UAMS College of Medicine Series

Showcase of Medical Discoveries:

A Focus on Aging
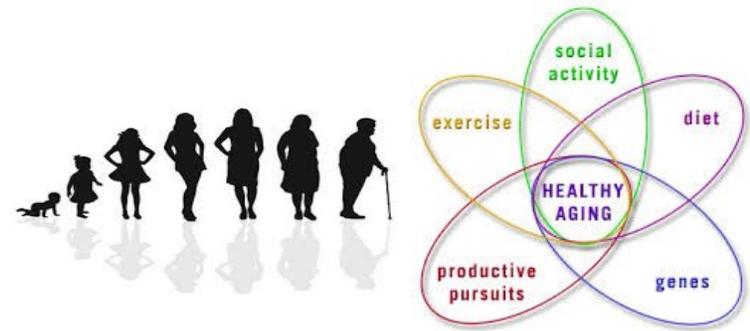
Wednesday, June 10, 2015
4:30—6:00 p.m.

A Wine & Cheese Reception Featuring
UAMS Investigators Discussing their
Research and Discoveries.

Winthrop P. Rockefeller Cancer Institute
10th Floor Rotunda

Poster #1

Suppression of Complement Factor H Increases Deposition of Membrane Attack Complex and Vessel Growth in Mouse Model of CNV

Nalini S. Bora, Valeriy V. Lyzogubov and Puran S. Bora

The role of complement Factor H (FH), the key regulator of the alternative pathway (AP) was investigated in wet, age-related macular degeneration (AMD) using a murine model of choroidal neovascularization (CNV). CNV was induced in C57BL/6 mice using Argon laser. Animals received FH siRNA, control siRNA and vehicle separately via the subretinal route. Suppression of FH was confirmed by RT-PCR and Western blotting. For CNV size evaluation animals were perfused with FITC-dextran, images of injured sites were captured using confocal microscopy of flat mounted RPE-choroid-sclera. Additionally, RPE-choroid-sclera flat mounts were stained for MAC. Subretinal injection of FH siRNA decreased FH mRNA and protein expression in RPE-choroid. Immunohistochemical analysis demonstrated that suppression of FH leads to increased MAC deposition on RPE cells. Compared to the animals receiving control siRNA and vehicle alone, a sharp increase in the size of CNV complex was observed in the animals in which FH was suppressed. Our results demonstrate that FH inhibition leads to increased MAC deposition on RPE cells and predisposes to early and more intensive vessel growth in experimental wet AMD. Collectively, these results raise the possibility that inhibition of the AP of the complement system could be a successful therapeutic strategy to treat wet AMD.

Poster #12

P49/STRAP Regulates the Expression of MicroRNA and MicroRNA Clusters

Xiaomin Zhang, Gohar Azhar, Steven C. Rogers, Stephen R. Foster, Emmanuel Williams and Jeanne Y. Wei

Objective: p49/STRAP is a transcription cofactor that has been implicated in cardiac aging. p49/STRAP interacts with serum response factor (SRF) and participates in the regulation of SRF-target genes, which include muscle-specific genes and microRNA genes. MicroRNAs are short, endogenous, single-stranded RNA molecules that regulate various cellular functions. Certain microRNAs and microRNA clusters are important regulators of aging and senescence. Since p49 protein level increases in the aging heart, we hypothesized that p49 might impact microRNA expression, thereby affecting cardiac aging and senescence. Methods: Recombinant p49 adenovirus was used to treat C2C12 cells. The RNA samples were isolated and analyzed with Exiqon microRNA assays. Results: We observed that 39 microRNAs were differentially expressed with at least 1.2-fold change in p49 treated cells versus control adenovirus treated cells (p <0.05, n=3). 20 microRNAs were up-regulated, while 19 microRNAs were down-regulated. 18 microRNA clusters were affected. Five clusters matched those microRNA clusters that are significantly impacted in the heart during aging in our previous study. These clusters are miR-379-410, miR-23-27b-24-1, miR-466-467-669, miR-29b-1-28a and miR-290-295. The SRF-binding site in the promoter region of these microRNA clusters was examined, and the microRNA target genes were analyzed.

Conclusion: p49/STRAP is a transcriptional regulator of microRNA and microRNA cluster genes. Overexpression of p49/STRAP altered the expression of a set of microRNA clusters that are associated with cardiac aging. The increased expression of p49/STRAP that is observed in aged mice may impact microRNAs and microRNA clusters and contribute to functional and morphological changes in aging and senescence.

