Summer 2010 Volume 2, Number 1

AAB CARDIOVASCULAR RESEARCH INSTITUTE
Department of Medicine

CVRI Beat

UNIVERSITY of ROCHESTER MEDICAL CENTER
MEDICINE of THE HIGHEST ORDER

Upcoming Scientific Events:

American Heart Association Scientific Sessions in Chicago, IL on November 13-17, 2010

Director's Column

Our three year anniversary at the Aab CVRI is fast approaching! The Cardiovascular Research Institute continues to expand, with new faculty, new staff, and new ideas.

Watch for the Aab CVRI Scientific Advisory Board meeting here on September 24, 2010. Our distinguished advisors include: Dr. Eric Olson from the University of Texas Southwestern, Dr. Edward Fisher from the New York University School of Medicine, Dr. Jose "Pepe" Jalife from the University of Michigan Medical Center, Dr. Aldon"Jake" Lusis from the University of California at Los Angeles Medical Center, and Dr. Alain Tedgui from Institut National de la Santé et de la Recherche Médicale in France. Dr. Olson will present the keynote address for our Advisory Board meeting with a speech entitled: "Molecular Pathways of Muscle Development and Disease."

The Aab CVRI Scientific Advisory Board will advise us on our research projects and future directions. They will also discuss our plans for recruiting into areas including platelets and thrombosis, heart failure, and vascular inflammation. We have heard talks from invited speakers including Dr. Junichi Sadoshima, Dr. Akiko Hata, Dr. Aarif Khakoo, Dr. Wolfgang Bergmeier, and Dr. Dennis Bruemmer in the past few months during our Wednesday Seminar Series.

The Howard Hughes Medical Institute (HHMI) graduate student program will begin this fall. The Med-into-Grad program encourages graduate students to consider research into medically significant areas. We will begin this year with four outstanding graduate students, who will take courses, rotate through labs herein within the Aab CVRI, and visit cardiology clinics at the medical center. This fall will see the construction of new facilities inside our vivarium. The shelled space to house more cages and a new procedure room will help us to increase the number of mice we can house.

We are planning a picnic in the early fall to welcome all CVRI staff, students, and faculty after the long, hot summer. More details to follow!
-- Charlie Lowenstein, MD

Technical Corner

By: Dr. Keigi Fujiwara
Director, Imaging Core

Many cultures have a proverb that means, "Seeing is believing." In science, this is also true. Microscopes were invented to see what our naked eyes couldn't see, but how small a thing can we see using a light microscope? The limit of resolution depends on the wavelength of visible light, and for non-luminous specimens, the theoretical limit of resolution is about 0.1 μm. As long as we use visible light, structural details smaller than this cannot be resolved, and this resolving power was achieved several decades ago. For this reason, many biomedical researchers felt that light microscopes, as a research tool, had reached their maximum level of usefulness. Then in the late 1970's, we saw rebirth of light microscopy based on fluorescence. Unlike non-luminous specimens, fluorescent specimens emit light. This allowed us to detect biological structures that are less than 0.1 μm in size, molecular interactions, and even a single molecule. The key word here is "detect." Fluorescent specimens emit light against a dark background. Similar to observing (detecting) stars in the night sky (although their shapes cannot be resolved),

fluorescent objects below the limit of resolution can be observed (detected) by fluorescence microscopy. Combined with techniques of digital imaging and image processing, fluorescence microscopy is a powerful tool for today's biomedical research. In fact, certain specialized methods of fluorescence microscopy defied the limit of resolution of a light microscope and can now resolve distances down to several nanometers.

The Aab CVRI Imaging Core houses state-of-the-art fluorescence microscopes. The most heavily used is the standard type of epi-fluorescence microscope (Olympus BX51) with a CCD color camera, which provides basic information regarding subcellular localization of various proteins in fixed cells and tissues. Because images seen in an ordinary microscope are a mixture of in-focus and out-of-focus images, the latter reduces the contrast (or sharpness) of the in-focus image. To remove out-of-focus images from fluorescence images, a laser scanning confocal microscope was invented, which uses a small pin hole to remove light from out-of-focal areas. Our confocal unit (Olympus FV300) is mounted on an inverted microscope (Olympus IX70) and is equipped with Argon, Krypton, and He/Ne lasers for excitation at 488 nm, 568 nm, and 633 nm, respectively, for capturing green, red, and far-red fluorescent images. The scope's software is capable of 3D image reconstruction and viewing, pixel analysis for co-localization, fluorescence intensity measurement, and image processing.

