YALE UNIVERSITY SCHOOL OF MEDICINE

Vascular Biology and Therapeutics Program

Annual Report 2014-2015
On the Cover:
Submitted by Yibing Qyang, Ph.D. – Self Explanatory.
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Annual Report 2014 – 2015

Message from the Director

The past academic year has been filled with exciting scientific developments with many VBT faculty publishing in top notch journals in 2015. Clearly this is a testimony to the excellence and high quality of work here at Yale within the VBT program.

The interactions of VBT with the Cardiovascular Research Center (CVRC) is growing with continued collaborations, joint meetings, papers and grants. Finally, I would like to applaud Carol Muzzey and her team for organizing a fantastic retreat!

PROGRAM OPERATIONS

VBT Steering Committee

The Steering Committee serves as the principal advisory and leadership group for the program for the program. The current membership of the Steering Committee is listed in Table 1.

Administrative Operations

Ms. Carol Muzzey serves as the Program Manager and is assisted by Ms. Diane Strumpf. The program is served by the Central Administration Business Office.

<table>
<thead>
<tr>
<th>VBT Steering Committee</th>
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<tbody>
<tr>
<td>Jeffrey R. Bender, M.D., Robert I Levy Professor of Medicine (Cardiology) and Professor of Immunobiology; Associate Chief of Cardiovascular Medicine</td>
</tr>
<tr>
<td>Alfred L.M. Bothwell, Ph.D., Professor of Immunobiology and Director of Graduate Studies</td>
</tr>
<tr>
<td>Anne Eichmann, Ph.D., Professor Medicine (Cardiology) and Professor of Cellular and Molecular Physiology</td>
</tr>
<tr>
<td>Themis Kyriakides, Ph.D. Associate Professor of Pathology and of Biomedical Engineering</td>
</tr>
<tr>
<td>Laura Niklason, M.D., Ph.D., Professor Anesthesia and Biomedical Engineering</td>
</tr>
<tr>
<td>Jordan S. Pober, M.D., Ph.D., Bayer Professor of Translational Medicine; Director, Human and Translational Immunology Program; Vice-Chair Department of Immunobiology for the Section of Human and Translational Immunology</td>
</tr>
<tr>
<td>Nancy H. Ruddle, Ph.D., Professor Emeritus of and Senior Research Scientist in Epidemiology</td>
</tr>
<tr>
<td>W. Mark Saltzman, Ph.D., Goizueta Foundation Professor of Biomedical Engineering, Chemical &amp; Environmental Engineering and Physiology</td>
</tr>
<tr>
<td>William C. Sessa, Ph.D., Director Vascular Biology &amp; Therapeutics, Professor and Vice Chair of Pharmacology and Professor of Medicine (Cardiology)</td>
</tr>
<tr>
<td>Martin Schwartz, Ph.D., Robert W. Berliner Professor of Medicine (Cardiology) and Professor of Biomedical Engineering and of Cell Biology</td>
</tr>
<tr>
<td>Michael Simons, M.D., RW Berliner Professor of Medicine and Cell Biology, Director of Yale Cardiovascular Research Center</td>
</tr>
<tr>
<td>George Tellides, M.D., Ph.D., Professor of Surgery (Section of Cardiac Surgery) and of Investigative Medicine; Chief of Cardiothoracic Surgery, Veterans Affairs Medical Center</td>
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</tbody>
</table>
Program Faculty Membership
All faculties at Yale with a significant interest in vascular biology and/or therapeutics are eligible to join VBT. VBT members in academic year 2014-2015 are:

<table>
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<tr>
<th>Table 2 VBT Membership</th>
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<tbody>
<tr>
<td>Jeffrey R. Bender, MD, Robert I. Levy Professor of Medicine (Cardiology) and Professor of Immunobiology; Associate Chief, Cardiovascular Medicine, Director, Yale Cardiovascular Research Center</td>
</tr>
<tr>
<td>Anton Bennett, PhD, Professor of Pharmacology and of Comparative Medicine; Co-Director, Program in Integrative Cell Signaling and Neurobiology of Metabolism; Director, BBS Minority Affairs</td>
</tr>
<tr>
<td>Alfred L.M. Bothwell, PhD, Professor of Immunobiology</td>
</tr>
<tr>
<td>Demetrios Braddock, MD, PhD, Associate Professor of Pathology; Medical Director, Precipio Diagnostics</td>
</tr>
<tr>
<td>David A. Calderwood, PhD, Associate Professor of Pharmacology and of Cell Biology</td>
</tr>
<tr>
<td>Hyung J Chun, MD, FAHA, Associate Professor of Medicine (Cardiology); Director, YPB Fellows’ Cardiovascular Clinic</td>
</tr>
<tr>
<td>Alan Dardik, MD, PhD, FACS, FAHA, Professor of Surgery (Vascular); Vice Chair for Faculty Affairs, Chief, Vascular Surgery, VA Connecticut Healthcare Systems, West Haven, CT</td>
</tr>
<tr>
<td>Barbara E. Ehrlich, PhD, Professor of Pharmacology and of Cellular and Molecular Physiology</td>
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<tr>
<td>Anne Eichmann, PhD, Ensign Professor of Medicine (Cardiology) and Professor of Cellular and Molecular Physiology</td>
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<tr>
<td>Tarek Fahmy, PhD, Associate Professor of Biomedical Engineering and of Immunobiology</td>
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<tr>
<td>Carlos Fernandez-Hernando, PhD, Associate Professor of Comparative Medicine and Pathology</td>
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<tr>
<td>Richard Flavell, PhD, FRS, Sterling Professor of Immunobiology; Investigator, Howard Hughes Medical Institute; Chairman, Department of Immunobiology</td>
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<tr>
<td>Armar Geirsson, MD, Associate Professor Surger, Clinical Instructor in Surgery (Cardiothoracic)</td>
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<tr>
<td>Frank J. Giordano, MD, Associate Professor of Medicine (Cardiology)</td>
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<tr>
<td>Daniel R. Goldstein, MD, Professor of Medicine (Cardiology) and of Immunobiology, Director Center for the Biology of Aging and Cardiovascular Diseases</td>
</tr>
<tr>
<td>Daniel Greif, MD, Assistant Professor Medicine (Cardiology)</td>
</tr>
<tr>
<td>Jaime Grutzendler, MD, Associate Professor of Neurology and of Neurobiology; Director, Center for Experimental Neuroimaging (YCEN)</td>
</tr>
<tr>
<td>Murat Gunel, MD, FACS, FAHA, Nixdorff-German Professor of Neurosurgery and Professor of Genetics and of Neuroscience, Co-Director, Yale Program on Neurogenetics, Director, Neurovascular Surgery</td>
</tr>
<tr>
<td>Karen Hirschi, PhD, Professor of Medicine (Cardiology) and of Genetics</td>
</tr>
<tr>
<td>Jay Humphrey, PhD, John C. Malone Professor of Biomedical Engineering</td>
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<tr>
<td>John Hwa, MD, PhD., Associate Professor of Medicine (Cardiology); Director of Cardiovascular Pharmacogenetics</td>
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<tr>
<td>Yasuko Iwakiri, PhD, Assistant Professor of Medicine (Digestive Disease)</td>
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<tr>
<td>Suk-Won Jin, PhD, Associate Professor (Adjunct) of Medicine (Cardiology)</td>
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<tr>
<td>Martin S. Kluger, PhD, Research Scientist in Immunobiology</td>
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<tr>
<td>Diane Krause, MD, PhD, Professor of Laboratory Medicine, of Cell Biology and of Pathology; Assoc. Director, Yale Stem Cell Center; Assoc. Director, Transfusion Medicine Service; Medical Director, Clinical Cell Processing Laboratory; Medical Director, Advanced Cell Therapy Laboratory</td>
</tr>
<tr>
<td>Sanjay Kulkarni, MD, FACS, Associate Professor of Surgery (Transplant) and of Medicine (Nephrology), Director, Center for Living Organ Donors, Scientific Director, Yale Transplant Research Unit, Surgical Director, Kidney &amp; Pancreas Transplantation</td>
</tr>
<tr>
<td>Themis Kyriakides, PhD, Associate Professor of Pathology and of Biomedical Engineering, Director Graduate Programs</td>
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<tr>
<td>Patty J. Lee, MD., Associate Professor of Medicine (Pulmonary), Director of Research (Pulmonary)</td>
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<tr>
<td>Name</td>
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<tr>
<td>Joseph A. Madri, MD, PhD, Professor of Pathology and Director of Education</td>
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<tr>
<td>Arya Mani, MD, Associate Professor of Medicine (Cardiology) and of Genetics; Director, Cardiovascular Genetics Program</td>
</tr>
<tr>
<td>Kathleen Martin, PhD, Associate Professor of Medicine (Cardiology) and of Pharmacology</td>
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<tr>
<td>Laura R. Ment, MD, Professor of Pediatrics (Neurology), Associate Dean for Admissions and Financial Aid, Director, START Program</td>
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<tr>
<td>Wang Min, PhD, Professor of Pathology</td>
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<tr>
<td>Stefania Nicoli, PhD, Assistant Professor of Medicine (Cardiology)</td>
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<tr>
<td>Laura E. Niklason, MD, PhD, Nicholas Greene Professor of Anesthesiology and Professor of Biomedical Engineering, Division Chief, Vice Chair, Research</td>
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<td>Nancy Hartman Ruddle, PhD, Professor Emeritus of and Senior Research Scientist in Epidemiology (Microbial Diseases)</td>
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<td>William C. Sessa, PhD, Director, Vascular Biology &amp; Therapeutics Program; Alfred Gilman Professor of Pharmacology and Professor of Medicine (Cardiology), Vice Chairman, Pharmacology</td>
</tr>
<tr>
<td>Michael Simons, MD, Robert W. Berliner Professor of Medicine (Cardiology) and Professor of Cell Biology</td>
</tr>
<tr>
<td>Albert J. Sinusas, MD, Professor of Medicine (Cardiology), Director, Translational Research Imaging Center, Director, Cardiovascular Imaging</td>
</tr>
<tr>
<td>Jeffrey L. Sklar, MD, PhD, Professor of Pathology and of Laboratory Medicine; Director, Molecular Diagnostics Program; Director, Molecular Genetics Pathology Fellowship; Director, Molecular Tumor Profiling Laboratory; Director of Molecular and Genomic Pathology</td>
</tr>
<tr>
<td>Edward A. Snyder, MD, Professor of Laboratory Medicine; Associate Chair, Clinical Affairs (Therapeutic); Director, Apheresis/Transfusion Service; Director, Blood Bank; Director of Membership, Yale Cancer Center; Editor, Lab News</td>
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<tr>
<td>Bing Su, PhD, MPH, Associate Professor of Immunobiology</td>
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<tr>
<td>Yajaira Suarez, PhD, Assistant Professor of Comparative Medicine and of Pathology</td>
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<tr>
<td>George Tellides, MD, PhD, Professor of Surgery (Cardiac Surgery); Chief of Cardiothoracic Surgery, Veterans Affairs Medical Center</td>
</tr>
<tr>
<td>Jean-Leon Thomas, PhD, Associate Professor of Neurology</td>
</tr>
<tr>
<td>Daniela Tirzui, PhD, Research Scientist in Medicine (Cardiology)</td>
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<tr>
<td>Agnès Vignery DDS, PhD, Senior Research Scientist in Orthopaedics and Rehabilitation and in Cell Biology</td>
</tr>
<tr>
<td>Dianqing (Dan) Wu, PhD, Professor of Pharmacology</td>
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<tr>
<td>Lawrence H. Young, MD, Professor of Medicine (Cardiology) and of Cellular and Molecular Physiology; Vice-Chairman, Department of Medicine</td>
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<tr>
<td>Jun Yu, MD, Research Scientist in Medicine (Cardiology)</td>
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# PROGRAM ACTIVITIES

## Seminar Series

The VBT Monday afternoon seminars continue to serve as an intellectual focus of the vascular biology community at Yale. The series also serves as a venue for assistance in the recruitment of faculty with research in vascular biology to various departments at Yale. The seminars are run by Dr. Themis Kyriakides. A list of seminar speakers and their titles are shown in Table 3.