Comprehensive Research Informatics Suite (CRIS) for Aging Research

Christine Rodgers, Umit Topaloglu Ph.D., Paula Roberson, Ph.D., Gohar Azhar, M.D., Arny Fernando, Ph.D., Robert Wolfe, Ph.D., Jeanne Y. Wei, M.D., Ph.D.,

A novel component of our Claude D. Pepper Older Americans Independence Centers (OAIC) at the Institute of Aging is the adoption of common research biomedical informatics systems. Based on the initiative from Winthrop P Rockefeller Cancer Institute, we have been developing the Comprehensive Research Informatics Suite (CRIS); a comprehensive set of open source software tools for electronic management of clinical trials and associated data. All components of the CRIS are web based, enabling sharing and integration of clinical research information for single and multi-site trials. All applications are integrated into a portal that allows a single point of access with a registered UAMS username and password. All CRIS applications reside on a cluster server with failover capability behind the UAMS firewall, and thus have the benefit of high security, fire protection, and routine backup. CRIS modules are widely adopted across UAMS and ACHRI, and currently enhancing the suite to support their vanguard work in translating genomics and proteomics research into clinical practice.

## UAMS Center for Osteoporosis and Metabolic Bone Diseases

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S.C. Manolagas, R.L. Jilka, R.S. Weinstein, C.A. O'Brien, M. Almeida, and H. Zhao, P.K. Roberson

The Osteoporosis and Metabolic Bone Disease Center was established in 1994, with the recruitment of authorities in the field from around the world. In a short time, it became an internationally renowned center of excellence and a premier facility for research in osteoporosis and other metabolic bone diseases, as well as a patient referral center for treatment of these conditions. The primary objective of the research by the center's interdisciplinary faculty has been the understanding of the cellular and molecular mechanisms behind the development of osteoporosis and other bone diseases in women after menopause and women and men with old age, as well as in patients developing osteoporosis as a side-effect of therapy with steroids. Using such an understanding, center investigators are developing more effective therapies for treatment of this common metabolic bone disease. The Center's faculty has the combined research experience of almost 200 years, has a collective record of more than 1,200 publications, and represent a highly synergistic team with complimentary expertise in molecular and cellular biology, molecular genetics, the biology of bone as a tissue, and the clinical diagnosis and treatment of osteoporosis.

Advances made by the Center's faculty have improved understanding of the mechanisms of steroid hormone action on bone and how physiologic, pathologic, or iatrogenic changes in hormone levels can lead to increased fracture risk. Estrogens, androgens, and glucocorticoids alter the cellular composition of bone by regulating the supply and lifespan of osteoclasts and osteoblasts. Additionally, they influence the survival of osteocytes, long-lived cells that are entombed within the mineralized matrix and mediate the homeostatic adaptation of bone to mechanical forces. Altered redox balance is a proximal underlying mechanism of some of these effects, and sex steroid deficiency or glucocorticoid excess contributes to the aging of the skeleton.

Poster #3

Optimal Infusion Rate of Amino Acids for Improving Whole Body Net Protein Balance in Cancer Patients
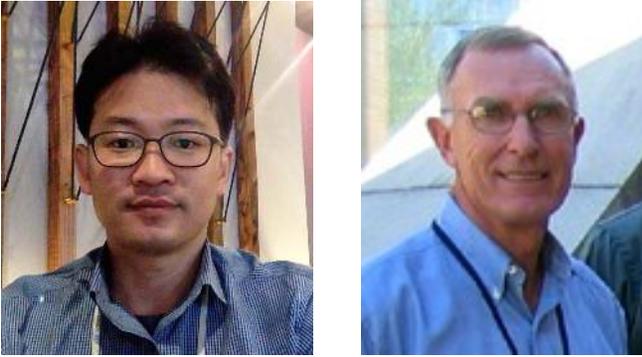
Il-Young Kim, Scott E. Schutzler, Donald L. Bodenner, Robert H. Coker, Arny A. Ferrando and Robert R. Wolfe

