hypertension*

Keivan Zandinejad, MD
Case Western Reserve University
Mentor: Jonathan S. Stamler, MD
Title: *S-Nitrosylation Therapy to Treat Hypoxia-Induced Pulmonary Arterial Hypertension*

We invite you to celebrate with us as the winners are honored for their outstanding initiatives at the 2013 YIA Ceremony to be held during the American Thoracic Society International Conference (ATS•2013). Our program will take place on:

Monday, May 20, 2013
12:45 PM – 2:15 PM
Ceremony and Luncheon

The Ritz-Carlton, Philadelphia Hotel
Plaza Ballroom
Ten Avenue of the Arts, Philadelphia, PA

For more information about the 2013 YIA Award Ceremony and to register for this event, please email jfreeman@medtelligence.net.

Meanwhile, please enjoy perusing this edition of Spotlight, which highlights some of the important people and events in the YIA Program. In this issue, we've included interviews with 2012 award winners Kenny Schlosser, PhD, and Kelly J. Shields, PhD.

Warm regards,

Ronald J. Oudiz, MD
Program Chairman

Kenny Schlosser, PhD, 2012 YIA Winner

Dr. Schlosser is a 2012 Young Investigators Award Program winner for his project, *Role of Extracellular Circulating MicroRNAs in Idiopathic Pulmonary Arterial Hypertension*. He is a Fellow at Ottawa Hospital Research Institute in Ottawa, Canada. Spotlight spoke with him recently about his project and his future plans.

continued on page 2

The ENTELLIGENCE Program is supported through an educational grant from Actelion Pharmaceuticals US, Inc.

Table of Contents

Kenny Schlosser, PhD *(continued from page 1)*

Spotlight: What is the biggest reward associated with winning the ENTELLIGENCE award?

Dr. Schlosser: Speaking from the perspective of a postdoctoral fellow, just having the opportunity to write a research grant at this early stage in my career is a great learning experience and reward in itself. The subsequent recognition of winning, and generous funding to pursue these new research ideas freely, is icing on the cake.

Spotlight: What is the major challenge you face in conducting your ENTELLIGENCE project?

Dr. Schlosser: The circulating miRNAs we're interested in studying are present in plasma at much lower levels than miRNAs from cells or tissues, which makes their quantification inherently more difficult. A particular challenge is the lack of well-established 'housekeeping' genes in plasma to facilitate the normalization of miRNA levels. Therefore, preliminary studies will be necessary to identify and validate suitable plasma reference genes.

Spotlight: What are your short- and long-term professional goals?

Dr. Schlosser: I earned my PhD in a basic science field by developing synthetic nucleic acids with novel engineered functions, which is quite different from my current postdoctoral research pursuits. Therefore, in the short term I'm taking full advantage of the rich mentoring environment provided by my supervisor (Dr. Duncan Stewart) and the Ottawa Hospital Research Institute, in order to broaden my skills in translational medical research. In the long term, I hope to leverage these new skills to lead a productive cross-disciplinary research lab, investigating the roles and potential therapeutic merits of both natural and non-natural nucleic acids in pulmonary arterial hypertension.

Abstract

Role of Extracellular Circulating MicroRNAs in Pulmonary Arterial Hypertension

Background: Pulmonary arterial hypertension (PAH) is characterized by deterioration of the underlying structure of the lung vasculature, and the resulting increase in pulmonary vascular resistance leads to right heart failure and death. Despite improvements in treatment, the overall prognosis for PAH remains poor. The precise cause of PAH remains unclear, but there is increasing interest in small, non-coding RNA molecules known as microRNAs (miRNAs). MiRNAs associate with specific protein complexes and control gene expression by directing the translational inhibition or degradation of target messenger RNAs. To date, over 1000 highly conserved mammalian miRNAs have been annotated, and many have been shown to act as key regulators of fundamental biological processes, including cell proliferation, apoptosis, and inflammation; these processes have all been implicated as possible pathobiologic mechanisms of PAH. MiRNAs have traditionally been thought to exist and function exclusively within cells; however, stable extracellular miRNAs have more recently been discovered in the blood, which has led to speculation of an entirely new type of paracrine and/or hormonal function. These circulating miRNAs have been isolated from plasma under both normal and pathophysiological conditions, including various cancers and cardiovascular disease, but their identification and functional significance in lung vascular diseases like PAH have yet to be thoroughly investigated.