<table>
<thead>
<tr>
<th>Table 3 – VBT 2014-2015 Seminar Series</th>
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<tr>
<td><strong>OCTOBER 2014</strong></td>
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<tr>
<td>J. Silvio Gutkind, PhD, Chief Oral and Pharyngeal Cancer Branch, Chief, Cell Growth Regulation Section, Chief, Molecular Carcinogenesis Section, NIH-National Institute of Dental and Craniofacial Research, “The P13K-mTor Signaling Circuitry in Cancer and Tumor Angiogenesis: The Hippo in the Room?”</td>
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<td><strong>NOVEMBER 2014</strong></td>
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<tr>
<td>Patricia D’Amore, PhD, Charles L. Schepens Professor of Ophthalmology, Professor of Pathology, Co-Director, HMS Ophthalmology AMD Center of Excellence, Vice-Chair of Basic Research, Department of Ophthalmology, Harvard Medical School, “VEGF in the Adult: Implications for Anti-VEGF Therapy”</td>
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<td><strong>DECEMBER 2014</strong></td>
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<td>Timothy Hla, PhD, Director of Vascular Biology, Professor of Pathology and Laboratory Medicine, Weill Cornell Medical College, “RNA regulons and angiogenesis”</td>
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<tr>
<td>Christopher K. Breuer, MD, Professor of Surgery, Deputy Vice Chair for Research, Nationwide Children's Hospital Co-Director, Tissue Engineering Program, CRM-CBT, The Ohio State University College of Medicine, “Tissue Engineered Vascular Grafts: an update”</td>
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<td><strong>FEBRUARY 2015</strong></td>
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<tr>
<td>Diane Krause, MD, PhD, Professor of Laboratory Medicine, of Cell Biology and of Pathology, Assoc. Director, Yale Stem Cell Center, Assoc. Director, Transfusion Medicine Service, Medical Director, Clinical Cell Processing Laboratory, Medical Director, Advanced Cell Therapy Laboratory, Yale University, “Role of MKL and guanine exchange factors in megakaryopoiesis”</td>
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<tr>
<td>Christopher K. Glass, MD, PhD, Professor of Cellular and Molecular Medicine, Professor of Medicine “Exploiting and understanding effects of natural genetic variation on cell-specific gene expression”</td>
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<td><strong>MARCH 2015</strong></td>
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<tr>
<td>Christopher D. Kontos, MD, Associate Professor of Medicine, Duke University School of Medicine, “Vascular Cell and Skeletal Muscle Crosstalk in Peripheral Artery Disease”</td>
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<tr>
<td>Joao Pereira, PhD, Assistant Professor Department of Immunobiology, Yale University, “Mesenchymal and vascular niches controlling B lymphocyte development and egress from bone marrow” “Mechanisms of Abdominal Aortic Aneurysm Formation”</td>
</tr>
<tr>
<td>W. Robert Taylor, MD, PhD, Marcus Chair in Vascular Medicine, Director, Division of Cardiology, Emory University School of Medicine, “Mechanisms of Abdominal Aortic Aneurysm Formation”</td>
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<td><strong>APRIL 2015</strong></td>
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<tr>
<td>James Dahlman, PhD, Post-doctoral Fellow, Broad Institute, MIT, “New platforms for in vivo endothelial cell gene editing”</td>
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<tr>
<td>Didier Y. Stainier, PhD, Director, Developmental Genetics, Max Planck Institute for Heart and Lung Research, Bad Nauheim, Germany “Cardiovascular development in zebrafish”</td>
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<tr>
<td>Dean Y. Li, MD, PhD, Chief Scientific Officer for U Health Sciences, Associate VP for Research, Director, Molecular Medicine Program, Interim Co-Chair, Department of Physiology, Vice Dean for Research, School of Medicine, University of Utah Health Sciences, “Insights into oncogenes from endothelial signaling”</td>
</tr>
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Retreat
The annual retreat continues to be an extremely popular activity, bringing together over one hundred ninety scientists from the laboratories of VBT faculty members. This past year, the retreat was held on October 30, 2015 at the Grace Murray Hopper Auditorium, West Campus. The retreat continued the poster session competition with prizes for the best posters by a graduate student and a post-doctoral fellow. The Keynote Address at this years’ retreat was M. Luisa Iruela-Arispe, Ph.D., JCCC Cancer Center California, Los Angeles, CA “Developmental Origins of Vascular Disease” The retreat Program is listed in Appendix 1.

Yale-Cambridge Program in Cardiovascular Disease
The research alliance with Cambridge has continued as an important activity, with 15 faculty from Yale visiting Cambridge in September 2014 for a two day scientific meeting. The program for this retreat is listed in Appendix 2.

Tissue Engineering Group
This biweekly forum, sponsored by VBT and organized by Dr. Themis Kyriakides, brings together investigators from Yale Medical School and Yale’s central campus to exchange updates in research in progress and to foster new research collaborations.

VBT Research in Progress (RIP) Talks
A monthly series organized by Marty Kluger, Ph.D. and Jun Yu, M.D. featuring presentations by graduate students and postdoctoral fellows working in VBT laboratories.
Jeffrey R. Bender, M.D.
Robert I. Levy Professor of Medicine (Cardiology) and Professor of Immunobiology, Associate Chief, Cardiovascular Medicine, Director, Yale Cardiovascular Research Center

1. Overall Goal(s) of the Research Program of the Laboratory:
My laboratory has had a longstanding interest in inflammation and immunity, as they relate to vascular physiology and pathology. The interactions between mononuclear leukocytes and endothelial cells play major roles in atherogenesis, acute and chronic manifestations of atherosclerosis, angiogenesis and allograft rejection. We have extended these studies to evaluating effects of ovarian steroid hormones on endothelial function. The work is performed at the cellular, molecular, and pre-clinical animal model levels.

2. Publications:
(Publications July 1, 2013– June 30, 2014)
Anton M. Bennett, Ph.D.
Professor of Pharmacology and of Comparative Medicine; Co-Director, Program in Integrative Cell Signaling and Neurobiology of Metabolism; Director, BBS Minority Affairs

1. Overall Goal(s) of the Research Program of the Laboratory:
The broad research interests of this laboratory are to define the molecular mechanisms, physiological and pathophysiological roles of the protein tyrosine phosphatase (PTP) family of enzymes. Using mouse genetic approaches we are studying how PTPs are involved in regulating metabolism. This work suggests new modes of control through which PTPs participate in metabolic signaling and potentially the progression of obesity and diabetes. We are also investigating the pathophysiological mechanisms of the PTPN11 gene, which encodes for the PTP, SHP-2. PTPN11/SHP-2 mutations are found in ~50% of Noonan syndrome cases, which represents the largest non-chromosomal cause of congenital heart disease. The broad goal of this project is to identify the signaling mechanisms induced by the mutant form of PTPN11/SHP-2 that leads to congenital heart disease.

1. **Overall Goal(s) of the Research Program of the Laboratory:**
The goals of the lab are to understand the mechanisms that both T cells and tumor cells utilize to cross endothelial cell barriers into parenchymal tissue.

2. **Specific Research Accomplishments in the last 12 months:**
We have identified a complex role for the wnt antagonist Dkk-1 in regulating the immune system and in affecting tissue permeability. Environmental antigens or pathogens can trigger activation of platelets which has potent regulatory consequences for the immune response. The signaling pathways utilize the SGK-1 kinase and p38 to drive a Th2 immune response.

3. **Significance of Key Findings Relevant for the Mission of VBT:**
This work has identified platelets as the primary in vivo source of circulating Dkk-1 in peripheral blood and begun to identify molecular mechanisms of action. Platelet activation results in increased expression of P-Selectin which enhances a physical interaction with neutrophils and lymphocytes. This results in migration to target tissues and an inflammatory reaction. This represents a direct molecular connection between the coagulation and immune system. The consequences on the immune response are quite profound.


1. Overall Goal(s) of the Research Program of the Laboratory:
The broad goal of research in the Calderwood lab is to understand the molecular basis of signaling
to and from integrin adhesion receptors. We use a combination of structural, biochemical and cell
biological approaches to investigate how protein-protein interactions regulate integrin activation
state, provide links from integrins to the actin cytoskeleton, control cell migration and
morphogenesis, and assemble into signaling complexes. More recently, in collaboration with the
Boggon lab at Yale, we have also become interested in cerebral cavernous malformation (CCM)
proteins and their interactions.


Uchil PD, Pawliczek T, Reynolds TD, Ding S, Hinz A, Munro JB, Huang F, Floyd RW,
Yang H, Hamilton WL, Bewersdorf J, Xiong Y, Calderwood DA, Mothes W. TRIM15 is a focal
adhesion protein that regulates focal adhesion disassembly. J Cell Sci. 2014 Sep 15;127(Pt
Central PMCID: PMC4163643.

Huet-Calderwood C, Brahme NN, Kumar N, Stiegler AL, Raghavan S, Boggon TJ,
Calderwood DA. Differences in binding to the ILK complex determines kindling isoform adhesion
10.1242/jcs.155879. Epub 2014 Aug 1. PubMed PMID:25086068; PubMed Central PMCID:
PMC4179494.

Bancroft T, Bouaouina M, Roberts S, Lee M, Calderwood DA, Schwartz M, Simons M,
Sessa WC, Kyriakides TR. Up-regulation of thrombospondin-2 in Akt1-null mice contributes to
compromised tissue repair due to abnormalities in fibroblast function. J Biol Chem. 2015 Jan
PubMed Central PMCID: PMC4281743.

