Dr. Gregory Ordway, Interim Chair  
Department of Biomedical Sciences

As we progress into our second year as the Department of Biomedical Sciences, each and every employee is encouraged to look for newer and/or innovative ways that we can improve our Department’s research, teaching, service, and finances. As we near the end of June 30, 2013, we are encouraged to maintain good stewardship with our finances. As always, everyone is encouraged to please provide feedback to my office regarding any problems or concerns.

Many thanks to those graduate and undergraduate students who participated in the Appalachian Student Research Forum and the ETSU Boland Undergraduate Research Symposium. I commend each of these students for their willingness to represent the Department and for their research endeavors. Award results and abstracts associated with these presentations and forums are listed in this newsletter. In addition, much appreciation is extended to the Faculty Sponsors of these students. A special “Thank You” to the Faculty who donated their time to serve as judges at these events.

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Joe Wu, Graduate Student, who received a Graduate Student Research Award from the School of Graduate Studies in the amount of $792.13, for his project titled, “Induction of a novel cardioprotective metabolic pathway by H1F1α.” Funding availability is 7/1/2013-6/30/2014.
PAPERS/ABSTRACTS/PUBLICATIONS:

Beach, Justin and Scott Champney. *An Examination Of The Inhibitory Effects Of Antibiotic Combinations On Molecular Targets In Staphylococcus Aureus.* Presented at the 2013 Appalachian Student Research Forum, ETSU, April 4, 2013. (Poster) **Awarded 3rd Place in the Biomedical & Health Sciences Division for Graduate/Doctoral Candidates.**

Burt, Rees, Bridget M. Graves, Chaunfu Li, David L. Williams, Santiago P. Fregoso, Donald B. Hoover, and Robert Wondergem. *9-Phenantrol Inhibits Calcium Oscillations In HL-1 Mouse Cardiomyocyte.* Presented at the 2013 Appalachian Student Research Forum and Boland Undergraduate Research Symposium, ETSU, April 3-4, 2013. (Poster) **Awarded 3rd Place in the Biomedical & Health Sciences Division for Undergraduate Students.**


Crawford, Jason L., Michelle J. Chandley, Jessica D. Crawford, and Gregory A. Ordway. *Molecular Analysis Of Gfap Positive Astrocytes From Neocortical Gray Matter Of The Anterior Cingulate Cortex In Autism Spectrum Disorder.* Presented at the 2013 Appalachian Student Research Forum, ETSU, April 4, 2013. (Poster) **Awarded 3rd Place in the Biomedical & Health Sciences Division for Undergraduate Students.**

Crawford, Jessica D., Michelle J. Chandley, Brandon L. Waters, Jason L. Crawford, and Gregory A. Ordway. *Elevated Glial Fibrillary Acidic Protein (Gfap) Protein Expression In White Matter From Autism Spectrum Disorder Subjects.* Presented at the 2013 Appalachian Student Research Forum, ETSU, April 4, 2013. (Oral) **Awarded 3rd Place in the Biomedical & Health Sciences Division for Graduate Students/Doctoral Candidates.**

Daniels, Christopher R., Xia Zhang, Suman Dalal, Stephanie Cunningham, Chuanfu Li, William Joyner, Mahipal Singh, and Krishna Singh. *Exogenous Ubiquitin Plays A Protective Role In The Heart Against Ischemia/Reperfusion Injury: Role Of Cxcr-4 In Fibroblast Function.* Presented at the 2013 Appalachian Student Research Forum, ETSU, April 4, 2013. (Poster) **Awarded 1st Place in the Biomedical & Health Sciences Division for Graduate Students/Doctoral Candidates.**

Perry, Evan, Eliot Smith, and David Johnson. *Expression Of Recombinant Human Neutrophil Cathepsin G.* Presented at the 2013 Appalachian Student Research Forum, ETSU, April 4, 2013. (Poster)

Sabri, Mohammad, Jaime Parman-Ryans, and Antonio Rusiñol. *Proximity-Labeling Of Mouse Fibroblasts Proteins That Are Near-Neighbors Of Lamin A And Lamin A-D50 (Progerin).* Presented at the 2013 ETSU Boland Undergraduate Research Symposium, April 2, 2013. (Poster) **Awarded 1st Place in the Biomedical & Health Sciences Division for Undergraduate Students.**