Hypothesis: We hypothesized that PAH is associated with altered levels of circulating miRNAs, which reflect disease-specific mechanisms of vascular injury and/or remodeling.

Material and Methods: High-content PCR (polymerase chain reaction) arrays will be used to measure miRNA levels in plasma isolated from human and experimental models of PAH. The SU5416/chronic hypoxia and/or monocrotaline rat models will be used to gain further insight into the temporal regulation of circulating miRNAs, and the degree to which changes in plasma miRNA levels correlate with changes in key organs, including the lung and heart. The effects of specific miRNA inhibition (via antagomir or anti-miR technology) or supplementation (via synthetic miRNA mimics) will also be investigated in these *in vivo* models.

Aims: We aim to 1) characterize the global plasma miRNome of PAH patients vs. healthy controls, 2) identify specific plasma miRNAs with altered expression patterns that are conserved between human and experimental models of PAH, and 3) determine if these plasma miRNAs play a causal, adaptive, or bystander role in this disease.

Implications: The identification and characterization of extracellular circulating miRNAs may provide new biomarkers of PAH, insight into novel mechanisms underlying the disease pathobiology, and potential targets for therapeutic intervention.

ENTELLIGENCE Milestones

Year established: **2005**

Review cycles completed: **8**

Awards: **42**

Funding: **$3,425,000**

Kelly J. Shields, PhD, 2012 YIA Winner

Dr. Shields is a 2012 Young Investigators Award Program winner for her project, *The Role of Perivascular Adipose Tissue in Pulmonary Arterial Hypertension*. She is an Assistant Professor of Medicine at Temple University School of Medicine in Philadelphia, PA. Spotlight spoke with her recently about her project and her future plans.

Spotlight: What is the biggest reward associated with winning the ENTELLIGENCE award?

Dr. Shields: The entire process, from grant submission and acceptance to the current laboratory work, has been very rewarding and a great experience. As a translational scientist, it is exciting to translate some of the work we have done on previous animal models involving a different disease model, and applying these findings to PAH. The ENTELLIGENCE award has allowed me the opportunity to expand my research potential, not only by learning a different disease state along with a new animal model and novel technical methods; but also developing administrative skills, including managing budget expenses on a larger scale.

Spotlight: What is the major challenge you face in conducting your ENTELLIGENCE project?

Dr. Shields: Prior to the ENTELLIGENCE award, I had not worked in the area of PAH; however, upon seeing some of the demographic similarities between the autoimmune disease lupus and idiopathic PAH, I thought this would be an appropriate translation of my current work to another disease profile. My challenges have shifted from initially bringing myself up to speed on the current research and animal models in PAH and garnering preliminary data, to more recent challenges, such as protocol development for our rat model. At present, the main challenge is to start teasing apart the findings of this longitudinal model, evaluating areas to develop in greater depth with my remaining funding, and preparing abstracts and manuscripts for submission based on my findings with this award.

Spotlight: What are your short- and long-term professional goals?

