



Texas A&M University

CERH Highlights

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**Research
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Dr. Robert Burghardt**

FROM THE DIRECTOR

Philip E. Mirkes, Ph.D.

Greetings to all CERH members:

2005 was a busy year for the Center and now that the Center Renewal has been submitted, I think it is appropriate to review events of the last year.

In late August, we learned that the new Director of NIEHS, David Schwartz, was drafting new guidelines for NIEHS Core Centers and that these guidelines would apply to all proposals submitted in 2006 and beyond. The major change going forward is an increased emphasis on translational bench-to-bedside research. The NIEHS also has set forth new requirements for Centers, which include the need for three ES funded grants by at least two PIs in order to be eligible to apply; a new Integrated Health Sciences Facility Core; up to 25% of budget can be allocated to Pilot Projects; increased flexibility in center structure and organization; and a COEP is now optional. Although the changes in the Core Center guidelines are significant, particularly the mandatory Integrated Health Science Facility Core, we are already well along in making the necessary changes to our current Center structure.



To respond to the NIEHS emphasis on translational research, the annual CERH retreat, held again at the Del Lago Resort, was dedicated to presentations by CERH investigators who are already engaged in translational research or whose research is "ready" for translation. I want to thank Drs. Donnelly, Phillips, Safe, Lupton, Harris, Wu and Dees for their excellent presentations, which provided important information needed to structure the new Integrated Health Science Facility Core. Also, thank you to all the CERH members who attended and provided critical input, and to Anna Kjolen and Ashley Riggs for organizing all aspects of the retreat.

Be sure to cite Center support (P30-ES09106) on all relevant publications.

In December the CERH hosted its first Holiday Party at the MSC. Although the weather was cold and rainy outside, the banquet hall was warm and the food was tasty and plentiful. For those of you who could not attend, hopefully you will be able to join us next year for this event. This is one of the few opportunities for Center members to gather socially during the year, so I invite everyone to attend this function in the future.

The annual CERH Scientific Symposium entitled “Folic Acid: From Prevention to Intervention. How a B-Vitamin can Impact Human Health” was held on December 16 at the College Station Hilton Conference Center. Speakers included Dean R. Appling, University of Texas at Austin; Laura E. Mitchell, Center for Environmental and Genetic Medicine, IBT; Robert S. Chapkin, Texas A&M University; and Philip S. Low, Purdue University. This event provides Center members the opportunity to interact with the speakers, as well as the opportunity to share their latest research results through the poster session.

Having now completed the competitive renewal for the CERH, it is clear that we have truly outstanding facility cores that provide state-of-the-art expertise and technologies to Center members. In this issue of CERH Highlights, we feature several research projects supported by the Genomics Facility Core (directed by Dr. Robert Chapkin). Through his efforts, the core is able to award free CodeLink Genome Micorarrays (compliments of GE Healthcare) and free array processing (compliments of the Genomics Core) to CERH investigators. We hope to continue these awards in coming years, so look for the call for proposals.

At the heart of the CERH is the outstanding research of Center members who are constantly developing new approaches to the study of toxicant-induced diseases. This issue of CERH Highlights also features an article by Dr. Robert Burghardt describing a “cellulomics” approach to identifying subcellular targets as well as mechanisms and chronology of toxicant-induced cellular injury. If you want to learn more about “cellulomics” and how it might facilitate your research, contact Dr. Burghardt.

Finally, I want to thank all of the Center members for their continuing support and dedication, and to congratulate all the CERH members who received awards in 2005 (see page 7).



Phil Mirkes



GENOMICS FACILITY CORE

In December 2004, the Genomics core held a CodeLink Microarray Award competition. Several labs applied for the award of 6 free CodeLink Genome microarrays compliments of GE and free array processing compliments of the Genomics core. Three awardees were selected from the excellent proposals. The awardees were the labs of Drs. Weston Porter, Yanan Tian and Alan Parrish. In November 2005, a CodeLink symposium was held at the CERH, featuring representatives of GE discussing the new features of the CodeLink platform. In addition, the three laboratories that received the complementary arrays presented an overview of their research progress.