Ellis SJ, Lostchuck E, Goult BT, Bouaouina M, Fairchild MJ, Lópe-Ceballos P,
Calderwood DA, Tanentzapf G. The talin head domain reinforces integrin-mediated adhesion by
Central PMCID: PMC4230843.

Simpson MA, Bradley WD, Harburger D, Parsons M, Calderwood DA, Koleske AJ. Direct
interactions with the integrin β1 cytoplasmic tail activate the Abl2/Arg kinase. J Biol Chem. 2015
25694433; PubMed Central PMCID:PMC42375489.

Draheim KM, Li X, Zhang R, Fisher OS, Villari G, Boggon TJ, Calderwood DA. CCM2-
CCM3 interaction stabilizes their protein expression and permits endothelial network formation. J
PubMed Central PMCID: PMC4384732.
Hyung Chun, MD, FAHA
Associate Professor of Medicine (Cardiology)

1. Overall Goal(s) of the Research Program of the Laboratory:
The Chun Laboratory studies a number of signaling processes that regulate vascular function in health and disease. Research is focused on identifying signaling pathways that play key roles in the context of cardiovascular disease and development, including pulmonary arterial hypertension, atherosclerosis and cardiovascular development.

2. Specific Research Accomplishments in the last 12 months:
We continue to investigate the signaling mechanisms that are involved in pathogenesis of multiple cardiovascular disease processes, including pulmonary arterial hypertension, atherosclerosis, and diabetic endothelial dysfunction. Moreover, we are actively investigating the crosstalk of various G protein coupled receptor signaling pathways that are intricately involved in vascular development. Key highlights include:

1. Demonstration that selective inhibition of histone deacetylase IIa, via activation of the transcription factor MEF2, can ameliorate experimental models of pulmonary arterial hypertension
2. Demonstration that endothelial apelin-APJ signaling is critical to the glucose lowering effect of apelin signaling
3. Demonstration of crosstalk between APLNR and CXCR4 GPCRs, two GPCRs that are selectively expressed in the vascular endothelium, via a novel microRNA mediated mechanism

3. Significance of Key Findings Relevant for the Mission of VBT:
We continue to explore vascular signaling pathways that are important in both vascular disease states as well as maintenance of vascular homeostasis. Better understanding of these signaling mechanisms will provide greater insights into the mechanisms of disease pathogenesis, as well as identify novel venues for potential therapies aimed at treating vascular diseases such as atherosclerosis and pulmonary arterial hypertension.


Alan Dardik, M.D., Ph.D.
Professor of Surgery (Vascular)

1. Overall Goal(s) of the Research Program of the Laboratory:
   The Dardik laboratory continues to study the healing and function of blood vessels as used in patients having vascular bypass surgery as well as arteriovenous fistulae (AVF). We are currently trying to understand the fundamental molecular mechanisms by which vein graft adaptation and AVF maturation result in positive remodeling and successful adaptation to the arterial environment, yet often proceed, in the long-term, to neointimal hyperplasia and graft and AVF failure.

2. Specific Research Accomplishments in the last 12 months:
   The laboratory continues to study the mechanisms by which Eph-B4 controls venous adaptation to the arterial circulation, continuing to focus on our model of the mouse AVF that recapitulates human AVF maturation. Currently we are examining Eph-B4 interaction with downstream signalling pathways including caveolin-1, Akt, and eNOS. We continue to collaborate closely with several other labs in the VBT programs, as well as with collaborators at UCL.

3. Significance of Key Findings Relevant for the Mission of VBT:
   Our laboratory continues to identify mechanisms of venous remodeling and adaptation to the arterial circulation.

1. Overall Goal(s) of the Research Program of the Laboratory:
My laboratory studies vascular development, with particular interests in mechanisms that direct patterning and guidance. Specialized endothelial cells (EC) called tip cells located at the extremities of growing capillary sprouts mediate guided vascular patterning. Tip cells exhibit characteristic features, including extension of filopodia that explore the tip cell environment, lack of a lumen and a slow proliferation rate. Following behind tip cells, other EC termed stalk cells form the capillary lumen and proliferate. Our research is focused on molecular events guiding angiogenesis and arteriogenesis:

2. Specific Research Accomplishments in the last 12 months:
We have identified two major guidance receptor pathways regulating sprouting angiogenesis. Inducible, endothelial cell specific deletion in mice of Neuropilin1 Nrp1, or of the Slit2 receptors Robo1 and 2, leads to major angiogenesis defects in the vasculature of the developing postnatal retina, and in mouse models of ocular neovascular disease (Aspalter et al., Nat Comm 2015, Rama et al., Nat Med 2015). These results suggest that blocking Nrp1 or Robo1 and 2 function could represent powerful novel means to block angiogenesis in ocular neovascularization.

We have also shown that blood flow-mediated vascular mural cell recruitment plays a major role in directing sympathetic neuron noradrenergic differentiation in zebrafish embryos (Fortuna et al., Cell Reports 2015).

3. Significance of Key Findings Relevant for the Mission of VBT:
Our findings provide new insight into fundamental mechanisms directing angiogenesis and arteriogenesis.


1. Overall Goal(s) of the Research Program of the Laboratory: The Fernández-Hernando laboratory studies the molecular regulation of lipoprotein metabolism and how its deregulation contributes to cardiometabolic diseases such as atherosclerosis. Our specific goals are to understand how microRNAs and other non-coding RNAs regulate cellular cholesterol metabolism and the identification of novel genes that controls cellular cholesterol metabolism using high-throughput screening approaches.

2. Specific Research Accomplishments in the last 12 months: Discoveries over the past 12 months include: (1) Identification of miR-148a as a major posttranscriptional regulator of LDLR and ABCA1 expression, key transporters that regulate cellular cholesterol uptake and efflux. Importantly pharmacological inhibition of miR-148a reduces circulating LDL-C and increases plasma HDL-C; (2) Characterization of Akt1 and Akt2 functions during the progression of atherosclerosis, (3) Identification of miR-199 as important regulator of receptor-mediated endocytosis and (4) Characterization the role Akt1 in liver regeneration.

3. Significance of Key Findings Relevant for the Mission of VBT: Our research can form the basis of new therapies for treating dyslipidemia and cardiovascular related disorders including atherosclerosis.


1. **Overall Goal(s) of the Research Program of the Laboratory:**
My laboratory investigates blood vessel morphogenesis, the maintenance of the adult blood vessel and diseases of the vasculature. We initially determined the patterns of smooth muscle and adventitial cell differentiation, proliferation and migration in the developing pulmonary arterial wall in the mouse and the role of the platelet derived growth factor pathway in these processes. Currently, we are studying the morphogenesis of the walls of the aorta and the intracerebral vasculature, and comparing and contrasting their development with that of the pulmonary artery. In addition, little is known about the maintenance of blood vessels, and we aim to determine the patterns and underlying mechanisms of cell turnover, proliferation and migration in the adult vessel wall. Moreover, diseases of the vasculature are thought to largely involve a recapitulation of developmental programs, and we are investigating animal models of vascular diseases that involve defective vascular walls, such as atherosclerosis, arterial stenosis, intracerebral hemorrhage and pulmonary hypertension (PH). In addition, we have recently extended our studies to lung fibrosis which is an important cause of PH. Finally, we are studying clinical samples obtained from patients with vascular and lung diseases and relating them to our findings in animal models.

2. **Specific Research Accomplishments in the last 12 months:**
We previously determined that distal arteriole smooth muscle cells (SMCs) in hypoxia-induced PH derive from pre-existing proximal pulmonary artery smooth muscle and undergo stereotyped stages of dedifferentiation, distal migration down the uncoated endothelial cell tube, proliferation and finally redifferentiation. During the last year, we identified novel “primed” smooth muscle progenitors and in each pulmonary arteriole, with hypoxia-induced PH, one of them migrates distally, dedifferentiates and clonally expands, giving rise to distal SMCs. Furthermore, in mice exposed to hypoxia, enhanced lung platelet derived growth factor-B and primed cell Kruppel-like factor (KLF)4 are required for distal arteriole muscularization and PH. Finally, in PH patients, KLF4 is markedly upregulated in pulmonary arteriole smooth muscle, especially in proliferating SMCs. Thus, therapeutic strategies targeting these novel progenitors promise to have profound implications for pulmonary hypertension and perhaps other vascular diseases. This work was published in 2015 in *Science Translational Medicine*, 7:308ra159 with accompanying press attention in *The Scientist* and was a critical component of my receiving the Springer 2015 Junior Investigator Award from the North American Vascular Biology Organization.

We have also made seminal discoveries regarding blood-brain barrier formation and aortic wall development and disease (e.g. supravalvular aortic stenosis and atherosclerosis).

3. **Significance of Key Findings Relevant for the Mission of VBT:**
Abnormalities of the blood vessel wall are fundamental to many devastating vascular pathologies. Insights from our research will help design novel therapeutic strategies for these diseases.


Misra, A, Sheikh, AQ, Kumar, A, Luo, J, Hinton, RB, Smoot, L, Kaplan, P, Urban, Z, Qyang, Y, Tellides, G, Greif, DM*. Integrin beta3 inhibition is a therapeutic strategy for supravalvular aortic stenosis. Revised manuscript under third review at *Journal of Experimental Medicine*, (*Corresponding author).*
Jaime Grutzendler, MD
Associate Professor of Neurology and of Neurobiology; Director Center for Experimental Neuroimaging (YCEN)

1. Overall Goal(s) of the Research Program of the Laboratory:
The overall interest of our laboratory centers around cells in the so called "neuro-glio-vascular unit" (neurons, endothelium, astrocytes, pericytes, microglia, oligodendrocytes and NG2 cells). Our goal is to learn about the dynamic properties of these cells in vivo and how cell-cell interactions are disrupted in brain injury, vascular and neurodegenerative pathologies.