Cancers are often accompanied with loss of muscle mass, referred to as cancer cachexia, with poor outcome and quality of life. To determine effects of different amino acid (AA) doses on changes in whole body net protein balance (NB) [i.e., protein synthesis (PS) − protein breakdown (PB)] and its interaction with glucose/insulin infusion, eight cancer patients [65±4yr, 83±10kg, 51±6kg fat free mass] were randomly assigned to one of three amino acid doses (0.04, 0.08, or 0.13g/kg/day; 2/3/3 subjects, respectively) and studied under two conditions [i.e., each AA dose with or without insulin/glucose infusion] during 7-hour primed continuous infusions of L-[^{2}H$_5$]phenylalanine & L-[^{2}H$_2$]tyrosine. Whole body protein kinetics was expressed as changes above the fasted state. Due to lack of effect of insulin/glucose, protein kinetics was collapsed at each AA dose. AA infusion improved NB in a dose dependent manner [0.091±0.002, 0.187±0.014, and 0.337±0.034 g/kg ffm/min, $p < 0.001$]. The higher NB with increasing AA dose was achieved largely through corresponding increases in PS [0.081±0.022, 0.152±0.013, and 0.303±0.034 g/kg ffm/min, $p < 0.001$] without significant changes in PB. In conclusion, NB was increased with increasing doses of AA through increases in PS without effect of insulin/glucose infusion.

Poster #10

Scent-Trained Canine Prospectively Detects Thyroid Cancer in Human Urine Samples

Arny A. Ferrando, Andrew M. Hinson, and Donald L. Bodenner, MD

In a prior study, we demonstrated that canines could reliably discriminate between urine samples obtained from subjects previously diagnosed with either metastatic thyroid carcinoma (PTC) or benign thyroid disease1. The aim of this study was to prospectively detect thyroid cancer in undiagnosed patients that presented to our thyroid clinic. Prior to testing, a canine was imprinted with urine and thyroid tissue obtained from several different patients with PTC, and trained over 6 months to discriminate between PTC and benign urine samples.

Urine was collected from 59 subjects who presented to our thyroid clinic with one or more thyroid nodule(s) suspicious for thyroid cancer. Only PTC (conventional type) (n=15) and benign thyroid disease (n=19) confirmed by final surgical pathology were included in the final analysis.

In the lab, a gloved handler, blinded to the sample status, presented each unknown sample in a 3-mL cryotube to the canine. The handler verbally communicated the canine's alert (if PTC, the canine laid down) to a blinded study coordinator who recorded the response.

The canine's alert matched the final surgical pathology diagnosis in 30 out of 34 cases (88.2% accuracy, 2 false negatives and 2 false positives), yielding a sensitivity of 86.6% (59.5-98.0) and specificity of 89.5% (66.8-98.4). Kappa coefficient (which takes into account the amount of agreement that can be attributed to chance) was 0.76, indicating good agreement.

Based on preliminary data, scent detection by trained canines serves as a non-invasive, inexpensive and highly specific adjunct to current diagnostic practices.

Poster #9

Interleukin-1 Promotes Proteostatic Dysfunction and Aggregation in Alzheimer's and Parkinson's

W. Sue T. Griffin, Paul Parcon, Ahmad Aldwaikat, JoAnn Biedermann, and Robert E. Mrak

A growing body of evidence establishes neuroinflammation as central in pathogenesis of both Alzheimer's (AD) and Parkinson's (PD). Here we show evidence that pro-inflammatory cytokine IL-1 regulates the synthesis and function of two of the major components of protein degradation and/or recycling. Using immunohistochemistry and immunofluorescence, we probed the intercellular localization of NEDD8 and parkin in cell culture and human brain from AD, PD, and controls. We measured IL-1 and Parkin levels in an AD mouse model and measured Parkin levels in response to IL-1 in primary rat and human (NT2) neuronal cultures. In an animal model of AD, IL-1 and E3 ubiquitin ligase parkin increased with age. We show that neuronal IL-1 treatment increases parkin expression and mediates translocation of its activator NEDD8. Importantly, in human brain, NEDD8 immune-reactivity is absent in the nucleus and parkin is aggregated and increased in both AD and PD compared to that in age-matched controls. Our results are consistent with the idea that IL-1 is a driver of neddylation dysfunction and parkin aggregation in AD and PD. Thus, we predict that early intervention to appropriately regulate IL-1 expression and activity would prevent or delay protein recycling dysfunction in neurodegenerative disease.