Dr. Yanan Tian's group is interested in the cross talk between various cytokines and the regulation of drug metabolizing enzymes. As such, proinflammatory cytokines and bacterial endotoxin have long been known to suppress drug metabolism mediated by cytochrome P450 3A4 (CYP3A4). However, the mechanism for the suppression remains poorly understood. Pregnane X receptor (PXR, NR1I2) is an orphan nuclear receptor that binds to various ligands, and regulates the expression of many genes including CYP3A4. Using CodeLink™ microarray assay, we found that PXR ligand rifampicin and endotoxin co-treatment resulted in drastic induction of the NcoR2/SMRT mRNA in human primary hepatocytes. Furthermore, the endotoxin and TNF- α markedly suppressed the rifampicin-induced CYP 3A4 gene expression as determined by the code-link microarray. These findings lead to further experiments which identified NcoR2 as the interactive protein with PXR through interaction between the PXR-ligand binding domain and classic L/IXXII motif of NcoR2. Taken together, these findings provide a potential mechanism for the long standing observation that inflammatory substances, such as endotoxin, suppress drug metabolizing capacity.

Dr. Alan Parrish's group is interested in the mechanisms underlying the increased incidence and severity of acute renal failure. Using an in vitro model, it was demonstrated that aging was associated with an inherent susceptibility of renal tissue to ischemic and nephrotoxic insult; importantly, life-long caloric restriction attenuated this increased susceptibility to injury (Figure 1). In an attempt to identify molecular pathway(s) underlying this response, microarray analysis was performed using the CodeLink™ Rat Whole Genome Bioarray slides.

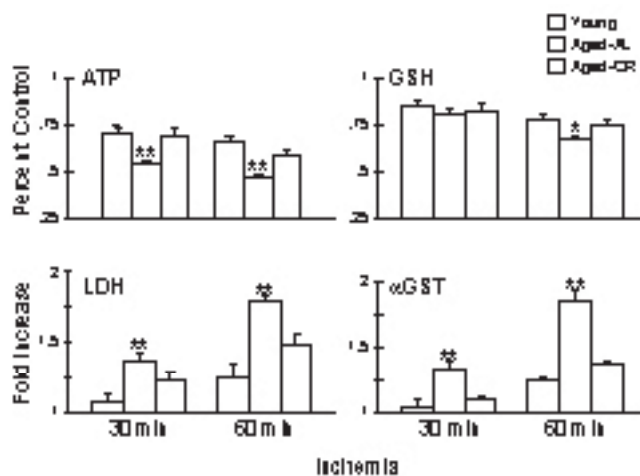


FIGURE 1: The Impact of Aging on the Susceptibility of the Kidney to Ischemic Injury. Kidney slices harvested from young (4 mon), aged-ad libitum (24 mon) or aged-caloric restriction (24 mon) were challenged with ischemia (100% N₂ for 30 or 60 min). Viability was assessed by intracellular ATP and GSH content or leakage of lactate dehydrogenase (LDH) and α -GST into the culture media. The results were compared to control slices (cultured in 95:5 O₂: CO₂) from each respective group. Each data point represents the mean \pm SD for 4 animals (4 slices per animal).

Subsequent gene expression analysis was performed using GeneSpring software. Using a 2-fold difference as a cut-off and a Welch ANOVA (Benjamini and Hochberg False Discovery Rate 0.05), 131 genes were identified whose pattern of expression suggested that caloric restriction attenuated a change induced by aging. The expression of 42 genes was reduced during aging but rescued by caloric restriction. The expression of 89 genes was increased during aging but attenuated by caloric restriction, including MMP-7 (Figure 2). We found significant similarities between this list of genes and the Gene Ontology lists related *host pathogen interaction* and *defense immunity protein activity*. In summary, we have identified several changes in gene expression that may be associated with the increased susceptibility of aging kidney to ischemic acute renal failure.