To image live cells, the Imaging Core has a microscope with a stage climate chamber which controls temperature (room temp-37°C), humidity (normally up to 60%), and CO_2 (0-10%) of the enclosed space around the entire stage, objective lenses, and condenser of an Olympus IX81 inverted microscope. Cultured mammalian cells may be maintained on the scope stage for an extended period of time (>48 hours). The microscope is motorized so that focusing and stage motion is controlled and programmed by a computer. It is equipped with a Hamamatsu CCD camera and light sources including Halogen, Xenon, and Argon laser and is capable of recording the fluorescence resonance energy transfer (FRET), total internal reflection fluorescence (TIRF), epifluorescence, and DIC/phase contrast/bright field images. The entire scope, including stage movement, lens selection, filter selection, and selection of light sources, is operated by the SlideBook software which also has deconvolution (i.e. a computational version of confocal imaging) capability. Live cells expressing fluorescent proteins can be studied by this microscope, generating excitingly new image information on cells that make up the cardiovascular system.

Recently, Dr. Craig Morrell's Lab has added intravital microscopy capability. This includes an inverted Nikon Eclipse Ti-S fluorescent microscope with a digital imaging camera and NIS-Elements software for data analysis. This allows for the high-speed acquisition of live fluorescent in vivo images and software to analyze and quantify your data. It is commonly used to image leukocyte-endothelial interactions or thrombosis in real-time, but other creative uses are also possible. For more information on the use of this microscope, please contact Dr. Morrell.

Every day, investigators of the Aab CVRI generate precious image data, but only a very small fraction of them are used in publications and the rest are archived in researchers' computers, never to become a part of scientific knowledge. The Imaging Core is in the process of creating an image database so that images captured by each investigator may be shared with other scientists in the CVRI. By looking at what's been already done in the past, unnecessary duplications can be avoided, but more importantly, old data can be reevaluated later in the light of newly discovered facts on life and disease. One forgotten fact is that images usually contain more information than investigators have originally intended to get, and such information can be extracted from them later. An open source, web-based software called CAISIS will be used to do this job. The software is already used to create diagnostic and other database for patients in the U. of R. Medical Center. The Aab CVRI Image Database will have various image data (microscope images, video images, and even images of gels) with appropriate meta data.

For more detail, please contact Dr. Craig Morrell on intravial microscopy and Dr. Keigi Fujiwara on other topics.

"In Focus"

By: Dr. Burns Blaxall

Heart failure (HF) is a debilitating disease with poor prognosis, and our laboratory is pursuing several projects to both understand and treat HF. In HF pathogenesis, adrenaline receptors (β-ARs), which normally regulate rate and force of heart contraction, are chronically desensitized. This occurs in part due to elevated levels of an enzyme (GRK2) that is recruited to the β-ARs by the G-protein Gβγ. Prior studies have demonstrated that interfering with Gβγ signaling downstream of β-AR activation is cardioprotective in HF models. In collaboration with URMC investigator Dr. Alan V. Smrcka, we recently identified small molecule inhibitors of Gβγ signaling. Our results currently in press in Circulation Research demonstrate that systemic delivery of these compounds can disrupt pathologic molecular changes underlying HF. Further, we demonstrate that these small molecules reduce the progression of HF in two distinct mouse models. Our study provides rationale for further development of Gβγ-targeting compounds as a therapeutic approach for HF. Fortunately the NIH has recently provided new R01 funding to allow further progress toward this goal.

Comings and Goings

Welcome to our newest Aab CVRI Personnel:

Enakshi Chakrabarti (Morrell Lab)
Christine Christie (Berk Lab)
Lori Cox (Blaxall Lab)
Geun-Young Kim (Berk Lab)
Allison Pytlak (Taubman Lab)
Janice Gerloff (Berk/Korshunov Lab)
Yuichiro Takei (Abe Lab)
Daria Vorojeikina (Abe Lab)
Qian Zhou (Miano Lab)
Lindsay Marchetti (Berk Lab)
April Rice (Shared Tech)

Best Wishes and a Fond Farewell to:

We wish a very fond farewell and best of luck to those who have moved on…

Tom Spangenberg has departed the Abe Lab after being accepted into graduate school at Upstate Syracuse. Tom has decided on a career as a physical therapist.

Mike O'Dell has departed the Berk Lab to continue his technical research career as a Tech Associate in the Cancer Center.

Leisha Robert, the CVRI shared tech, took a new position at URMC as a Lab Tech III in Dr. Jim Palis's lab at The Center for Pediatric Biomedical Research

Heidi Michaloski has departed the Berk Lab after being with us for two years as she was accepted into graduate school; it is a joint degree with VT and Georgetown. Heidi has decided to study in the field of Biomedical Technology Development and Management.

Prashanthi Menon completed her PhD after five years in the Berk Lab and begins her postdoctoral fellowship in the Beth Israel Deaconess Medical Center/Harvard Medical School in Boston.

Elaine Smolock, PhD departs the Berk Lab to join her husband as he continues his surgical training in Houston. Elaine will continue her NRSA Fellowship at Methodist Research Institute in Houston.

Fabrice Jaffre, PhD completed his postdoc in the Blaxall lab and has a new position in Germany at the University of Munich as a postdoctoral fellow.