Poster #4

Transcriptional Analysis of Doxorubicin-induced Cardiotoxicity in Elderly Breast Cancer Patients


Todorova, V., PhD; Siegel, E.R., MS ;Makhoul, I., MD; Wei, J., MD; and Klimberg V.S., MD

Doxorubicin (DOX), a widely used anti-cancer drug is known for its cardiotoxicity. DOX cardiotoxicity is cumulative-dose-dependent and begins with the first dose of chemotherapy. Older age is one of the factors that can significantly increase the risk of developing DOX-induced cardiomyopathy even at low dose. To date, no biomarker for early presymptomatic detection of DOX cardiotoxicity has been validated. Our previous data indicated that blood cells can be used as a surrogate tissue for identification of biomarkers for DOX cardiotoxicity. Therefore, this study aimed to identify the age-related patterns of gene expression in blood cells induced by a single dose of DOX-based chemotherapy of 33 women with breast cancer divided into 3 age-groups: > 56 years, 48-55 years and < 47 years of age. The gene expression was examined before the start and after the first cycle of chemotherapy using microarrays. The results showed that one single dose of DOX induced a significant dysregulation of 235 genes of which 66 genes exhibited overlapping but distinct age-related pattern. Enriched functions included similar to those reported for B-cell development, EIF2 signaling and NFAT regulation, as well as for immunological and respiratory diseases, cardiac dilation, and liver cirrhosis. Age-associated changes in the gene expression induced by DOX have not been reported previously. Our study identified trends that are relevant for the age-related sensitivity to chemotherapy toxicity and lays foundation for further studies to identify potential biomarkers of DOX-cardiotoxicity in elderly.

Therapeutic Potential of ARE-inducers in Immune Dysregulation during Aging
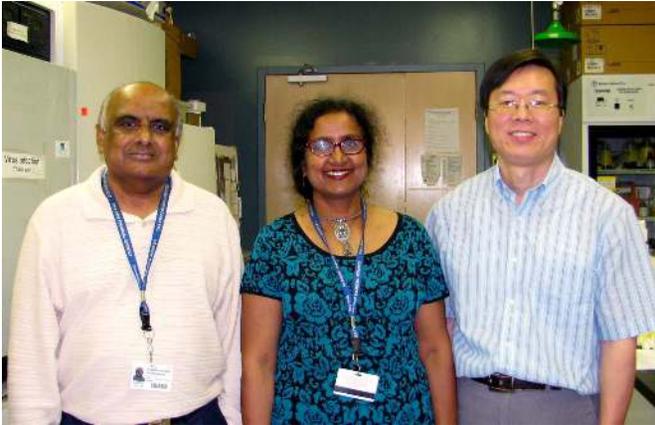

Subramaniam Ponnappan, Ph.D; Xuming Zhang, Ph.D.
and Usha Ponnappan, Ph.D.

The regulated degradation of ubiquitin tagged proteins by the ubiquitin proteasome system (UPS) is one of the important pathways for the removal of aberrant, misfolded, and oxidatively modified proteins, as well as for the maintenance of cellular protein homeostasis or "proteostasis". Proteostasis is severely challenged under conditions of oxidative stress, which occurs during advanced age and microbial infections. Our studies have demonstrated significant decline in both proteasomal proteolysis and functional chaperone activity in T lympho-cytes during aging, impacting proteostasis. This functional decline is accompanied by the accumulation of oxidatively modified protein substrates and ubiquitinated proteins. Anti-oxidant response element (ARE)- inducers such as D3T, alleviate proteasomal dysfunction in $CD4^+$ T cells from the elderly, along with effectively decreasing free radical generation and carbonylated protein modification, by virtue of induction of phase-II enzymes and proteome maintenance genes. To assess whether exposure to ARE-inducers impact response to influenza virus infection in the aged, we used a murine model of influenza infection employing mouse adapted A/PR8/34 strain of the influenza A virus. Our studies for the first time demonstrate that agents that impact the UPS and anti-oxidant defense systems, by virtue of activating ARE, also regulate host defense against viral infection.

When good chaperones turn bad: Proteasome blockage by aggregated proteins affecting multiple neurodegenerative disease models
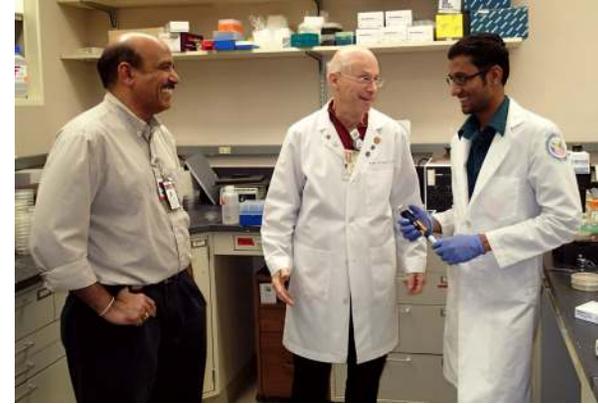
Srinivas Ayyadevara, Sundaram Balasubramaniam, Yuan Gao, Li-Rong Yu, Boris Zybaylov, Ramani Alla and Robert Shmookler Reis