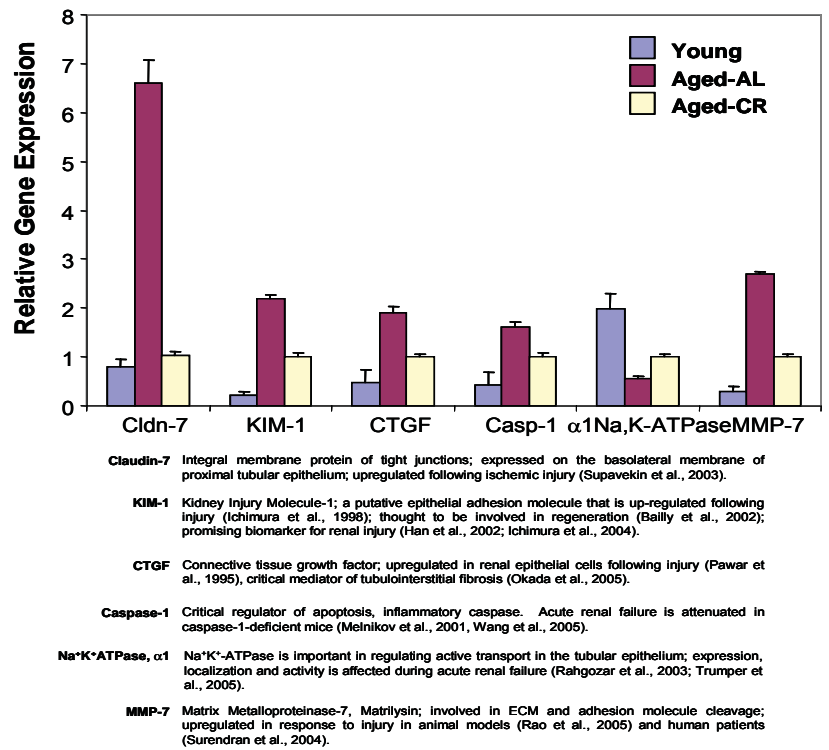


FIGURE 2: The Impact of Aging on Renal Gene Expression. A) RNA was isolated from young (5 mon), aged-ad libitum (24 mon), or aged-caloric restriction (24 mon) rats and assessed using the CodeLink whole rat genome array. Each data point represents the normalized mean±SD for 4 animals.

Recently, awards were made for the 2006 Microarray slide competition. Drs. Rajesh Miranda (College of Medicine, TAMUS-HSC), Weimin He (Institute of Biosciences and Technology, TAMUS-HSC) and Aurl Jayaraman (Chemical Engineering, TAMU) each received a set of 6 microarray slides, including complimentary processing.



PILOT PROJECTS

In 2005, 16 applications for CERH Pilot Project Grants were submitted. Following external review, 6 grants were funded in the amount of \$25,000 each. As usual, the grants were of high-quality and represented the diversity of the Texas A&M University System. The research funded promises to further the goals of the CERH and the NIEHS. The six awardees were:

Dr. Brad Amendt, Institute of Biosciences and Technology, TAMUS-HSC

Dr. Lori Bernstein, College of Medicine, TAMUS-HSC

Dr. Kai Elgethun, Department of Geography, TAMU

Dr. Anatoliy Gashev, College of Medicine, TAMUS-HSC

Dr. Joe Sharkey, School of Rural Public Health, TAMUS-HSC

Dr. Thomas McDonald, School of Rural Public Health, TAMUS-HSC

Dr. Gashev provides an overview of his research project:

The lymphatic system plays important roles in maintaining body fluid, macromolecular balances, eliminating products of metabolic exchange from the tissues, immune function, lipid absorption, and movement of tumor cells. To accomplish these tasks, lymph must be moved from the interstitium throughout a network of lymph vessels and nodes and emptied into the central veins. Experiments have shown that intrinsic contractions of the lymphatics are critical to the generation of lymph flow in many tissues. These contractions are modulated by hydrodynamic factors like intraluminal pressure and flow as well as different vasoactive substances and pharmacological agents. However, the mechanism(s) of lymph transport and its regulation has not been well established. Furthermore very little is known about how the lymphatic function and contractile mechanisms in aging are modulated. Histological studies have shown that age-modulated structural changes occur in lymphatic wall. We propose that age-modulated changes in lymphatic contractile apparatus will lead to decreased lymphatic contractility. The nature of age-modulated changes in functional activity of organs and tissues varies in different regions of body. The central hypothesis of this proposal is that aging will differentially modulate the functional activities of various lymphatics that produce greater differences in local conditions of lymph and fluid dynamics. This might be the basis for increased age-modulated variability of lymphatic contractile behavior and for accelerated regression of lymphatic transport function in some organs and tissues in comparison with others.

The general objectives of this proposal are to start investigations of lymphatic intrinsic contractile activity during aging and to evaluate the age-dependent changes in mechanisms involved in the regulation of the active lymph pump. Understanding the lymphatic biology during aging will give insight into mechanisms underlying the contractile function of lymphatics, which will be useful to develop therapies for disease conditions.