Wu, Joe and Gary Wright. *The Purine Nucleotide Cycle: A Novel Pathway Induced By Hif1α.* Presented at the 2013 Appalachian Student Research Forum, ETSU, April 4, 2013. (Poster) **Awarded 2nd Place in the Biomedical & Health Sciences Division for Graduate Students/Doctoral Candidates.**
AWARD RESULTS—ASRF/Boland Research Symposium, APRIL 2-4, 2013:

ASRF AWARD WINNERS: Poster Presentations
Undergraduate Students:
- First Place: Mohammad Sabri*
- Second Place: Kaitlyn Hinshaw
- Third Place: Rees Burt

Graduate Students:
- First Place: Christopher Daniels*
- Second Place: Joe Wu
- Third Place: Justin Beach

ASRF AWARD WINNERS: Oral Presentations
Graduate Students:
- Third Place: Jessica Crawford

DBMS Faculty Judges:
Dr. Alok Agrawal, Dr. Jeffrey Ardell, Dr. Cherie Bond, Dr. Sharon Campbell, Dr. Scott Champney & ASRF Task Force, Dr. Tom Eca, Dr. David Johnson, Dr. Michael Kruppa, Dr. Philip Musick, Dr. Mitch Robinson, Dr. Krishna Singh, and Dr. Doug Thewke.

GRANT REVIEW STUDY SECTION MEETINGS:
- Dr. Gregory Ordway, Professor & Interim Chair, invited to serve on the American Foundation for Suicide Prevention (AFSP) Study Review Committee, April 11-12, 2013.
- Dr. Alok Agrawal, Professor, invited to serve on the NIH-ACTS Study Section Review Committee, June 4-7, 2013, Chicago, IL.

INVITED PRESENTATONS BY FACULTY:
- **DR. RICHARD KOCHTISZWA**, Professor, has been invited to participate in the XVIII International Congress of the Polish Pharmacological Society symposium to be held in Kazimierz Dolny, Poland, May 22-25, 2013. His seminar is entitled, "The Changing Face of Neurotoxins." The oral presentation will be given as part of the intrameeting 23rd Neuropsychopharmacological Days.

- **DR. ROBERT SCHELLER**, Professor, was invited to present his research findings at the 11th German Chlamydia Workshop in Würzburg (DCW). The workshop was hosted at the Gartenpavillon of the Juliusspital, April 10-12, 2013. The aim of the workshop—discuss all issues of Chlamydia infection to microbiologists, immunologists, clinicians, veterinarians, and cell biologists. Dr. Schellerg’s presentation title: "Using an Experimental Murine Model to Evaluate the Role of Persistent/Stressed Chlamydia in Treatment Failure and Disease Progression."
BUILDING, EQUIPMENT, OSHA NEWS
TJ Neal, Departmental Coordinator

♦ EQUIPMENT
The inventory of lab equipment is still in process. As we move, shift, and surplus equipment, please ensure that TJ Neal, who coordinates inventory functions for the department, is informed of these changes.

♦ HEALTH & SAFETY NEWS
The Department of Health & Safety has an array of online training modules that deals with generic safety and wellness issues. All personnel are encouraged to visit the healthsafety.etsu.edu and review the Emergency Procedures and Fire/Life Safety Procedures modules.

♦ BUILDING COORDINATION
Know the Building Coordinators who are responsible for assisting with building issues, performing monthly fire and life safety inspections, reporting problems, assisting with fire drills, etc.

  Bob Montgomery—Building Coordinator for all College of Medicine Buildings (791-0046)
  TJ Neal—Building Coordinator DBMS & —1st Floor B Wing (439-2040)

2013 Caduceus Awards—Congratulations to:
M1—Outstanding Course of the Year—Medical Human Gross Anatomy
  Course Director: Dr. Thomas Kwasigroch
M1—Professor of the Year—Dr. Paul Monaco
M2—Outstanding Course of the Year—Microbiology
  Course Director: Dr. Russ Hayman
M2—Professor of the Year—Dr. Robert Schoborg

Conference/Workshop Attendees—April 2013
11th German Chlamydia Workshop, Würzburg—April 10-12, 2013
  Dr. Robert Schoborg
Experimental Biology Conference, Boston, MA—April 20-24, 2013:
  Dr. Caroline Abercrombie  Bridgette M. Browe
  Dr. Jeffrey Ardell  Rees Burt
  Dr. Eric Beaumont  Evan Perry
  Dr. Michelle Duffourc
  Dr. Thomas Ecay
  Dr. Krishna Singh
  Dr. Mahipal Singh

7th Progeria Research Foundation International Workshop, Bethesda, MD—April 24-26, 2013:
  Dr. Phil Musich
  Dr. Yue Zou

OSHA

♦ LAB PERSONNEL TRAINING
It is time for laboratory personnel to begin their annual online OSHA training. The online modules can be accessed at healthandsafety.etsu.edu.

