LE&RN Postdoctoral Fellowship Awards Program

2014 Grant Recipients

Maike Frye, Ph.D.
Uppsala University
"Functional Characterization of Primary Lymphedema Gene GATA2"

Lymphatic vasculature constitutes a network of vessels critical for the maintenance of tissue fluid homeostasis. Failure of the lymphatic system leads to chronic tissue swelling, called lymphedema, but it is also linked to defects in immune function, obesity, atherosclerosis and cardiovascular disease. Improved knowledge of the mechanisms regulating lymphatic vessels, in particular identification and characterization of the key regulatory genes, is essential for developing novel therapeutic strategies for diseases associated with impaired lymphatic function. This project investigates the role of GATA2 transcription factor, a newly identified causative gene of primary lymphedema, in lymphatic vascular development. Since loss of GATA2 function is associated with a human disease characterized by lymphatic failure, we expect that this molecule is a novel key regulator of lymphangiogenesis and characterization of its function is thus expected to increase our understanding of lymphatic development and pathophysiological mechanisms involved in lymphedema.

Katarzyna Koltowska, Ph.D.
The University of Queensland
"Characterisation of a Novel Modulator of Vegfc/Vegfr3 Signaling during Lymphatic Development"

The lymphatic vasculature maintains fluid balance in our tissues. When lymphatic vessels fail to form, patients suffer from fluid accumulation leading to swelling of the limbs and a debilitating, progressive disease known as lymphedema. Our laboratory investigates the genetic factors that cause inherited lymphedema and that control formation of new lymphatic vessels. The Vegfc/Vegfr3 molecular pathway is known to be central in controlling lymphatic vessel formation. I have used genetics in zebrafish embryos to discover a new unstudied, gene that regulates Vegfc/Vegfr3 signalling. Here, I will study the role of this new gene in detail. This will allow us to better understand how the lymphatic vasculature network forms and should serve as an avenue to find new therapeutic targets and approaches in lymphedema and vascular diseases.

LRF Postdoctoral Fellowship Awards Program

2012 Grant Recipients

Alireza Haghighi Kakhki, M.D., Ph.D.
University of Pittsburgh
"Genetic Basis of the Yellow Nails-Lymphedema-Pleural Effusion Syndrome (OMIM 153300)"

The aims of this proposal are 1) to identify the gene that causes autosomal recessive Yellow Nail-Lymphedema-Pleural Effusion (YNS; OMIM 153300) using a combination of homozygosity mapping and next generation sequencing in a large Jordanian kindred; 2) to test for heterogeneity of the YNS syndrome in three other consanguineous families from the Middle East to see if their YNS is caused by the same mutation, by other mutations in the same gene, or is caused by mutations in another gene by direct sequencing of the gene in other families and singleton cases from the same population: and 3) to test whether YNS appearing as a sporadic in populations from the U.S. and Italy is caused by mutation in the YNS gene identified in the Middle Eastern population, or mutation in a different gene by direct sequencing of the YNS gene in patient from the U.S. and Italy. This will identify a new gene causing heritable lymphedema, characterize the genetic heterogeneity within the YNS syndrome, and will be the first identified lymphedema gene that leads to highly penetrant lung phenotype, pleural effusion, that may give us an entry to the study of the role of the lymphatics in the lung. If time and resources permit, we will begin to characterize the role of the YNS gene through a series of gene expression studies and immunohistochemical studies in human and mouse skin and lung biopsies.

Yiqing Yang, Ph.D.
University of Pennsylvania
"The Interaction between Lymphatic Vessels and Secondary Lymphoid Organs in Development"

Secondary lymphoid organs play a unique and crucial role in the organization and function of the adaptive immune system, and are the site where T cells recognize the antigens presented by dendritic cells (DCs) and other antigen presenting cells (APCs) to activate the adaptive immune response.

Lymphatic vessels play an essential role in secondary lymphoid tissue function and adaptive immunity, as efferent and afferent lymphatic vessels connect the relatively solitary lymph nodes with the lymphatic system: afferent lymphatic vessels transport antigens and APCs from distant regions to lymph nodes (LNs), while efferent lymphatic vessels provide the route by which lymphocytes entered LNs and Peyer's patches (PPs) to search for their cognate APC re-enter the vasculature to fulfill their effector function.

Efficient adaptive immune responses rely upon the ability of the lymphatic vascular network to interface with and support secondary lymphoid tissues, but the roles of lymphatic vessels in the development of secondary lymphoid organs have not been well studied.

Our laboratory has recently generated mice lacking CCBE1, a novel lymphangiogenic factor responsible for human Hennekam syndrome and required for all lymphatic growth in mice. These mice exhibit a complete lack of lymphatic vessels with no defects in other organs or blood vessels, and therefore are an ideal model in which to dissect the role of lymphatic vessels during LN and PP formation and maintenance.