RESEARCH HIGHLIGHT

Robert C. Burghardt

The overall goal of this project is to develop and implement a novel integrative biology approach (i.e., “cellulomics”) to investigate chemical-induced stress and injury mechanisms, identify new biomarkers of exposure, and at the same time, develop more robust strategies to define exposures of concern and potential threats to human health. We will exploit technological advances in imaging tools that provide opportunities for the study of molecular mechanisms of toxicant action at the cell-, tissue- and organ-level. Studies completed by Dr. Burghardt and Dr. Rola Barhoumi over the past several years with primary cultures and cell lines suggest that combination of non-invasive functional imaging tools with molecular approaches offer great potential to identify subcellular targets as well as mechanisms and chronology of toxicant-induced cellular injury. It has also been possible to gain insight into molecular mechanisms of altered cellular homeostasis in the absence of lethal injury to cells. Model PAH compounds selected for this investigation include benzo[a]pyrene (BaP), benzo[e]pyrene (BeP), 5-methylchrysene (5MCr), and 3-methylcholanthrene (3MCo).

Tissue and subcellular partitioning of PAHs will be modeled using their inherent fluorescence in conjunction with precision-cut tissue slices and confocal or multiphoton microscopy. Cellulomics assessment of cellular homeostasis will be performed by monitoring multiple functional endpoints in exposed cells of mouse models by laser cytometry. Endpoints include, but are not limited to, intracellular Ca^{2+} homeostasis as both a sentinel and an effector of cellular injury caused by exposure to environmental chemicals, along with a combination of other functional cellular homeostasis parameters (i.e., Ca^{2+} -mediated signal transduction, quantitative assessment of intracellular glutathione (GSH) levels, mitochondrial Ca^{2+} levels and membrane potential ($\Delta\Psi_m$), NO production and reactive oxygen species (ROS) generation, lipid peroxidation, plasma membrane potential, P450 isozyme induction, gap junction-mediated intercellular communication (GJIC), and apoptosis/necrosis). Spatial and temporal assessment of these endpoints will be used to identify the functional consequences of exposure including the adaptive cellular responses. Collaborative studies with Dr. A. Jay Gandolfi at the University of Arizona were undertaken to integrate advances in precision-cut tissue slices and our cellulomics assessment of cultured cells. Preliminary confocal and multiphoton studies were conducted with liver slices to confirm the utility of this approach. We first evaluated and optimized several probes including SYTO10 and DEAD Red as a live/dead cell screen, Oregon Green BAPTA-1 and fluo-4 for intracellular Ca^{2+} , tetramethylrhodamine methyl ester (TMRM) and rhodamine 123 for assessment of

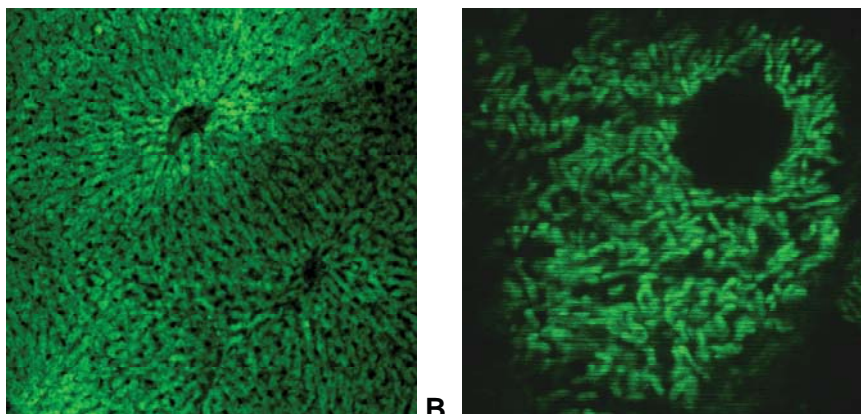


Figure 1. A) A precision-cut liver slice maintained for 24 hr in dynamic culture followed by loading with the mitochondrial probe, rhodamine 123. Width of field is 1000 μm . B) High magnification of a single hepatocyte showing abundant mitochondria with high $\Delta\Psi_m$. The intensity and uniformity of probe loading provides direct evidence of functional mitochondria that exhibit a high $\Delta\Psi_m$ driven by the electrochemical proton gradient necessary to maintain the negatively charged probe within mitochondria. Width of field is 15 μm .