2. Specific Research Accomplishments in the last 12 months:
   - We have characterized for the first time in vivo the molecular, structural and functional features of brain vascular mural cells. Our studies conclude that pericytes are not contractile as previously thought and also revealed interesting physiological properties of these mysterious cells
   - We continue to make progress on the understanding and potential translation of our recent discovery of a novel mechanism of microvascular recanalization independent of the fibrinolytic system that we have named angiophagy. (Nature 2010 and Science Trans Med 2014). We are looking for potential applications of our findings in stroke and vascular dementia

3. Significance of Key Findings Relevant for the Mission of VBT:
In our recent Neuron paper, we conclusively identify and functionally characterize in vivo the perivascular mural cells that, through their contractile properties, control neurovascular coupling and microregional cerebral blood flow. We conclude the following: Capillary pericytes are morphologically and functionally distinct from precapillary and post-capillary myocytes. Capillary pericytes do not express smooth muscle actin (SMA) in mice or in humans. In vivo spontaneous vasomotility occurs in vessels with SMA expression, regardless of their diameter or branch order, but does not occur in pericyte-covered vessels, all of which lack SMA. Single cell in vivo optogenetic activation causes vessel constriction by smooth muscle cells, while identical light activation causes no constriction by capillary pericytes. Sensory stimulation evokes vessel dilation in smooth muscle-covered vessels and minimal secondary passive dilation in pericyte-covered capillaries. Cortical spreading depolarization induces vasomotility in smooth muscle-covered vessels but not pericyte-covered capillaries. Ischemia reperfusion and spreading depolarization can induce focal transient obstructions in smooth muscle-covered vessels, which may lead to "no-reflow" after reperfusion. Overall these findings conclusively demonstrate that capillaries are not the sites of cerebral blood flow control and that pericytes are not contractile. Blood flow is controlled by smooth muscle cells on arterioles and pre-capillary arterioles under normal and pathological states.

4. Publications
   - RA. Hill, L. Tong, P. Yuan, S. Murikinati, S. Gupta, J. Grutzendler. Regulation of regional cerebral blood flow is mediated by smooth muscle cell contractility and not by capillary pericytes. Neuron. 2015 Jul 1;87(1)
Karen Hirschi, Ph.D.
Professor of Medicine (Cardiology) and Genetics

1. Overall Goal(s) of the Research Program of the Laboratory:
A primary interest of our laboratory is to understand, at the cellular and molecular level, the events leading to blood vessel formation. We are specifically interested in the regulation of vascular cell commitment, differentiation and cell cycle progression, and defining signaling pathways that modulate these processes. We also aim to understand how some vascular cells acquire specialized functions, such as the generation and/or maintenance of multi-lineage stem/progenitor cells.

1. Overall Goal(s) of the Research Program of the Laboratory:
The primary goal of my laboratory is to understand better and mathematically model the roles of mechanobiological mechanisms in tissue-level homeostasis, adaptation, and pathogenesis. Specific interests include arterial changes in response to hypertension, altered flow, and aging, the design and assessment of tissue engineered vascular grafts, venous changes when used as arterial grafts, and the progression of large artery diseases such as aortic and cerebral aneurysms. We are also interested in exploiting genetically modified mice to elucidate individual contributions of extracellular matrix constituents (e.g., fibrillin-1, fibulin-5, fibulin-4) to tissue level arterial structure and function.

2. Specific Research Accomplishments in the last 12 months:
Among the many different findings, four particularly important ones were the (i) use of four different mouse models of compromised elastic fiber integrity to delineate potential reasons for arterial stiffness as well as aneurysmal enlargement, (ii) establishment of a new theoretical framework for modeling long-term vascular adaptation and disease progression via a concept of “mechanobiological stability”, (iii) identifying a new hemodynamic metric for identifying when and where intraluminal thrombus may form within regions of disturbed flows in large arteries and aneurysms, and (iv) formulation of a computational model for aiding in the design of polymeric scaffolds for tissue engineered vascular grafts.

3. Significance of Key Findings Relevant for the Mission of VBT:
From a vascular biology perspective, we put forth a new hypothesis of dysfunctional mechano-sensing as causative in thoracic aortic aneurysms and dissections that is guiding our next series of studies. From the perspective of therapeutics, the formulation of a new theoretical framework and computational model of the in vivo development of tissue engineered vascular grafts has potential to speed up the search for optimal scaffold properties. We just received a new R01 based on these promising results and hope that this will impact therapeutics.


Baeyens N, Mulligan-Kehoe MJ, Corti F, Simon DD, Ross TD, Rhodes JM, Wang TZ, Mejean


Ramachandran AB, Sankaran S, Humphrey JD, Marsden AL (2015) Computational simulation of the adaptive capacity of vein grafts in response to increased pressure. J Biomech Engr 137 (ePub ahead of print) PMCID PMC4321118

Ferruzzi J, Bersi MR, Uman S, Yanagisawa H, Humphrey JD (2015) Decreased energy storage, not increased material stiffness, characterizes central artery dysfunction in fibulin-5 deficiency independent of sex. J Biomech Engr 137: (ePub ahead of print) PMCID PMC4321117


(italicized names are trainees)
1. Overall Goal(s) of the Research Program of the Laboratory:
The major focus of our laboratory is to decipher the mechanisms leading to diabetic platelet dysfunction. Our overall hypothesis is; “aldose reductase is a major transducer of the hyperglycemic response, resulting in mitochondrial dysfunction and damage and a prothrombotic state”. To test our hypotheses we study human patients and human tissues (clinical, pathophysiology, pharmacology, cell biology, molecular biology, and bioinformatics) in combination with animal studies. Our ultimate goals are to identify patients at increased risk for atherothrombosis, and to develop novel therapies targeting atherothrombosis.

2. Specific Research Accomplishments in the last 12 months:
Thrombosis is a well-recognized complication of diabetes mellitus (DM). Mitochondria play a central role in platelet metabolism and activation. Mitochondrial dysfunction is evident in DM. The molecular pathway for hyperglycemia-induced platelet mitochondrial dysfunction in DM platelets is unknown. Using both human and humanized mouse models, we have demonstrated that hyperglycemia-induced aldose reductase (AR) activation, and subsequent reactive oxygen species (ROS) production, leads to increased p53 phosphorylation (Ser15), which promotes mitochondrial dysfunction, damage and rupture by sequestration of the anti-apoptotic protein Bcl-xL. In a glucose dose dependent manner, severe mitochondrial damage leads to loss of mitochondrial membrane potential and platelet apoptosis (cytochrome c release, caspase 3 activation and phosphatidylinerine exposure). Apoptotic platelets leads to increased thrombosis. The degree of mitochondrial dysfunction and damage determines whether hyperactivity (mild damage) or apoptosis (severe damage) will ensue. A second series of studies have focused on miRNA regulation of VWF. VWF levels are increased in DM and are associated also with increased thrombosis. We discovered another interesting hyperglycemia induced pathway (in endothelial cells) leading from ROS (through AR) to reduced miR-24 and increased VWF. Further studies are underway assessing how the hyperglycemia induced mitochondrial damage affects platelet and endothelial metabolism, how autophagy may serve a protective role, and how other miRNA may be affected by DM. These signaling components provide novel therapeutic targets for DM thrombotic complications.

3. Significance of Key Findings Relevant for the Mission of VBT:
- Identification of the role played by aldose reductase in platelet mitochondrial damage and platelet apoptosis
- Identification of miR-24 dysregulation in diabetes mellitus


1. Overall Goals of the Research Program of the Laboratory:
The main areas of my research include vascular remodeling in liver diseases, liver fibrosis and liver regeneration. We are especially interested in how hemodynamic changes are associated with liver fibrosis and liver regeneration and how endothelial cell dysfunction leads to liver fibrosis. The goals are to understand the basic mechanisms of flow-induced changes in the liver and to apply this knowledge to develop therapeutic strategies for liver fibrosis/cirrhosis and portal hypertension.

2. Specific Research Accomplishments in the last 12 months:
We have demonstrated Nogo-B promotes liver fibrosis and portal hypertension. We have also found the mechanisms of arterial thinning in portal hypertension with liver cirrhosis.

3. Significance of Key Findings Relevant for the Mission of VBT:
Research conducted by our laboratory focuses on development of therapeutic strategies that modify vasculature/endothelial cell function in a manner to prevent liver fibrosis in portal hypertension.

Publications (from July 1, 2013 to June 30, 2014):
Martin S. Kluger, Ph.D.
Research Scientist, Department of Immunobiology

1. Overall Goals of the Research Program of the Laboratory:
Endothelium controls the passage of fluid, solutes and macromolecules between the blood and tissue, a process critical for vertebrate life. Blood capillary endothelial cells (EC) form the principal component of this permselective barrier and paracellular exchange across capillaries, unlike venules, is prevented by intercellular tight junctions. We seek to understand how inflammatory cytokine signals disrupt capillary endothelial cell tight junctions leading to dysfunctional capillary barriers. In particular, we study how tumor necrosis factor activates signaling pathways in EC that affect tight junctional molecular complexes involving claudin-5, JAM-A or ZO-1. Our work aims to advance understanding of how capillary barrier dysfunction leads to organ failure in blood sepsis or in other systemic inflammatory response syndromes (e.g., as induced by cardiopulmonary bypass).

2. Specific Research Accomplishments in the Last 12 Months:
- With pilot grant funding from the Yale west campus Center for Molecular Discovery, we adapted electrical cell-substrate impedance sensing (ECIS) for high throughput siRNA screening of the human kinome. This work, being performed in collaboration with the inventors of ECIS at Applied BioPhysics, Inc (Troy, N.Y) has generated novel hits that are potential therapeutic targets for treating capillary leak.
- Extended studies from our HDMEC model, which is perhaps the most thoroughly characterized human system for the study of endothelial TJs, by inducing TJ function in human endothelial colony forming cells derived from cord blood in a publication reporting the first use of CRISPR/cas9 on primary, non-immortalized EC.
- Mentoring Richard Pierce, MD, a research fellow in the Yale Pediatric Basic Science Training Program, established procedures and methods at Yale for generating cultures of microvascular ECs from human lung. These cultures are a new tool for modeling barrier function in an organ targeted in conditions like sepsis or SIRS.
- Completed a 4th successful year co-directing (with Dr. Jun Yu) the well-attended VBT Research-In-Progress Series, a seminar series that promotes research interactions among different VBT laboratories housed in the Amistad Research building. This year graduate students and post-docs presented research accomplishments from the Fernandez, Kyriakides, Min, Niklason, Pober, Saltzman, Sessa, Suarez, Tellides, Wu and Yu labs.

3. Significance of Key Findings Relevant for the Mission of VBT:
Our topics are translational in nature and our focus on the roles for tight junctions in controlling the capillary barriers of peripheral tissues represents a paradigm-shifting perspective over current thinking overstating the role of adherens junctions.

4. Publications:
1. **Overall Goal(s) of the Research Program of the Laboratory:**
The overall goals of my research are to characterize bone marrow derived stem and progenitor cells, and to define the molecular mechanisms (signal transduction, biomechanical, and epigenetic) that regulate the self-renewal and differentiation of these cells. Our recent emphasis has been on megakaryocyte development and platelet formation with a recent focus on platelet function. We are studying the roles of G-proteins, the SRF signal transduction pathway, and RNA binding proteins in order to better understand and treat hematopoietic diseases including myelodysplasia, myeloproliferative disease and leukemia, as well as vascular diseases related to thrombus formation and acute infarction. Projects include work with embryonic stem cells as well as hematopoietic stem cells from mice and humans. Our work provides insights not only into normal blood cell development, but also to the pathogenesis of benign and malignant hematological diseases.