Dr. Shields: In the short term, I will be evaluating the best use of my resources for completing the proposed work to generate abstracts and manuscripts. I have applied for additional funding through various grant mechanisms, and I am using this opportunity as a step toward independent research. My long-term goal is to continue to investigate the role of the complement cascade in tissue (dys)function and in particular, the pathological contribution of small visceral adipose depots to systemic inflammation in PAH and lupus. We have many important questions to answer with this line of research and my hope is that we can begin to develop appropriate therapeutic intervention.

continued on page 4

Scientific Congresses where YIA winners (2005-2012) have presented their research

- American Heart Association
- American Society of Gene and Cell Therapy
- American Thoracic Society
- Aspen Lung Conference
- European Respiratory
- Grover Conference
- International Society of Heart and Lung Transplantation
- Pediatric Academic Societies
- Pittsburgh International Lung Conference

2013–2014 Conferences with Pulmonary Vascular Disease Content

American Thoracic Society
Philadelphia, PA
May 7–22, 2013
www.thoracic.org

European Society of Cardiology
Amsterdam, The Netherlands
August 31–September 4, 2013
www.escardio.org

European Respiratory Society
Barcelona, Spain
September 7–11, 2013
www.ersnet.org

The American College of Chest Physicians
Chicago, IL
October 26–31, 2013
www.chestnet.org

American Heart Association
Dallas, TX
November 16–20, 2013
www.myamericanheart.org

American College of Cardiology
Washington, DC
March 29–31, 2014
www.cardiosource.org

Kelly J. Shields, PhD *(continued from page 3)*

Abstract

The Role of Perivascular Adipose Tissue in Pulmonary Arterial Hypertension

Background: Pulmonary arterial hypertension (PAH) is a rare disease characterized by ever-increasing pulmonary vascular resistance and significant vascular remodeling. Although the classic indicators of PAH progression are well recognized, the initiating factors involved in the pathogenesis of PAH are not. Interest in the relationship between perivascular inflammation and pulmonary artery (PA) remodeling, along with the role of localized inflammation in smaller visceral adipose depots in cardiovascular disease (CVD), is growing. We previously found complement proteins C3 and C4 (C3/C4) deposited in the vascular wall and surrounding perivascular adipose tissue (PVAT) in a murine model of CVD in the absence of luminal deposition or plaque development. We determined that C3/C4 bind to collagen and elastin within the vascular wall of murine aorta, suggesting that the complement system may play a critical role in the pathogenesis of vascular stiffness and atherosclerosis through a mechanism initiated at the adventitia or the PVAT, rather than the endothelial surface. The same pro-inflammatory environment may exist surrounding the PAs, contributing to PAH.

Hypothesis: We hypothesize that late-stage PAH and PA vascular remodeling are directly associated with increased PA PVAT volume, and that this small adipose depot influences the extent of complement protein deposition and the upregulation of pro-PAH protein expression within the PA vascular wall and the PA PVAT.

Materials and Methods: First, we will quantify the volume of PA PVAT using microCT and correlate these findings with the extent of vascular remodeling and PA PVAT hypertrophy due to PAH progression, measured through morphological changes using scanning electron microscopy. Second, we will characterize the deposition of C3/C4 in the PA vascular wall and PA PVAT using established immunohistochemistry and histology techniques while evaluating the association with vascular remodeling. Finally, we will identify unique and novel pro-PAH proteins and inflammatory cell populations found in the PA PVAT, using proteomics and molecular histology, and correlate these findings with vascular remodeling at specified time intervals.

Aims: We aim to 1) Quantify the PA PVAT volume and evaluate the ultrastructural and morphological remodeling of the PA vascular wall during the development of PAH, 2) Characterize the deposition of complement proteins C3 and C4 during the development of PAH, and 3) Determine the functional state of PA PVAT through proteomic analysis of serum, PA, PA PVAT and lung tissue

Implications: Currently, no studies have evaluated the potential role of PA PVAT and C3/C4 in the development of PAH or characterized this visceral adipose depot. Differences in PA PVAT volume and function may contribute to the pathophysiology of PAH through production and deposition of complement proteins and pro-PAH protein expression. Elucidation of the molecular and cellular mechanisms responsible for the development of PAH and vascular remodeling is critical for the development of subclinical disease measures and therapeutic interventions. In successfully completing the experiments outlined in this proposal, we will determine the role of PA PVAT volume hypertrophy in the progression of PAH and vascular remodeling.

ENTELLIGENCE Young Investigator Award Program Timeline