PLUS Application

Project Proposal and Mentoring Plan Example #1

Submission Year: 2019

Project Proposal:
Background: Pulmonary Arterial Hypertension (PAH) is a severe and progressive disease that results in death due to increased pulmonary vascular resistance that eventually leads to right heart hypertrophy and failure. Obstruction of pulmonary arteries is caused by arterial remodeling and muscularization, intimal fibrosis, in situ thrombosis with neovascularization and occasionally by the formation of plexiform lesions, all of which result in increased pulmonary arterial pressure. Both pulmonary arterial smooth muscle and endothelial cells undergo changes in intracellular signaling that leads to a proliferative, apoptosis resistant phenotype that causes remodeling and occlusion of the pulmonary vasculature.

Sphingosine-1 Phosphate (S1P) is a bioactive sphingolipid that regulates cell proliferation, differentiation, motility and vascular tone. Sphingosine kinase 1 (Sphk1) is a conserved enzyme that phosphorylates sphingosine to generate S1P. The Sphk1/S1P signaling pathway is involved in increased tumor cell proliferation and migration in multiple types of cancers and has been shown to promote tumor cell proliferation under hypoxic conditions. Our laboratory has demonstrated that Sphk1 expression is increased in the lungs and pulmonary artery smooth muscle cells (PASMCs) of PAH patients and in the lungs of rodent models of hypoxia mediated pulmonary hypertension (HPH). Consistent with this observation S1P levels are enhanced in the lungs of PAH patients and in the pulmonary arteries of rodents with HPH.

Importantly, Sphk1 has a mitochondrial transport signal (MTS) and has been shown to locate to the outer membrane of the mitochondria during activation of the mitochondrial unfolded protein response (mtUTR). In cancer cells activation of the mtUPR promotes cell survival by enhancing proliferation and inhibiting apoptosis. Furthermore, hypoxia activates the mtUPR, which may be a maladaptive response to stress that leads to survival of cancer cells. Activation of the mtUPR has been suggested to promote PAH. However, little is known about the role of mitochondria in the initiation/progression of PAH. Our unpublished observations suggest that S1P promotes activation of the mtUPR in both human pulmonary artery endothelial cells and smooth muscle cells (HPAECs and HPASMCs, respectively) but via activation of different signaling mediators.

Project Objectives:
We hypothesize that activation of the Sphk1/S1P signaling axis promotes activation of the mtUPR, which enhances hypoxia induced proliferation of HPAECs and HPASMCs. This hypothesis will be tested by investigating the following objectives:
Objective 1. Identify which mtUPR pathways are activated in HPAECs versus HPASMCs.
Objective 2. Determine if Sphk1/S1P activation of the mtUPR occurs via receptor mediated signaling versus S1P acting as an intracellular signaling mediator.
Objective 3. Determine if genetic deletion of Sphk1 in pulmonary endothelial or smooth muscle cells prevents HPH *in vivo* using rodents and define the role of the mtUPR.

Research Design:

Objective 1: Identify which mtUPR pathways are activated in HPAECs versus HPASMCs. Cells will be treated with S1P (1, 6, 12, and 24h) under normoxic (21% O_2) or hypoxic (3% O_2) conditions.

General methods: Western blot and real-time PCR will be used to detect activation and expression of signaling mediators that indicate activation of mtUPR (p-eIF2a, HSP70, HSP60 and LONP1). ATF-5 nuclear localization (indicates mtUPR activation) will be measured by immunofluorescence. PCNA and BrdU assay will be used to determine the effect on cell proliferation. Anti-apoptotic signaling will be analyzed by immunoblotting for BCL2. To determine if activation of the mtUPR is necessary for S1P induced cell survival and proliferation, siRNA will be used to knock-down expression of proteins that are essential for mtUPR activation.

Objective 2. Determine if Sphk1/S1P activation of the mtUPR occurs via receptor mediated signaling versus S1P acting as an intracellular signaling mediator. Sphk1 will be overexpressed in HPAECs and HPASMCs. The effect on activation of the mtUPR will be analyzed as described in the general methods. Expression of the S1P transporter, Spns2 or of the S1P receptor, will be knocked down using siRNA to determine if S1P secretion and receptor engagement is necessary for Sphk1 induced activation of the mtUPR.

Objective 3. Determine if genetic deletion of Sphk1 in pulmonary endothelial or smooth muscle cells prevents HPH *in vivo* using rodents and define the role of the mtUPR. Preliminary studies from our laboratory suggest that smooth muscle specific deletion of Sphk1 ameliorates HPH. Employing our general methods, we will determine if mtUPR is activated in normoxic versus hypoxic animals in isolated PASMCs and PAECs. We will analyze PAECs to determine if deletion of smooth muscle Sphk1 has protective effects on the pulmonary endothelium. The same experiments will be performed in animals with Sphk1 deleted in endothelial cells. In all experiments, hemodynamic analysis and H and E staining of pulmonary arteries will be used to assess the degree of pulmonary hypertension.