2. **Specific Research Accomplishments in the last 12 months:**
Work relevant to VBT over the past 12 months has been focused on regulation of platelet function. These studies have not yet been published. The findings are summarized briefly below.

3. **Significance of Key Findings Relevant for the Mission of VBT:**
Novel discoveries in platelet activation: The laboratory will be submitted 3 papers for publication over the next 4 months with new insights into regulation of platelet production and activation.

**E-cadherin**
Dr. Alexandra Teixeira, who recently completed her PhD studies in my laboratory, discovered that E-cadherin protein is present in megakaryocytes and platelets, and that loss of e-cadherin compromises platelet activation in vitro and platelet clotting function in vivo.

**ARH-GEF12**
Siying Zou, a graduate student in the laboratory, has shown that LARG, also known as ARHGEF12, acts downstream of agonists that act via Ga12/13 in both primary murine and human platelets. Loss of LARG activity leads to compromised platelet function, hemostasis and thrombosis accompanied with reduced RhoA activation and myosin light chain phosphorylation. We recapitulated the mouse data in human platelets in vitro using a LARG inhibitor. In addition, mice with decreased LARG formed smaller and fewer thrombi in a pathological stroke model suggesting that pharmacological inhibition of this guanine exchange factor could be useful therapeutically. These are the first data to ascribe a role of LARG in platelet function.

**ARH-GEF3**
Siying Zou also performed a tremendous amount of work that shows that despite its very strong association on GWAS studies with reduced platelet numbers and increased platelet size, Arhgef3 does not seem to play a critical role in platelet formation or function.


1. Overall Goal(s) of the Research Program of the Laboratory:
My laboratory investigates mechanisms of lung injury and cytoprotection during oxidant stress. Specifically, we have focused on the lung endothelium as a central mediator of lung injury and repair responses. We identified the importance of the stress-response protein heme oxygenase-1 (HO-1) and its gaseous reaction product, carbon monoxide (CO), in resisting oxidant-induced endothelial cell death via mitochondrial pathways. We found that a family of signaling molecules, mitogen-activated protein kinases (MAPKs), mediates HO-1's and CO’s protective effects as well as optimal IL-13-induced lung inflammation / remodeling and, more recently, critical innate immune responses. The innate immune system consists of pattern-recognition receptors called toll-like receptors (TLRs), of which TLR4 is the LPS-responsive receptor. We discovered that TLR4 is required for lung structural cell survival in aging and oxidant challenges. These studies represent important paradigm shifts in our understanding of TLRs and lung biology and are now the basis of translational studies in people with acute lung injury and age-related chronic lung disease, such as chronic obstructive pulmonary disease (COPD). In the process of our investigations, we were the first to demonstrate the utility of intranasal, lung-targeted and endothelial-targeted silencing RNA (siRNA) constructs in vivo. In parallel, we have also generated endothelial-targeted transgenic and knockout mouse models to specifically interrogate the role of the endothelium in lung disease. Our coordinated use of siRNA technology and genetic approaches in both cell and mouse models offer immense insight into disease pathogenesis and may identify novel therapeutic targets for a range of lung diseases.

2. Specific Research Accomplishments in the last 12 months:
Her key accomplishments for the year include creating lung-endothelial targeting vectors that can be used in vivo, showing for the first that mitochondrial turnover and fission are critical determinants of COPD and pulmonary vascular remodeling and identifying a novel innate immune pathway, MIF-CD64, in the pathogenesis of vascular injury. She also assembled a group of multi-disciplinary investigators to study COPD in the context of immunologic aging. The investigators are from Yale Pathology, Comparative Medicine, Program on Aging, Rheumatology and Infectious Diseases. Their collaborations in the past year alone have resulted in a submitted P01, “Immune Mechanisms in the Aging Lung,” an In Press monograph, “Aging Lung: Mechanisms and Clinical Sequela” and an In Press chapter in the seminal gerontology text - Hazzard’s textbook of Gerontology, 7th Edition.

3. Significance of Key Findings Relevant for the Mission of VBT:
Dr. Lee’s work aligns well with the mission of VBT by introducing new vascular biology reagents, specifically in lung vasculature, and forming multi-disciplinary research groups to study novel aspects of lung biology.

4. Publications: (Publications July 1, 2014–current)


1. **Overall Goal(s) of the Research Program of the Laboratory:**

This past year we have continued investigating specific mouse strains, which mimic the wide range of responses to hypoxia observed in the human very low birth weight premature infant population. We have found these strains to exhibit significant differences in selected signaling nodes (GSK-3β and HIF-1α), growth factors and neurotrophins that have been shown to be involved in the responses to hypoxia.

In a recent study, we have found that a decreased expression of a particular transcription factor (Sox10) correlates with a poor response to chronic hypoxia in mouse pups, resulting in multiple neurodevelopmental deficits. Sox10 regulates oligodendrocytogenesis, differentiation and myelination, known modulators of synaptic transmission and neuronal impulse. We have identified a small molecule (minocycline) that induces Sox10 and improves cognitive behavior in mice following hypoxic insult. These studies should lead to a more complete understanding of the proteins and pathways involved and provide us with needed insights for rational drug design.

2. **Specific Accomplishments in the last 12 months:**

We have also continued our investigations of Hippo pathway signaling components (YAP/TAZ) via endothelial cell and fibroblast adhesion molecules (CD44, CD31 and VE-cadherin & N-cadherin) and extracellular matrix components in brain microvascular endothelial cell and fibroblast proliferation and apoptosis. We found that endothelial cells and fibroblasts lacking CD44 escaped from contact inhibition and exhibited abnormal proliferation and apoptosis and exhibited increased expression of Survivin.

3. **Publications:**


   Tsuneki, M., **Madri J.A.**, Saku T., Cell-extracellular interactions in oral tumorigenesis: the
Arya Mani, M.D.
Associate Professor of Medicine (Cardiology) and of Genetics; Director Cardiovascular Genetics Program

1. Overall Goal(s) of the Research Program of the Laboratory:
My laboratory’s major focus is the identification of genetic causes of major cardiovascular disorders and the elucidation of their pathophysiology. To achieve this, we have built strong ties at national and international levels with major cardiovascular centers. The goal is to identify and recruit patients and families with diverse cardiovascular disorders that have strong genetic components. Following identification and characterization of theses mutation we carry out physiological studies in mutation carriers, in vitro and in mouse models of the disease.

2. Specific Research Accomplishments in the last 12 months:
My laboratory identified a nonconservative mutation in highly conserved kinase-like domain \textit{DYRK1B} gene in subjects with early onset coronary artery disease and metabolic syndrome. Functional characterization of the disease gene revealed that non-mutant protein encoded by \textit{DYRK1B} inhibits the SHH (sonic hedgehog) and Wnt signaling pathways and consequently enhances adipogenesis. Furthermore, \textit{DYRK1B} promoted the expression of the key gluconeogenic enzyme glucose-6-phosphatase. The R102C allele showed gain-of-function activities by potentiating these effects. Further examination showed that the mutation causes insulin resistance. Investigation of the skeletal muscles led to identification of novel targets that can be targeted pharmaceutically to improve insulin sensitivity.

3. Significance of Key Findings Relevant for the Mission of VBT:
- Identified the key role of Wnt signaling in regulation of de novo lipogenesis and cholesterol synthesis
- Established the role of Wnt/LRP6 in vascular smooth muscle cell differentiation and integrity of the vascular wall
- Recognized the role of Dyrk1B in adipogenesis, gluconeogenesis and its altered function in development of coronary artery disease and diabetes
Identified the genetic causes of patent ductus arteriosus in humans and ductus
Identified the genetic causes of slow atrial fibrillation

A Case for Inclusion of Genetic Counselors in Cardiac Care: A Case for Genetic Counselors.
Martin KA, Mani MV, Mani A. New targets to treat obesity and the metabolic syndrome. *Eur J Pharmacol*. 2015;763.. PMID: 26001373


1. Overall Goal(s) of the Research Program of the Laboratory:

The primary goals of the Martin lab are to understand the signaling mechanisms that regulate VSMC phenotype in order to suggest therapeutic strategies for intimal hyperplasia, graft arteriosclerosis, atherosclerosis, and hypertension.

2. Specific Research Accomplishments in the last 12 months:

A major interest in our lab has been signaling through the mTOR pathway in vascular smooth muscle cells, since this is the pathway targeted by the highly effective stent therapeutic rapamycin. We have recently discovered that the mTORC1 pathway promotes VSMC differentiation through regulation of multiple signaling cascades and transcriptional and epigenetic effectors. This year, we determined that the mTORC1 inhibitor rapamycin, through feedback activation of Akt2, upregulates the activity of a key transcription factor, GATA-6, in VSMC. We find that Akt2 phosphorylation of GATA-6 promotes VSMC differentiation and anti-proliferative effects. Genetic loss of Akt2 resulted in severe intimal hyperplasia, which could be rescued by in vivo gene therapy with GATA-6 in a mouse model. The therapeutic effect was greatest when using GATA-6 with a phospho-mimetic mutation, highlighting the importance of this Akt2 phosphorylation in vivo. This study reveals important findings about VSMC biology, signal transduction, Akt isoforms, and has implications for rapamycin therapeutic approaches.

In collaboration with Dr. Hwa, we have demonstrated a unique relationship between hyperglycemia, miR-24, and VWF. This study provides insights into the etiology and mechanisms by which hyperglycemia in diabetes promotes cardiovascular, particularly atherothrombotic, disease.

3. Significance of Key Findings Relevant for the Mission of VBT:

The work in our own laboratory has identified TET2 as an exciting new epigenetic master regulator of VSMC phenotype. In contrast to myocardin, the master transcriptional coactivator in VSMC differentiation, we find that TET2 coordinately regulates differentiation and de-differentiation genes to promote a healthy smooth muscle phenotype. In collaboration with Dr. George Tellides, we have found that TET2 is repressed in human atherosclerotic samples. With Dr. Jun Yu, we have found that TET2 is repressed following vascular injury, and, most importantly, that TET2 overexpression can rescue intimal hyperplasia in vivo. These findings have therapeutic implications for multiple cardiovascular diseases involving smooth muscle. As VBT is a multi-disciplinary and highly collaborative program, we are enthusiastic about our additional VBT collaborations with the Hwa lab, in which our expertise in signal transduction has helped them in making exciting new discoveries about the effects of glucose on platelets in diabetics and normal subjects. We have additionally collaborated with Drs. Karen Hirschi and Arya Mani on joint review publications this year.


differentiation after mTORC1 inhibition. *Science Signaling*, 2015 May 12; 8(376), ra44. (denotes equal contribution).