In this proposal I will use these newly generated CCBE1 knockout and conditional knockout animals and studies of the development of LNs and PPs to address these important questions. In my PhD studies I focused on immunology studies of T cell development, a background that is strongly complementary to the expertise in lymphatic vascular development in the Kahn lab and that will allow me to integrate the vascular and immune biology in this proposal. These studies are predicted to provide a better understanding of the roles of lymphatic vessels in the development of immune system, and give us new information important for the pathogenesis and treatment of lymphatic diseases, lymphedema and related disorders.

Additional Support for NIH-funded Postdoctoral Fellows in Lymphatic Research Awards

Carolyn Bayer, Ph.D.
University of Texas at Austin
"Molecular Photoacoustic Imaging using Multitargeted Contrast Agents"

Critical to improving the treatment of cancer is an increased understanding of the interactions between solid tumors and the surrounding lymphatic system. Lymphangiogenesis and cancer metastasis are the result of a complex interaction between the constituents of the tumors, including the tumor cells and the cells of the vasculature and lymphatics. The ability to noninvasively image the lymphatics before and after the treatment of an in vivo tumor model could provide information critical to understanding the treatment mechanism. Therefore, in vivo imaging studies of tumor response to treatment while monitoring the lymphatic, vascular and tumor anatomy, could lead to improved therapeutic techniques for cancer. To address this need, we propose to use the multiplex photoacoustic molecular imaging system being developed to study the lymphatics surrounding the described tumors while following the proposed treatment plans. Recent experimental results from this project have demonstrated the accumulation of two targeted nanorods contrast agents within in vivo tumors with differing cellular receptor overexpression . Current work seeks to improve bioconjugation methods to enhance the in vitro cellular uptake of the silica-coated gold nanorod contrast agents, and to reduce the overall size of the gold nanorods via novel chemical synthesis methods. To address the question of the impact of lymphatic involvement in the tumor growth and treatment response, we propose the following minor modification to our future studies. In addition to targeting HER2/neu and ?v?3integrin, we will add a third silica-coated gold nanorod contrast agent bioconjugated to a monoclonal antibody specific to the lymphatic endothelial hyaluronan receptor-1 (LYVE-1). Multispectral photoacoustic images will be acquired, and the resulting photoacoustic signals will be spectrally unmixed to distinguish the location of each unique contrast agent, correlating to the anatomical locations of the tumor cells, the lymphatics, and the vasculature. The tumors will be monitored for a change in the density of the lymphatics and vasculature supplying the tumor region, in response to treatment, using our multiplex photoacoustic imaging system. The resulting images will demonstrate proof of principle photoacoustic molecular imaging of both lymphatics and vasculature in vivo under therapeutic treatment. This unique approach would enable the study of the interactions between vasculature infiltration, lymphangiogenesis, and tumor cell growth and response to treatment in vivo to provide critical insight on the effect tumor treatment on lymphangiogenesis and lymphatic regression.

2010 Grant Recipients

Hélène Maby-El Hajjami, Ph.D.
University Central Hospital, Switzerland
"Development of transcriptional differentiation approaches for treatment of lymphedema-distichiasis syndrome"

The lymphatic vasculature maintains normal tissue fluid balance by transporting fluid and macromolecules from tissues back to the blood circulation, and it also plays an important role in human diseases such as lymphedema, inflammation and cancer. Lymphatic vasculature is a hierarchically organized network, composed of two functionally and molecularly distinct types of vessels: capillaries and collecting vessels. One of the outstanding questions in lymphatic vascular biology is how this hierarchy of lymphatic vessels is initiated and maintained. Answering this question has profound clinical implications, as damage to the collecting lymphatic vessels causes the majority of lymphedemas, which affects up to 30% of long-term breast cancer survivors. We focus our research on a rare hereditary form of lymphedema, lymphedema-distichiasis, caused by mutated form of forkhead transcription factor FOXC2. Here we propose to study the mechanisms and potential treatment approaches of collecting lymphatic vessel failure in lymphedema by identifying the regulators of FOXC2 transcription in lymphatic endothelium and by developing the transgenic mouse model to study the ability of FOXC2 to promote the differentiation of lymphatic vessels in vivo. We expect that our results will provide novel fundamental knowledge about the regulation of vascular specification during vertebrate development, and will help to design novel treatment strategies for primary and secondary lymphedema.

Li-Chin Yao, Ph.D.
University of California San Francisco, United States
"Development and maintenance of lymphatic endothelial cell specializations in mouse respiratory tract"

Lymphatic endothelial cells are gatekeepers of fluid and cell clearance from tissues. Defective lymphatic transport induces lymphedema and impairs immune responses to infection. To date, there is no effective cure for lymphedema and treatment is limited to manual lymph drainage therapies. Knowledge of the structure and function of lymphatics has lagged far behind corresponding studies of blood vessels. Our laboratory recently discovered that endothelial cells in initial lymphatics have discontinuous junctions uniquely different from the continuous junctions in collecting lymphatics and blood vessels. Junctions are also abnormal in tips of new lymphatics growing during inflammation. The functional significance of these unexpected changes is unknown.