Project contribution to advancement of professional goals:

I plan to become an expert in defining the molecular mechanisms that lead to the aberrant function of cells in the lung and how this promotes lung diseases such as pulmonary hypertension. My long-term career aspiration is to establish a translational science laboratory to identify treatment and management approaches. My previous training has equipped me with technical skills and with knowledge in the areas of cellular physiology and molecular biology and have provided me will the skills to investigate biological processes that lead to diseases in humans. In addition to progressing my current research project investigating the effect of Sphk1/S1P on mitochondria function in the progression of PH, participation in the PLUS program will give me the opportunity to acquire **additional skills necessary to develop *in vivo* tools** to answer research questions that will advance our fundamental knowledge about the basic cellular mechanisms and potential therapeutic targets that need to be characterized to promote better treatment of PH.

Dr. YYYYY's laboratory is an excellent environment for my mentored career development because of his expertise in pulmonary physiology and PH and his established collaborations with individuals who are experts in our field. My research project is distinct from other projects in the YYYYY laboratory and will be used to establish my independent research career.

Mentoring Plan:

Dr. XXXXX is a very talented investigator who clearly possesses the initiative, imagination and dedication to develop into an independent investigator. I am very pleased to act as a mentor for her career development and I will do everything I can to foster her career. I will provide recommendations and advice regarding progression of her research projects and appropriate steps in her career development. This includes advice and guidance on applications for extramural funding, assistance and advice in the writing of manuscripts and oral presentations and other professional activities. The goal is to help her to develop a unique niche in research that she can pursue as an independent investigator in the future. Her current project will provide her the opportunity to expand and broaden her focus on lung disease to investigating PH pathology, with additional publications in this area and with a greater breadth of technical expertise and scientific inquiry. By the time of completion of the PLUS program, I expect Dr. XXXXX to have the necessary expertise and independence to be able to compete successfully for her own research funding. I will fully support her using these studies as a basis to apply for her own independent funding. The overall goal is to prepare her to function effectively as an independent scientist and faculty member and to be able to establish and maintain her own independent research laboratory.

The following research and professional skills will be developed:

1.) This project will allow Dr. XXXXX to broaden her technical expertise into skills in live animal research using a broad range of modern *in vivo* technical approaches available to study lung pathophysiology.

2.) Dr. XXXXX is expected to design her own experiments under my supervision and to write manuscripts, abstracts, and research grant proposals. It is my expectation that she will be the primary author on manuscripts resulting directly from this project. She will also have the opportunity to collaborate on other studies going on in my laboratory. Dr. XXXXX plans to write an R01 grant application for her independent research support so that she may transition to a permanent faculty position.

3.) Dr. XXXXX will participate in and make scientific presentations at both local and national meetings on a regular basis throughout the program. I will encourage Dr. XXXXX's active participation in professional societies such as the American Thoracic Society as well as in activities within the Indiana University School of Medicine that enhance and expand her professional credentials.

4.) Dr. XXXXX will be exposed to a wide variety of research approaches and techniques by exposure to scientists of national stature who are invited to present at the Pulmonary seminar series and at many other departments and programs within IUSM, and by attending national scientific conferences. She will also be able to interact with other faculty in the Department of Medicine, which has many prominent and successful investigators with a broad range of active well-funded research programs.

5.) Dr. XXXXX will meet regularly with faculty, students and fellows participating in weekly lab meetings to discuss her results and research progress. At these meetings, she will present the results of her studies and describe her plans for upcoming experiments. Technical difficulties and experimental design will be discussed. Other members of my laboratory and collaborating laboratories will also present their results regularly at these meetings. Thus, Dr. XXXXX will have the opportunity to obtain feedback on her project and progress from other lab members as well

as myself, and to be exposed to other ongoing projects in the laboratory. Furthermore, I will meet individually with Dr. XXXXX at least once a month.

6.) Dr. XXXXX will have the opportunity to participate in mentoring doctoral/medical students and residents as well as pulmonary fellows in my laboratory.
I will fully support and encourage Dr. XXXXX's attempts to use the studies in in this project to build a basis for the development of her own independently funded research program. I will encourage and support her goal of establishing an independent research laboratory. Dr. XXXXX will be encouraged to submit applications for extramural funding to private foundations such as the American Lung Association and American Thoracic society, as well as to the NIH. By the summation of the PLUS program I expect her to submit an application for an NIH R01 award. I will also assist her in establishing herself as an independent investigator as she gains the credentials and funds to run her own independent research program.