Many neurodegenerative diseases form age-dependent, neurotoxic aggregates that contain both disease-specific proteins and common components (e.g., phosphorylated tau and TDP-43, seen in several diseases). We asked whether proteomic analyses of isolated aggregates might reveal other proteins shared by multiple neuropathies. A *C.elegans* model of Huntington's and other polyglutamine-array diseases, expressing Q40::YFP in muscle, accrues aggregates for about one week after hatching. These foci, affinity-purified on antibody-coated magnetic beads, were analyzed at high resolution by mass spectro-metry. Three Q40-associated proteins appeared to promote aggregation and cytotoxicity, since RNA-interference knockdown reduced or delayed those traits — not only in the Huntington model but also in two Alzheimer Disease models, nematodes with muscle or pan-neuronal $A\beta_{1-42}$ expression and behavioral phenotypes. The most abundant aggregated proteins are prion-like (rich in glutamine and asparagine), likely to form hydrophobic interactions with other random-coil proteins. The protein contributing most effectively to aggregation, despite making up <1% of aggregates, upon knockdown reduced Q40::YFP foci 86% ($P < 10^{-12}$), reduced β-amyloid staining 60% in Aβ-expressing tissue, and slowed age-dependent paralysis >30% ($P < 10^{-6}$). These effects absolutely required proteasome function, vanishing in the presence of a proteasome inhibitor or RNAi against a proteasome α subunit. Immunostaining indicates that proteasomes become "trapped" within aggregates, but are liberated upon silencing of this protein .

Poster #7

Nutrition and Lipid Metabolism in Aging

Elisabet Borsheim, Eugenia Carvalho, Nicholas M. Hurren, Leybi L. Ramirez

Our aim is to understand the mechanisms that drive hypertriglyceridemia (an elevated plasma triglyceride [TG] concentration) in aging, and to uncover novel treatment strategies for this condition. Coronary heart disease (CHD) remains the leading cause of death for men and women in the U.S. today, and hyper-triglyceridemia is an independent risk factor for CHD. An increased plasma TG concentration is a common finding with aging, as with overweight/obesity and inactivity. During postabsorptive conditions, most plasma TGs circulate as components of very low density lipoprotein (VLDL) particles secreted from the liver. In the postprandial state, the intestine packages dietary fat into huge TG-rich lipoproteins called chylomicrons, and these particles compete with VLDLs to have their TG hydrolysed by the enzyme lipoprotein lipase within muscle and adipose tissue capillary endothelia.

In our projects, we study the influence of nutrition on fasting and postprandial lipid metabolism in older adults: specifically, the acute and chronic effects of ingesting supplemental amino acids. Principal methodologies include stable isotope tracer techniques to quantify regional lipid kinetics *in vivo*, indirect calorimetry to assess whole-body substrate oxidation rates, nuclear magnetic resonance spectroscopy to quantify intracellular stores of lipid in the liver and skeletal muscle, molecular biology techniques to study mechanisms at the gene/protein level, and high-resolution respirometry to determine mitochondrial function within skeletal muscle and adipose tissue. Preliminary results suggest that amino acids may increase VLDL turnover.

Poster #6

Pilot Testing an Innovative Teaching Tool to Improve Dressing Assistance in Nursing Homes

Pao-Feng Tsai, PhD, RN; Cornelia Beck, PhD, RN; Thomas Jakobs, MS, PE; Mallikarjuna Rettiganti, PhD; Kerry Jordan, MSN, RN; Erik Jakobs, BA, BS; and Stephanie Kitch, BSN, RN

This study tested initial effects of an innovative technology intervention on caregivers' level of assistance (LoA) in dressing and nursing home residents' dressing independence. Nine caregiver-resident pairs completed the study. Control and experimental arms participated in a traditional one-hour education module. The experimental arm received an additional two-hour intervention using a new video simulator on a tablet computer that enabled certified nursing assistants (CNAs) to practice their LoA skills. Major outcomes—CNAs' scores on appropriateness of performing dressing LoA and residents' scores on Beck Dressing Performance Scale (BDPS)—were measured before and 6 weeks after the intervention. The results showed that the two arms were not significantly different in either appropriateness of dressing LoA or BDPS (p=.36, .25). A lack of effort to assist and low statistical powers explained these findings. Nevertheless, LoA predicted BDPS (p<.001). These data suggested if caregivers receive training LoA and make an effort to practice with their residents, residents will improve their dressing independence regardless of using simulator. Future studies should verify CNAs achieve a specific skill level and knowledge after training, and incorporate strategies to improve CNAs' intention to improve behavior.