1. Concise description of research program(s): Understanding of the fundamental molecular mechanisms for inflammation and vascular remodeling may lead to improved therapeutic strategies for treatment of vascular diseases. The goal in my lab is to dissect the signaling pathways in vasculature involved in vascular inflammation and remodeling related to human diseases such as atherosclerosis, stroke, graft transplant rejection and tumor metastasis.

2. Specific accomplishments in the last year:

A). Characterization of Trx2 function in heart: Thioredoxin 2 (Trx2) is a key mitochondrial protein which regulates cellular redox and survival by suppressing mitochondrial ROS generation and by inhibiting apoptosis stress kinase-1 (ASK1)-dependent apoptotic signaling. We found that Trx2 protein expression levels were reduced in hearts from patients with dilated cardiomyopathy (DCM), with a concomitant increase in increased ASK1 phosphorylation/activity. Trx2-cKO mice develop spontaneous DCM at 1 month of age with increased heart size, reduced ventricular wall thickness, and a progressive decline in left ventricular (LV) contractile function, resulting in mortality due to heart failure by ~4 months of age. The progressive decline in cardiac function observed in Trx2-cKO mice was accompanied by disruption of mitochondrial ultrastructure, mitochondrial membrane depolarization, increased mitochondrial ROS generation and reduced ATP production, correlating with increased ASK1 signaling and increased cardiomyocyte apoptosis. Moreover, chronic administration of a highly selective ASK1 inhibitor improved cardiac phenotype and reduced maladaptive LV remodeling with significant reductions in oxidative stress, apoptosis, fibrosis and cardiac failure. Our data support an essential role for mitochondrial Trx2 in preserving cardiac function by suppressing mitochondrial ROS generation and ASK1-dependent apoptosis. Inhibition of ASK1 represents a promising therapeutic strategy for the treatment of dilated cardiomyopathy and other cardiovascular diseases such as GA (Publication #3).

B). AIP1 in tumor angiogenesis: Studies from tumor cells suggest that tumor suppressor AIP1 inhibits epithelial-mesenchymal transition (EMT). However, the role of AIP1 in the tumor microenvironment has not been examined. We show that a global or vascular endothelial cell (EC)-specific deletion of the AIP1 gene in mice augments tumor growth and metastasis in melanoma and breast cancer models. AIP1-deficient vascular environment not only enhances tumor neovascularization and increases pre-metastatic niche formation, but also secretes tumor EMT-promoting factors. These effects from AIP1 loss are associated with increased VEGFR2 signaling in the vascular EC and could be abrogated by systemic administration of VEGFR2 kinase inhibitors. Mechanistically, AIP1 blocks VEGFR2-dependent signaling by directly binding to the phosphotyrosine residues within the activation loop of VEGFR2. Our data reveal that AIP1, by inhibiting VEGFR2-dependent signaling in tumor niche, suppresses tumor EMT switch, tumor angiogenesis and tumor pre-metastatic niche formation to limit tumor growth and metastasis (Publication #6).

C) New lab members: Drs. Qunhua Huang and Yeqi Wang have left. Visiting students Shu Tan, Wen Nie, Zongren Wang, visiting scientists Dr. Qiuling Xiang and Jiao Liu have joined the lab.
3. Significance of Key Findings Relevant for the Mission of VBT:
These studies provide insights into the mechanisms of vascular remodeling and tissue repair, providing potential new therapeutic targets for the treatment of vascular diseases.


1. **Overall Goal(s) of the Research Program of the Laboratory:**

Cardiovascular regenerative medicine has taken many avenues over the past three decades. One approach currently in clinical trials does not require any cells from the patient, and is an engineered tissue that is available "off-the-shelf".

Studies in vascular tissue mechanics showed several decades ago that the bulk of the mechanical properties of arteries derived not from the cellular components, but from the collagen- and elastin-based extracellular matrix. Using this principle, we have utilized banked human vascular smooth muscle cells to engineer implantable arteries. Our approach to vascular engineering involves seeding allogeneic vascular cells onto a degradable substrate to culture vascular tissues in a biomimetic bioreactor. After a period of 8-10 weeks, engineered tissues are then decellularized to produce an engineered extracellular matrix-based graft. The advantage of using allogeneic cells for graft production is that no biopsy need be harvested from the patient, and no patient-specific culture time is required. The acellular grafts can be stored for 6 months and are available at time of patient need. These grafts are being tested in 3, Phase I clinical trials in Europe and in the US. These tissue engineered vascular grafts have been tested most extensively as hemodialysis access in patients who are not candidates for autogenous arteriovenous fistula creation, with the first patient being implanted in December 2012 in Poland. Since that time, a total of 60 patients have been implanted with engineered, acellular grafts for dialysis access, 40 patients in Europe and 20 in the US. Patients utilize the grafts for dialysis access as soon as 4-8 weeks after graft implantation. This early experience supports the potential utility of this novel tissue engineered vascular graft to provide vascular access for hemodialysis.

The decellularization approach has also allowed us to generate scaffolds to support whole lung regeneration. Using rat, porcine and human sources of organs, lungs have been subjected to a range of decellularization procedures, with the goal of removing a maximal amount of cellular material while retaining matrix constituents. Next-generation proteomics approaches have shown that gentle decellularization protocols result in near-native retention of key matrix molecules involved in cell adhesion, including proteoglycans and glycoproteins. Repopulation of the acellular lung matrix with mixed populations of neonatal lung epithelial cells results in regio-specific epithelial seeding in correct anatomic locations. Survival and differentiation of lung epithelium is enhanced by culture in a biomimetic bioreactor that is designed to mimic some aspects of the fetal lung environment, including vascular perfusion and liquid ventilation. Current challenges involve the production of a uniformly recellularized scaffold within the vasculature, in order to shield blood elements from the collagenous matrix which can stimulate clot formation. In addition, we have developed methods to quantify barrier function of acellular and repopulated matrix, in order to predict functional gas exchange in vivo.

2. **Specific Research Accomplishments in the last 12 months:**

In the past twelve months, we have made encouraging progress in a number of areas. In the area of vascular engineering, we have refined a covalent modification of our engineered collagen-based grafts that retards platelet adhesion and activation, as a means of enhancing biocompatibility at small diameters. Ongoing rat aortic implantations show some evidence of resistance to thrombosis in vivo, as well as a potential beneficial effect on pannus ingrowth and anastomotic intimal hyperplasia.
In the area of lung regeneration, we have made significant technological strides in repopulation of decellularized lung vasculature with lung microvascular endothelium. Various markers of cell density, barrier function, and anti-thrombotic properties are similar to those of native lung. Furthermore, ongoing rat lung transplants of engineered constructs show a resistance to thrombosis, which had been a limiting factor for engineered lung function in prior years. One focus now is on the integrity of the alveolar basement membrane in engineered lung constructs, which is affected by protease digestion from both endothelial and epithelial cell types.

In the area of aging research, we continue to test several gene targets in C. elegans and in human cell culture. Our results indicate that over-expression of C. elegans genes BMK-1 and PCH-2 both confer an increase in lifespan, using separate pathways. These findings for PCH-2 were confirmed in human cell culture, and we are in the process of generating over-expressing mouse lines for the murine homologs of both of these gene products to determine the impacts on healthspan and lifespan in a mammalian system.

3. Significance of Key Findings Relevant for the Mission of VBT:
Progress in the area of covalent graft coatings may produce a graft that is functional at relatively low blood flow rates and small diameters typical of distal peripheral and coronary arterial grafting. Niklason just received a fundable score on an R01 application on which Alan Dardik is the co-Investigator. These novel coatings will be tested in porcine models of arterial grafting, which is a rigorous testbed for thrombosis since platelet activity in this species is quite robust.

In addition, our group is working on the development of completely gel-based, implantable microvascular systems that are composed of biological hydrogels with vascular endothelial cells lining pre-specified channels. Endothelium is either differentiated, or is derived from human iPS cells. Such gel-based microvascular constructs, if refined suitably, will be ideal for perfusing parenchymal cells having high metabolic demands such as pancreatic islets and hepatocytes. This, in turn, may be a useful experimental platform for in vivo cell delivery.


Jordan S. Pober, MD, PhD
Bayer Professor of Translational Medicine; Prof. and Vice-Chair, Dept. of Immunobiology

1. Overall Goal(s) of the Research Program of the Laboratory: Our laboratory studies interactions between the human immune and vascular systems. Specific areas of research are elucidating mechanisms by which vascular cells can promote inflammation and adaptive immune responses, such as those that occur in transplanted organs or in autoimmune diseases; mechanisms by which the immune system can cause large vessel pathologies such as allograft vasculopathy; mechanisms by which the immune system can cause microvessel pathologies such as capillary leak; and applications of vascular biology to improve tissue engineering, including approaches to avoid immune recognition of allogeneic endothelial cells

2. Specific Research Accomplishments in the last 12 months: During the last 12 months we further analyzed the effects of antigen presentation by endothelial cells on the recruitment and activation of effector memory T cells; we elucidated a novel mechanism by which complement can activate inflammatory and immune functions in vascular cells involving internalization of immune complexes; we characterized how TNF and other inflammatory cytokines disrupt capillary endothelial cell tight junctions, a process that distinguishes pathological capillary leak from functional venular leak; and we developed a method for applying CRISPR/Cas 9 genome editing to human endothelial cells as a first step in the process of making endothelial cells less immunogenic.

3. Significance of Key Findings Relevant for the Mission of VBT: VBT was founded to apply the insights of vascular biology to develop new diagnostic and therapeutic approaches to human diseases. Our findings are relevant for improving treatments in transplant, sepsis and tissue engineering.

1. **Overall Goal(s) of the Research Program of the Laboratory:**
   Our research laboratory is interested in illuminating the underlying basic science and translational opportunities relevant to heart development and dysfunction. Our efforts center on a novel population of cardiovascular progenitor cells that are able to produce the majority of cell types comprising mouse heart and blood vessel tissues, using murine embryos and murine embryonic stem (ES) cells as experimental systems. Additionally, we are using these cardiovascular progenitor cells to generate 3D engineered cardiac tissues in order to repair the injured heart in mouse and rat models.

   Another focus of our lab is the use of induced pluripotent stem (iPS) cells to develop novel experimental models of human genetic diseases for the purpose of elucidating causative mechanisms and identifying potential therapeutic interventions to treat those diseases. Through a close collaboration with several clinicians at Yale, we are able to obtain cells from a variety of tissues procured from patients with cardiovascular diseases. These cells include dermal fibroblast cells derived from skin punch biopsies, which are isolated and reprogrammed into iPS cells in our laboratory before being re-differentiated into mature cardiovascular cells. In this way, we have the ability to derive an unlimited amount of cardiovascular cells containing disease-causing genetic errors for use in our investigations into the specifics of cardiovascular disease mechanisms. In addition to identification of the mechanisms responsible for disease phenotypes, we also have interest and experience in producing candidate molecular intervention strategies using small molecule screening and homologous recombination-mediated gene correction. Using a multidisciplinary approach to the study of cardiac development, cardiac physiology, stem cell biology and small molecule screening, we hope to contribute to the understanding of cardiovascular disease mechanisms as well as the development of novel therapeutic interventions for these diseases.

