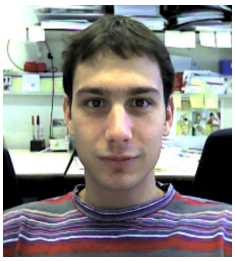
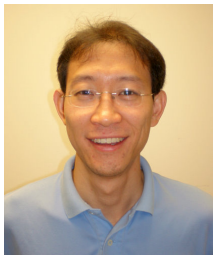
Lymphatic Research Foundation Fellows 2008—2010

Xabier Lopez Aranguren, Ph.D.



Institution: **Katholieke Universiteit Leuven (KULeuven)**
Project Title: **Molecular Biology and Therapeutic Potential of Multipotent Adult Progenitor Cell (MAPC) Derived Lymphatic Endothelial Cells**

Lymphatic vessels play an important role in highly prevalent diseases such as cancer, psoriasis and lymphedema and in physiological processes such as wound healing. Nevertheless, little is known about the lymphatic vessel formation and molecular regulation compared with blood vessels, in part, due to the lack of specific lymphatic endothelial cell (EC) markers. Only recently, different lymphatic EC markers (e.g. Prox-1, podoplanin, Lyve-1) and growth factors (e.g. VEGF-C, VEGF-D, HGF, etc.) were discovered enabling the isolation, culture and characterization of lymphatic ECs. In addition, the existence and origin of true lymphatic endothelial stem/precursor cells remains an open question and, therefore, their potential use for therapy in lymphedema and wound healing remains unexplored. Multipotent Adult Progenitor Cells (MAPCs) are adult stem cells with the potential to differentiate into cells of the three embryonic germ layers, including ECs. We recently showed that MAPCs are capable of being specified to arterial and venous ECs. In our preliminary experiments, human and rodent MAPCs give rise to cells with an expression pattern similar to that of lymphatic ECs. Therefore, in the current project, we intend to use MAPCs as a tool to study the molecular events involved in lymphatic EC differentiation. The research has 4 major objectives: (i) to optimize and characterize MAPCs as an in vitro system for lymphatic EC differentiation; (ii) to use this system to screen for genes/proteins that play a role during the differentiation process; (iii) to validate this screening system in a appropriate in vivo model (Xenpous tadpoles and zebrafish embryo's) and (iv) to evaluate the therapeutic potential of (un)differentiated MAPCs in rodent models of lymphedema and in a mouse model of physiological lymphangiogenesis (i.e. wound healing).



Sunkuk Kwon, Ph.D.

Institution: **University of Texas Health Science Center**
Project Title: **Near-infrared Fluorescence Optional Imaging of Lymph Function in a Preclinical Murine Model**

While investigators have intensively focused upon the role of lymph function in the genesis and resolution of edema for decades, none of the studies have demonstrated the ability to quantitatively and non-invasively image propulsive lymph flow in mice. Since preclinical investigation of potential pharmacological agents is typically performed in rodent models, imaging lymph function in preclinical models can foster the development of potential anti– and pro– lymphangiogenic drugs. Recently, I reported the ability to dynamically and non-invasively image lymph propulsion after intradermal injection of non-specific near-infrared (NIR) fluorophore, IC-Green, in the tail and dorsal aspect of the paw of living mice. Based upon these previous studies, we hypothesize that NIR optical imaging methods can be used to image lymph flow, function, and remodeling in lymphedema mice including transgenic mice with mutations in the forkhead transcription factor (FOXC2) or vascular endothelial growth factor receptor (VEGFR-3) in order to provide early diagnostics and information on response to therapy in lymph-related diseases. In this application, we seek to improve functional lymph imaging using a NIR fluorophore and evaluate lymph function with different variables that affect propulsive lymph flow. From the baseline lymph flow in intact mice, we seek to examine lymphatic architecture and function in lymphedema mice. Specifically, our aims are to: (1) Modify our current fluorescence optical imaging system to improve sensitivity and achieve greater magnification in order to develop a specific tool for interrogating lymphatic function in intact mice; (2) Quantify fluorescence imaging of baseline lymph function parameters in intact mice with the different variables thought to be crucial in lymphatic function such as injection volumes and pressures; and (3) Characterize propulsive lymph function and its physiological response on therapeutic intervention in lymphedema mice, and compare them with results from Specific Aim #2. If successful, we will be able to provide information on the connections between health and disease that impacts the lymphatic system, and to efficiently use our new understandings of lymphatic biology to identify and better manage disease in which the lymphatics play a role.

Damien Gerald, Ph.D.

Institution: **Beth Israel Deaconess Medical Center**
Project Title: **Investigation of RhoB's Function in Lymphatics: a Model to Improve Lymphedema Therapy**

Acquired lymphedema, characterized by the peripheral accumulation of intercellular fluid in tissues, is a consequence of lymph node resection, or infections. This disease, which has a significant impact on patient quality of life, still needs the development of an effective cure. In order to use multiple approaches for rational drug development, research on new animal models for lymphedema is necessary. Here, we have identified a gene whose loss leads to increased lymphangiogenesis. RhoB, a small GTPase, participates in stress-induced cell responses. RhoB knockout mice are viable and exhibit an interesting increase of lymphatic proliferation after wound healing. This suggests a negative role of RhoB in stressed-induced lymphangiogenesis. RhoB directly interacts with the transcription factor DB1/VEZF1, whose loss of one allele also increases lymphangiogenesis. These RhoB and DB1/VEZF1 knockouts provide us with a unique opportunity to investigate novel molecular mechanisms involved in lymphatic growth. Moreover we will test the function of lymphatics in these mice using well-established and new models of lymphedema, in order to determine whether RhoB and/or DB1 targeting has a positive impact on lymphedema formation and resolution. These studies will contribute to the understanding of lymphatic growth mechanisms, and set the stage for future therapeutic intervention targeted at these new molecules.