parameters (i.e., Ca^{2+} -mediated signal transduction, quantitative assessment of intracellular glutathione (GSH) levels, mitochondrial Ca^{2+} levels and membrane potential ($\Delta\Psi_m$), NO production and reactive oxygen species (ROS) generation, lipid peroxidation, plasma membrane potential, P450 isozyme induction, gap junction-mediated intercellular communication (GJIC), and apoptosis/necrosis). Spatial and temporal assessment of these endpoints will be used to identify the functional consequences of exposure including the adaptive cellular responses. Collaborative studies with Dr. A. Jay Gandolfi at the University of Arizona were undertaken to integrate advances in precision-cut tissue slices and our cellulomics assessment of cultured cells. Preliminary confocal and multiphoton studies were conducted with liver slices to confirm the utility of this approach. We first evaluated and optimized several probes including SYTO10 and DEAD Red as a live/dead cell screen, Oregon Green BAPTA-1 and fluo-4 for intracellular Ca^{2+} , tetramethylrhodamine methyl ester (TMRM) and rhodamine 123 for assessment of



mitochondrial function. An example of a liver slice cultured for 24 hr prior to loading with a mitochondrial membrane potential ($\Delta\Psi_m$)-sensitive probe (rhodamine 123) is shown in **Figure 1** confirming that cells are effectively loaded indicating the vitality of the entire slice. Similar experiments performed on liver slices loaded with the Ca^{2+} -sensitive fluorophore, fluo-4, AM revealed uniform cytosolic distribution of the

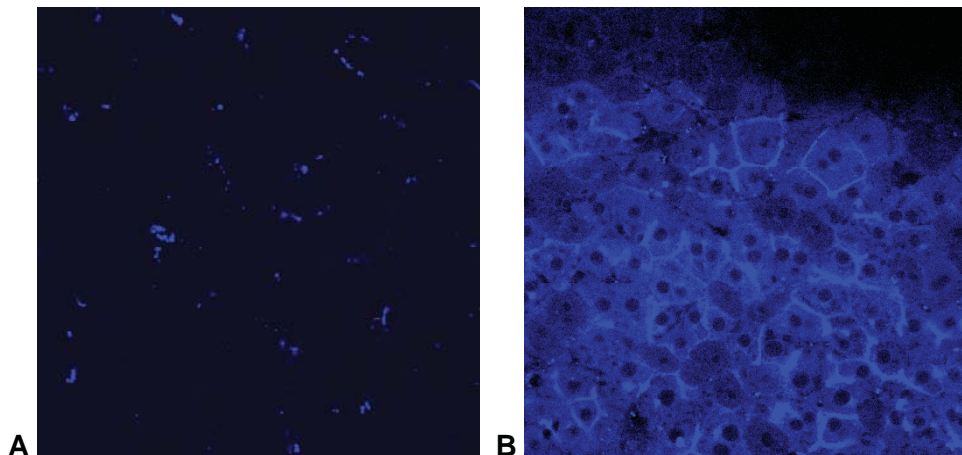


Figure 2. Precision-cut liver slices from C57BL/6 mice exposed in vitro for 4 hr to 30 μ M BaP and imaged with a BioRad 2000MP multiphoton microscope. All images were recorded with the same instrument settings. a) Background signal in liver slice. b) Liver slice. Width of each field is 130 μ m.

probe and complex Ca^{2+} oscillations upon addition of 1.0 nM vasopressin to the slice. Proof of concept was then generated to show that PAH uptake and partitioning into liver and kidney tissue can be detected in precision-cut liver slices following exposure of slices in vitro to BaP (**Figure 2**).

Dr. Jeffrey Catania, a recent graduate from Dr. Gandolfi's laboratory has recently joined my laboratory to continue progress on this project. It is hoped that cellulomics will become a tool for use by CERH investigators through the Image Analysis Core Facility.

CERH NEWS & HIGHLIGHTS

Dr. Stephen H. Safe received the 2005 Distinguished Achievement in Research Award from Texas A&M University and The Association of former students. This is our institution's highest professional honor. Congratulations to Dr. Safe.

The CERH was well-represented at the Texas A&M University System Junior Faculty Workshop in Corpus Christi. Drs. Bazer, Mirkes and Parrish participated in the Health and Biomedical Science panel which prominently featured CERH facilities and research.

Several CERH investigators were recently recognized with 2005 Vice Chancellor's Awards. Dr. Thomas Spencer received an award for Excellence in Individual Research while the Uterine Biology and Pregnancy Team of Drs. Fuller Bazer, Robert Burghardt, Gregory Johnson, Thomas Spencer and Guoyao Wu were awarded the Excellence for Team Research prize. In addition, Sudhakar Chintharlapalli, a student in Dr. Safe's laboratory, received the Excellence for Individual Graduate Student Research award.

Dr. Rola Barhoumi Mouneimne will receive a Colgate-Palmolive Grant for Alternatives in Research award at the 2006 Society of Toxicology meeting. Dr. Tiffany-Castiglioni is a co-investigator on the award.



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