2. **Specific Research Accomplishments in the last 12 months: (Publications July 1, 2014 – June 30, 2015)**

   We are reprogramming skin or blood cells from patients with cardiovascular diseases into pluripotent stem cells and then redifferentiate these stem cells into mature cardiovascular cells. In this way, we will be able to derive unlimited amount of cardiovascular cells with disease-causing mutations and study cardiovascular disease mechanisms. Specifically, we discovered a FDA-approved chemotherapeutic agent, vinblastine, which induced formation of actin filament bundles and inhibited hyperproliferation of smooth muscle cells (SMCs) derived from SVAS iPSCs. We will obtain mechanistic insight into how elastin and vinblastine inhibit the hyperproliferation of SVAS and WBS iPSC-SMCs and to screen for additional small molecules that ameliorate the hyperproliferative defect in SVAS and WBS and other vascular proliferative diseases.

   Heart failure caused by myocardial infarction remains a leading cause of morbidity and mortality in the developed world. We have established scaffold-free engineered heart tissues (EHT) with CPC and examine their contribution to heart repair and regeneration in animal models. Our studies have provided the first evidence that ESC-derived ISL1+ CPCs can effectively form new cardiac muscle in vitro and in vivo and improve heart function after implantation. ISL1+ CPCs will provide an abundant renewable cell source for basic research and will set the stage for using them for cell-based therapies for heart failure.

   Vascular disease due to arterial stenosis is the largest cause of mortality in the developed world. We have recently derived unlimited amounts of highly homogeneous functional vascular SMCs from hiPSCs (hiPSC-SMCs) and will investigate the therapeutic potential of hiPSC- and hESC-SMCs by...
developing TEBVs using biodegradable polyglycolic acid (PGA) scaffolds in a pulsatile bioreactor system and implanting them as aortic interposition grafts in nude rats, in collaboration with Dr. Laura Niklason. Development of TEBVs with significantly improved compliance using hiPSC-SMCs will enable us to take the next step towards developing autologous tissue engineered grafts for clinical intervention in vascular diseases.

3. Significance of Key Findings Relevant for the Mission of VBT
   - Generation of cardiovascular disease models using induced pluripotent stem (iPS) cells derived from patient skin fibroblast cells and screening of small molecules that can rescue disease phenotypes. Diseases currently studied: Supravalvular aortic stenosis (SVAS), Williams syndrome (WBS), Down’s syndrome and hypertrophic cardiomyopathy.
   - Derivation of cardiovascular cells from human embryonic stem (ES) and iPS cells, establishment of engineered heart tissues and blood vessels using these cells, and examination of their contribution to cardiovascular repair.

1. Overall Goal(s) of the Research Program of the Laboratory:
My laboratory investigates mechanisms of lung injury and cytoprotection during oxidant stress. Specifically, we have focused on the lung endothelium as a central mediator of lung injury and repair responses. We identified the importance of the stress-response protein heme oxygenase-1 (HO-1) and its gaseous reaction product, carbon monoxide (CO), in resisting oxidant-induced endothelial cell death via mitochondrial pathways. We found that a family of signaling molecules, mitogen-activated protein kinases (MAPKs), mediates HO-1's and CO’s protective effects as well as optimal IL-13-induced lung inflammation / remodeling and, more recently, critical innate immune responses. The innate immune system consists of pattern-recognition receptors called toll-like receptors (TLRs), of which TLR4 is the LPS-responsive receptor. We discovered that TLR4 is required for lung structural cell survival in aging and oxidant challenges. These studies represent important paradigm shifts in our understanding of TLRs and lung biology and are now the basis of translational studies in people with acute lung injury and age-related chronic lung disease, such as chronic obstructive pulmonary disease (COPD). In the process of our investigations, we were the first to demonstrate the utility of intranasal, lung-targeted and endothelial-targeted silencing RNA (siRNA) constructs in vivo. In parallel, we have also generated endothelial-targeted transgenic and knockout mouse models to specifically interrogate the role of the endothelium in lung disease. Our coordinated use of siRNA technology and genetic approaches in both cell and mouse models offer immense insight into disease pathogenesis and may identify novel therapeutic targets for a range of lung diseases.

2. Specific Research Accomplishments in the last 12 months:
Her key accomplishments for the year include creating lung-endothelial targeting vectors that can be used in vivo, showing for the first that mitochondrial turnover and fission are critical determinants of COPD and pulmonary vascular remodeling and identifying a novel innate immune pathway, MIF-CD64, in the pathogenesis of vascular injury. She also assembled a group of multi-disciplinary investigators to study COPD in the context of immunologic aging. The investigators are from Yale Pathology, Comparative Medicine, Program on Aging, Rheumatology and Infectious Diseases. Their collaborations in the past year alone have resulted in a submitted P01, “Immune Mechanisms in the Aging Lung,” an In Press monograph, “Aging Lung: Mechanisms and Clinical Sequela” and an In Press chapter in the seminal gerontology text - Hazzard’s textbook of Gerontology, 7th Edition.

3. Significance of Key Findings Relevant for the Mission of VBT:
Dr. Lee’s work aligns well with the mission of VBT by introducing new vascular biology reagents, specifically in lung vasculature, and forming multi-disciplinary research groups to study novel aspects of lung biology.

4. Publications: (Publications July 1, 2014–current)


Martin Schwartz, Ph.D.
Robert W. Berliner Professor of Medicine (Cardiology) and Professor of Biomedical Engineering

1. Overall Goal(s) of the Research Program of the Laboratory:
Our laboratory studies signaling by integrins and mechanotransduction in the vascular system. We are especially interested in how endothelial cells respond to forces from flowing blood. The goals are to understand basic mechanisms of signal transduction, and to apply this information to both atherosclerosis and flow-dependent artery remodeling.

2. Specific Research Accomplishments in the last 12 months:
A major accomplishment in the lab is the elucidation of an important connection between extracellular matrix remodeling and inflammation. This pathway involves the matrix protein fibronectin, which binds integrin α5β1, whose cytoplasmic domain binds and regulates a phosphodiesterase that suppresses the anti-inflammatory cAMP/protein kinase A pathway. In collaboration with Jay Humphrey’s lab, we have proposed a new model for aneurysm formation based on defects in mechanotransduction. In collaboration with Michael Simon’s lab, we have identified a role for syndecan 4 in endothelial sensing of flow direction and demonstrated the importance of cell alignment in inhibition of atherosclerosis.

3. Significance of Key Findings Relevant for the Mission of VBT:
These results advance our understanding of basic mechanisms by which endothelial cells respond to fluid shear stress and the downstream pathways by which flow triggers vascular remodeling and atherosclerosis.


1. Overall Goal(s) of the Research Program of the Laboratory:
Our laboratory is very interested in endothelial cell biology, signaling and regulation of post-natal angiogenesis/arteriogenesis and atherosclerosis.

2. Specific accomplishments in the last year: In the past year, we have made successful inroads into several areas of nitric oxide signaling, caveolin function and miRNAs.

3. Publications:


Goodwin JE, Feng Y, Velazquez H, Zhou H, Sessa WC. Loss of the endothelial glucocorticoid receptor prevents the therapeutic protection afforded by dexamethasone after

1. Overall Goals of the Research Program of the Laboratory
Our laboratory is interested in regulation of arterial and lymphatic morphogenesis and angiogenic growth factor signaling. These processes are investigated at all levels, including in vitro signaling studies, in vivo mouse transgenic and knock-out models and translational studies in larger animal models and early phase clinical trials.

2. Specific Research Accomplishments in the last 12 months
We have made significant advances in unraveling the role of VEGF and FGF signaling in the normal endothelium, novel mechanism of endothelial-to-mesenchyma transition and key molecular pathways regulating arteriogenesis and lymphangiogenesis.

3. Significance of Key Findings Relevant for the Mission of VBT
These findings advance our knowledge of molecular details of regulation of vascular development and signaling and should eventually enable the development of new therapeutic paradigms.

4. Key Publications


Simons M and Eichmann A. Molecular controls of arterial morphogenesis. Circ Res 2015; 116:1712-24; d.o.i. 10.1161/CIRCRESEARCHAHA.116.302953


Hegan PS, Lanahan AA, Simons M, Mooseker MS: Myosin VI and cardiomyopathy: Left ventricular hypertrophy, fibrosis, and both cardiac and pulmonary vascular endothelial cell defects in the Snell’s waltzer mouse. Cytoskeleton. 2015 Aug 12; 2015

Edward L. Snyder, M.D.
Professor Laboratory Medicine, Director, Apheresis/Cell Processing VBT Core Facility (Core D)

1. Overall Goal(s) of the Research Program of the Laboratory:
The Apheresis/Cell Processing Core Facility played a critical role in the Vascular Biology and Transplantation Program. The Cell Processing Core Laboratory is designed to support the needs of the VBT Program users who are performing basic science and clinical research involving mononuclear and other cell types, by providing five specific functions. First, the Apheresis section of the Cell Processing Core Laboratory procured and provided normal donor specimens in support of research projects. These samples, obtained under IRB approved protocols from fresh specimens, were made available to VBT membership. Second the VBT Core D Cell Collection and Processing Laboratory can provide, as requested, cell purification services. Third, the Cell Processing Core can provide large-scale processing capabilities in support of specific research studies involving human MNCs as well as CD34 positive and other cell types. Included within this section was the development of cell selection and culturing techniques to support the novel cell therapy protocols, as well as the pre-clinical validation of research procedures. The VBT Core resource provided the critical instrumentation and technical expertise in cell processing and cryopreservation, needed for the in vitro use of cells, or infusion of cells into animals. Fourth, the Core provided collections of MNC and could as, and if, needed, provide CD34+ cells from G-CSF stimulated donors. Fifth, the Apheresis/ Cell Processing VBT Core Facility maintained compliance with institutional, NIH, FDA, and AABB guidelines, and ensured that the protocols were safely and effectively applied. This objective includes training of new investigators in Compliance and Quality Control issues. Thus, this resource provided access to cell collection, selection, processing and culturing technologies, as well as services and scientific consultation to enhance the productivity of the VBT members. This technically sophisticated resource was critical to the VBT Section’s research progress.

2. Specific Accomplishments in the last 12 months:
In 2014 - 2015, Core D performed 23 MNC apheresis collections for VBT Program Leaders’ research.

3. Publications:
   Majhail NS, Snyder EL, Levine J. Significant improvement in survival after unrelated donor hematopoietic cell transplantation in the recent era. Blood and Bone Marrow Transplantation 2015:21;142-50.
Yajaira Suárez, Ph.D.
Assistant Professor of Comparative Medicine and Pathology.