The overall goal of this proposal is to extend our knowledge of the structure and function of specialized lymphatic endothelial junctions in development, health and disease. I hypothesize that the molecular composition of the specialized junctions determines the proper function of initial lymphatics and that abnormal lymphatic junctions are functionally defective, contributing to the pathophysiology of inflammation and development of lymphedema.

My three specific aims are to determine: (1) when specialized lymphatic endothelial junctions develop during pre/postnatal development; (2) whether endothelial junctions develop abnormally in new lymphatics that grow during inflammation; (3) whether treatment of sustained inflammation restores the normal junctional structure of initial lymphatics.

I will take advantage of our well-established mouse model of chronic inflammation induced by airway infection with the pathogen Mycoplasma pulmonis. Transgenic overexpression of VEGF-C will be also used. I will use confocal microscopy to determine the distribution of immunohistochemical markers of junctional molecules in lymphatics of mouse airways and lungs in three conditions: normal developmental; in inflammatory conditions; and during reversal and resolution of inflammation after treatment with antibiotics or anti-inflammatory drugs. These structural studies will be supported by functional and molecular biological studies of lymphatics, including transport of fluid and cells and qRT-PCR expression studies.

My long-term goal is to advance our understanding of the role of lymphatic vessels in chronic inflammation by elucidating the development and plasticity of endothelial junctions in initial lymphatics,. Such knowledge may suggest new strategies and therapeutic targets in human inflammatory diseases.

Guy Malkinson, Ph.D.
Weizmann Institute of Science, Israel
"Genetic analysis of lymphatic system development in zebrafish"

The lymphatic system is a network of blind-ended capillaries that protects and maintains the homeostasis of the body by filtering and draining lymphatic fluid. Lately, the lymphatic endothelium has become the subject of great interest especially because of its important role during pathological processes, such as the dissemination of tumor metastasis and lymphedema. Despite the importance of lymphangiogenesis, little is known about the mechanisms that control the early formation and differentiation of lymphatic vessels. Progress in understanding the development of the lymphatic system has been hampered by difficulties in observing lymphatic cells in-vivo and performing defined genetic and experimental manipulation of lymphatic endothelial cells.

Using multiphoton time-lapse imaging of living embryos, we previously demonstrated the existence of a bona-fide lymphatic vascular system in the zebrafish, and established this as an important new experimental and genetic model organism for studying lymphangiogenesis. In this proposal, we will draw on our previous experience characterizing the fish lymphatic vasculature to elucidate novel molecular and cellular mechanisms regulating early lymphangiogenesis. Specifically, we propose genetic and in-vivo imaging studies aimed at characterizing y114- a novel zebrafish mutant that fails to develop lymphatic vessels. Our specific aims are as follows:

1. Phenotypic characterization of y114: We will use long-term time-lapse imaging of homozygous mutants with EGFP-labeled endothelial cells to identify the specific steps of lymphangiogenesis affected by the mutation.
2. Identification of the mutated locus: We will identify the mutated locus responsible for the mutant phenotype by a combination of positional cloning and candidate gene approaches.
3. Functional characterization of y114 during lymphatic development. Studies in support of this aim will include spatiotemporal analysis of y114 expression as well as initial loss- and gain-of-function studies aimed at elucidating the function of the newly identified locus during lymphatic development.

Taken together these studies promise to offer key insights into the genetic programs and cellular behaviors that help shape the lymphatic vasculature. The analysis of the newly defined locus should shed light on previously uncharacterized lymphangiogenic components and possibly provide new drug targets for the treatment of pathological lymphatic conditions that lead to inflammation, autoimmunity and cancer.

2008 Grant Recipients

Xabier Lopez Aranguren, Ph.D.
Katholieke Universiteit Leuven, Belgium
"Molecular Biology and Therapeutic Potential of Multipotent Adult Progenitor Cell (MAPC) Derived Lymphatic Endothelial Cells"

Damien Gerald, Ph.D.
Beth Israel Deaconess Medical Center, United States
"Investigation of RhoB's Function in Lymphatics: a Model to Improve Lymphedema Therapy"

Sunkuk Kwon, Ph.D.
Baylor College of Medicine, United States
"Near-infrared Fluorescence Optical Imaging of Lymph Function in a Preclinical Murine Model"

2006 Grant Recipients

Rawad Mounzer, M.D.
Yale University School of Medicine, United States
"Lymphangiogenesis in Acute and Chronic Models of Inflammation"

Zhanna Nepiyushchikh, M.D.
Texas A&M University, United States
"Functional Roles of Myosin Light Chain in Lymphatic Pumping Activity"

Martin Schneider, M.D.
University of Leuven, VIB, Belgium
"Xenopus Laevis as a Model to Study Lymphangiogenesis in Health and Disease"

R Sathish Srinivasan, Ph.D.
St. Jude Children's Research Hospital, United States
"Lineage Tracing to Identify the Sources of Lymphatic Endothelial Cells"

Additional Support for NIH-funded Postdoctoral Fellows in Lymphatic Research Awards

Gregory Lam, M.D.
Duke University Medical Center, United States
"The Role of Tie2 and the Angiopoietins in EPC Biology"