Heather Martin, Tech Associate departs the Blaxall Lab to be with her husband who was accepted into Medical School in Rochester, Minnesota. Heather accepted a new position at the Mayo Clinic.

Hakjoo Lee, PhD, Research Assistant Professor will be leaving the Abe Lab in August to join Dr. Yisang Yoon's lab in the Department of Anesthesiology.

Masaaki Imamura, MD, PhD completed his post-doc training in the Miano Lab and is currently an Assistant Professor of Urology at Kyoto University.

Recent Publications

Sahni A, et al. (Alexis JD). UAP56 is an important regulator of protein synthesis and growth in cardiomyocytes. Biochem Biophys Res Commun.. 393(1); 106-110. 2010

Menon P, et al. (Berk BC). Impaired spine formation and learning in GPCR kinase 2 interacting protein-1 (GIT1) knockout mice. Brain Research. 317; 218-226. 2010

Shi F, et al. (Sottile J). Collagen I matrix turnover is regulated by fibronectin polymerization. Am J Physiol Cell Physiol. 298(5); 1265-1275. 2010

Srivastava K, et al. (Morrell CN). Platelet Factor 4 Regulation of Monocyte KLF4 in Experimental Cerebral Malaria. PLos One. 5(5); e10413. 2010

Miano JM. Role of serum response factor in the pathogenesis of disease. Laboratory Investigation. 2010 In Press

Smolock, et al. (Berk BC). Gas6-Axl Pathway: The Role of Redox-Dependent Association of Axl with Nonmuscle Myosin IIB. Hypertension. 56(1); 105-111

Menon P, et al. (Berk BC). GPCR Kinase 2 interacting protein 1 (GIT1) regulates osteoclast function and bone mass. Journal of Cell Physiology. 2010

Miano JM. Vascular smooth muscle cell differentiation. JBR. 24(3); 169-180. 2010

Nigro P, et al. (Berk BC). PKC{zeta} decreases eNOS protein stability via inhibitory phosphorylation of ERK5. Blood. 2010

O-Uchi J, et al. (Lopes C). Ion Channel Mechanisms Related to Sudden Cardiac Death in Phenotype-Negative Long-QT Syndrome Genotype-Phenotype Correlations of the KCNQ1 (S349W) Mutation. Journal of Cardiovascular Electrophysiology. 2010

Albinsson et al (Miano, JM). miRNAs are necessary for vascular smooth muscle growth and differentiation during development. Arterioscler.Thromb.Vasc.Biol. 30:1118-26, 2010.

Imamura, M, et al. (Miano JM). Expression and functional activity of four myocardin isoforms. Gene. 2010 In Press.

Casey LM, Pistner AR, et al. (Blaxall BC). Small Molecule Disruption of GBγ Signaling Inhibits the Progression of Heart Failure. Circ Research. 107. 2010

Liu B, et al. (Taubman MB). Protein kinase Cdelta mediates MCP-1 mRNA stabilization in vascular smooth muscle cells. Mol Cell Biochem. 2010

Grants/Awards

Dr. Burns Blaxall was elected to the Editorial Board of Circulation Research.

Dr. Nhat Tu Le was awarded an AHA Founders Affiliate award entitled “Pleitropic effects of statins and metformin and high throughout screening.”

Dr. Jin O-Uchi is the recipient of a Richard J Bing Young Investigator Award.

Dr. Xiaochun Long was awarded an AHA National Affiliate for her SDG application, “Role of TGF-beta-Regulated microRNAs on SMC Differentiation.”

Dr. Zheng-Gen Jin was awarded an AHA National Founders Affiliate for his GIA application, “PKD1 in regulation of VEGF-induced vascular permeability.”

Dr. Rashmi Ram was awarded an AHA National Founders Affiliate for her application, “A role for mEna in modulation of cardiomyocyte function and gap junction remodeling.”

Dr. Asad Zeidan was awarded an AHA National for his SDG Application, “CyPA and mechanical stretch project.”

Dr. Shi Pan received funding from Department of Defense for a stem cell grant, “Redox regulation in bone marrow failure.”

HHMI
HOWARD HUGHES MEDICAL INSTITUTE

Our new Howard Hughes Medical Institute (HHMI) graduate program officially begins with the arrival of four new students later this summer. The "Med-into-Grad" program here in the Aab CVRI is in Cardiovascular Sciences. To read about our program, and those of other institutions, go to www.hhmi.org/news/medintogradsum20091117.html Please recall that new student arrivals will need to rotate in labs here at the Aab CVRI so it is imperative that Aab CVRI faculty participate in accommodating interested students who would like to rotate through a lab. We will be formally introducing our newest students at the picnic planned for early fall.

New HHMI students are:
Melissa Martin
Kristina Modjeski
Stefan Chan
Qiuyu "Martin" Zhu

Existing students choosing to pursue HHMI studies are:
Oded Spindel
Eugene Chang