1. **Overall Goal(s) of the Research Program of the Laboratory:** The Suárez laboratory studies the contribution of non-coding RNAs, including microRNAs, to the regulation of endothelial cell and macrophage functions. Both cell types play major role in controlling both angiogenic and inflammatory responses and the interplay between these two cell types has been shown to be critical for several pathophysiological conditions like atherosclerosis, cancer (tumor growth), adipose tissue expansion and wound healing, among others.

2. **Specific Research Accomplishments in the last 12 months:** Several lines of evidence indicate that the regulation of microRNA levels by different stimuli may contribute to the regulation of stimulus-induced responses. The microRNA-17–92 cluster has been linked to tumour development and angiogenesis, but its function in endothelial angiogenic functions is unclear and the mechanisms that control its expression in response to angiogenic cues are unexplored. Our work provide compelling evidence that Elk1 activation is responsible for VEGF-induced transcription of the miR-17–92 cluster in endothelial cells. This stimulation is in turn necessary for endothelial cell proliferation and angiogenic sprouting and contribute to the endothelial angiogenic switch. Additionally, our present findings provide the first genetic evidence that endothelial miR-17–92 cluster is necessary for physiological retinal angiogenesis during development and for tumour induced angiogenesis. These findings provide fundamental insights into the molecular mechanisms by which angiogenic factors regulate the expression of microRNAs.

3. **Significance of Key Findings Relevant for the Mission of VBT:** Our research contribute to the understanding of the complex network involving miRNAs and their targets, leading to a coordinate pattern of gene expression that regulates the cellular and molecular mechanisms that control endothelial and macrophage functions. Our research can form the basis of new therapies for inflammation and angiogenesis.


George Tellides, M.D., Ph.D.
Professor of Surgery (Section of Cardiac Surgery) and of Investigative Medicine; Chief of Cardiothoracic Surgery, Veterans Affairs Medical Center

1. Overall Goal(s) of the Research Program of the Laboratory:
Vascular biology; Immunology; Transplantation

2. Specific Research Accomplishments in the last 12 months:
Described the effects of chronic graft rejection on host vascular disease.

3. Significance of Key Findings Relevant for the Mission of VBT:
Collaborations with Pober lab on mechanisms of graft arteriosclerosis, with Simons lab on mechanisms of endothelial to mesenchymal transformation, and with Humphrey/Schwartz labs on the role of mechanosensing in aortic disease.


1. Overall Goal(s) of the Research Program of the Laboratory:
The overall objective of our research activities is to understand the mechanisms and functions of signal transduction activated by chemoattractants and Wnts.

2. Specific Research Accomplishments in the last 12 months:
The key research accomplishment includes: 1) We solved the crystal structure of PIP5K1 and elucidate molecular mechanisms for its regulation by dimerization and by its interaction with Wnt signaling molecule dishevelled. 2) We identified FAM65 as being a novel RHOA regulator and characterize its important role in neutrophil polarization. 3) We characterized a previously unknown role of a ubiquitin-like protein Ubl4A in regulation of actin cytoskeleton dynamic and AKT activation in collaboration with Dr. Xiang’s lab. 4) We elucidated a role of SRF in neutrophil migration in collaboration with Stephanie Halene’s lab. 5) We characterized a mechanism for HGF to crosstalk with Wnt signalling in collaboration with Lloyd Cantley’s lab.


Lawrence H. Young, M.D.
Professor of Medicine (Cardiology) and of Cellular and Molecular Physiology; Vice-Chairman Department of Medicine

1. Overall Goal(s) of the Research Program of the Laboratory:
Key areas of our research are the molecular & cellular mechanisms regulating heart (metabolism in cv disease), autocrine-paracrine pathways (modulating cardiac cell signaling and function), and metabolic signaling pathways in atrial fibrillation. Processes under investigation include: regulation of heart metabolism/function; AMPK protection against solid organ ischemia; JNK pathway in reperfusion injury; cardiac autocrine/paracrine factors; MIF, DDT, CD74 signaling; novel myocyte autocrine proteins; and LKB1 role in cardiac growth and remodeling.

2. Specific Research Accomplishments in the last 12 months: Our work has defined a novel autocrine-paracrine pathway in the heart that is critical to the myocardial response to ischemia-reperfusion. D-dopachrome tautomerase is expressed in cardiac myocytes and the coronary vasculature, is secreted during ischemia and activates metabolic signaling in the heart. We have also shown that the LKB1 pathway is essential for early post-natal electrophysiological development, specifically regulating connexin and sodium channel expression and function. Finally, in collaboration with Dr. Tirziu we have elucidated the paracrine effects of endothelium derived nitric oxide in regulating miR182 that modulates myocardial hypertrophy during angiogenesis. This may represent an important mechanism in the development of physiological hypertrophy.

3. Significance of Key Findings Relevant for the Mission of VBT:
Our work continues to advance the understanding of the interaction between the cross-talk between the vasculature and myocardium.

   Qi D, Young LH. AMPK: energy sensor and survival mechanism in the ischemic heart. Trends in Endocrinology and Metabolism. 2015;26:422-429
APPENDIX 1

The Fourteenth Annual VBT Retreat
Schedule of Talks

8:00 - 8:30  
Registration  
Continental Breakfast

8:30 - 10:00  
SESSION I  
Jordan Pober, MD, Ph.D.

8:30 - 9:00  
Daniel R. Goldstein, MD  
"Novel pathway of age enhanced atherosclerosis"

9:00 - 9:30  
Dianqing (Dan) Wu, Ph.D.  
"From neutrophils to Cerebral Cavernous Malformation disease"

9:30 - 10:00  
Arndt Siekmann, Ph.D.  
Max Planck Institute for Molecular Biomedicine, Münster, Germany  
"Illuminating blood vessel formation in the zebrafish"

10:00 - 10:20  
Coffee Break

10:20 – 11:50  
SESSION II  
Session Chair – Mike Simons, MD

10:20 - 10:50  
Anne Eichmann, Ph.D.  
“Guidance of vascular patterning”

10:50 – 11:20  
William C. Sessa, Ph.D.  
“Exploring mechanisms of lipid uptake in endothelium”

11:20 – 11:50  
Alan Dardik, MD, Ph.D.  
“Venous adaptation to the arterial environment”

11:50 – 1:30  
Lunch and Poster Session

1:30 – 3:00  
SESSION III  
Session Chair – Jeffrey Bender, MD

1:30 – 2:00  
Laura Niklason, MD, Ph.D.  
“Clinical Outcomes with Engineered Arteries”
2:00 – 2:30  Yibing Qyang, Ph.D.
“Vascular disease modeling and tissue engineering using induced pluripotent stem cells”

2:30 – 3:00  Themis Kyriakides, Ph.D.
“Extracellular matrix and cell-biomaterial interactions in vascular applications”

3:00 – 3:20  Coffee Break

3:20 – 3:30  Introduction of Keynote Speaker
William C. Sessa, Ph.D.

3:30 – 4:30  Keynote Address
M. Luisa Iruela-Arispe, Ph.D.
JCCC Cancer Center California, Los Angeles
“Developmental Origins of Vascular Disease”

4:30 – 4:45  Announcement of Poster Contest Winners
William C. Sessa, Ph.D.
APPENDIX 2

The 13th Annual Yale – Cambridge Collaboration Meeting
This is a private meeting, for which all parties have signed a non-disclosure agreement.
This is a private meeting, for which all parties have signed a non disclosure agreement.
Monday 15 September 2014

08.00.  Breakfast                        Dining Hall

Session 2  Chair - Jordan Pober         Metabolism / Immunology

09.00.  Sir Stephen O’Rahilly           Obesity and insulin resistance: lessons from genetics
09.20.  Anton Bennett                    Signaling mechanisms by PTPN11/Shp2 in Noonan
09.40.  Frank Waldron Lynch             Dissection of the immune response to IL-2 in the
                                             Adaptive study of IL-2 dose on regulatory T cells in
                                             type 1 diabetes (DILT1D)
10.00.  Kevan Herold                     Beta cell death and dysfunction in subjects at risk
                                             for Type 1 diabetes

10.20.  Break                           

10.50.  Paul Lehner                      Genetic screens in human haploid cells identify
                                             novel retroviral silencing complexes
11.10.  Philip Askenase                  Two pathways for T cell regulation by extracellular
                                             miRNA - via exosomes and via chaperones
11.30.  Arthur Kaser                     IRE1 hyperactivation in Paneth cells as initiator of
                                             Crohn’s-like disease
11.50.  Martin Krieger                   The gut microbiota in systemic autoimmunity
12.10.  David Thomas                     A novel gene essential for host defence against
                                             salmonella typhimurium

12.30.  Lunch                           Dining Hall

Session 3  Chair - Patrick Maxwell       Immunology / Blood

14.00.  Mattia Frontini                 Blueprint - deciphering the epigenome of blood cells
14.20.  Diane Krause                    Megakaryocyte erythroid progenitors - a unique
                                             definable population
14.40.  Ana Cvejic                      Functional genomic screen of identified GWAS hits
                                             towards understanding haematopoiesis
15.00.  David Hafler                    From genetics to function
15.20.  Eoin McKinney                   Immunological exhaustion and autoimmunity
15.40.  Su Metcalfe                     Harnessing the therapeutic potential of LIF for
                                             remyelination of demyelinated axons

16.00.  Break                           

16.30.  Medimmune                       Round table discussion

19.00.  Reception                       Fellows Garden
19.30.  Dinner                          Dining Hall

This is a private meeting, for which all parties have signed a non disclosure agreement.
**Tuesday 16 September 2014**

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<td>Breakfast</td>
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<tr>
<td>09.00</td>
<td>Ziad Mallat - MHCII-restricted Ag presentation by pDCs drive pro-atherogenic immunity</td>
<td>Graham Storey Room</td>
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<td>09.20</td>
<td>Daniel Goldstein - Role of ageing on atherosclerosis</td>
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<td>09.40</td>
<td>Sanjay Sinha - Myocardin in vascular disease</td>
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<td>David Calderwood - Structural determinants of a CCM3:CCM2 interaction that stabilizes protein expression and permits endothelial network formation</td>
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<td>10.50</td>
<td>Mark Ormiston - Pulmonary hypertension in mouse models of Natural Killer cell deficiency</td>
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<td>11.30</td>
<td>Amer Rana - Development of induced pluripotent stem cell models of PAH for disease modelling and therapeutic screening</td>
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<td>11.50</td>
<td>Kaisa Maki-Petaja - Molecular imaging of vascular inflammation in rheumatoid arthritis</td>
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<td>12.10</td>
<td>John Bradley - Round up and close</td>
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<tr>
<td>12.15</td>
<td>Lunch</td>
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