OSHA—LAB INSPECTIONS
It is time to start planning for the upcoming Annual OSHA laboratory inspections. Review the checklist for inspections.

Last year we had an great year receiving outstanding reviews and inspection in all labs.
Welcome…. Dr. Kui Cui

Dr. Kui Cui joins the Department as a visiting Research Scholar. Dr. Cui attended Soochow University and Xuzhou Medical College in China. He is assisting Dr. Ming-Yang Zhu's research efforts.

CONGRATULATIONS TO CYNTHIA TAYLOR ON THE BIRTH OF HER GRANDCHILD

Landry Reese
6 lbs. 15 oz.
March 28, 2013

The Annual ETSU Staff Picnic will be held on May 24th from 1-3 at the Amphitheater on the main campus. Staff Awards will be announced, there will be music, outdoor games, door prizes, and a contest for the “Most Creative Blue and Gold Attire.” All Staff are encouraged to attend this annual event.

Anyone interested in donating non-perishable food to the Bucky’s Food Pantry, which is located on the main ETSU campus, can do so. Collection points on the VA Campus are Building 52, 119, and 178. A collection box is located on the main floor of Bldg. 178 (near the main entrance). This is an ongoing project and donations are accepted all the time.

Information about Bucky’s Food Pantry can be accessed at http://www.etsu.edu/foodpantry/.

Cindy Canter
Staff Senator/DBMS

Some attendees of the Experimental Biology Conference held in Boston, MA, April 20-24, 2013, enjoy pizza and time out. Pictured are: Dr. Caroline Abercrombie, Dr. Michelle Duffourc, and Bridgette Browe. The fourth person in upper left is April Joice-Coley, a friend of Caroline's from the University of Utah College of Pharmacy. (Picture contributed by Dr. Tom Ecay)

REES BURT RECEIVES ROBERT GUNN AWARD

Rees Burt received the Robert Gunn award for undergraduate research awarded by the Cell Physiology section of the American Physiological Society at the section's annual dinner Tuesday evening, April 23, 2013, in Boston at the Experimental Biology meeting. The prize consists of $300 plus registration fees for the meeting. The award culminated the competition among undergraduates from around the country for their poster presentations.

Robert Gunn, MD, was longtime chair of Physiology at Emory University School of Medicine. He was an ardent researcher and educator of physiology, and the award was established a few years ago by the Cell Physiology section following his untimely death from leukemia.

Please congratulate Rees.<b>burtr1@goldmail.etsu.edu</b>
DBMS SEMINAR SERIES

- April 9th — Joe Wu, Graduate Research Assistant — “The Purine Nucleotide Cycle: A Novel Pathway Induced by HIF1α”
- April 16th — Dr. Todd Reynolds, Associate Professor, Department of Microbiology, University of Tennessee-Knoxville — “Phosphatidylserine synthase in Candida albicans as a target for antifungals”
- April 22nd — Dr. James Kaper, Professor & Chair, Department of Microbiology and Immunology, University of Maryland School of Medicine — Student Invited Speaker — “Vibrio cholera and cholera: Vaccine development and in vivo gene expression”
- April 30th — Jessica Crawford, Graduate Research Assistant — “White Matter Glial Pathology in the cingulate cortex of autism spectrum disorder subjects”
- May 10th — Dr. Randy D. Blakely, Director, Vanderbilt-NIMH Silvio O. Cente Center for Neuroscience Research — “New Clues to the Regulation of Dopamine Signaling: A Can of Worms.” This is joint engagement with Psychiatry-Grand Rounds.

The Biochemical Nature of Disease Journal Club has been established. This club meets at noon on Thursdays and is a brown bag lunch. Topics are of speaker’s choosing. The paper to be discussed is sent via email on Monday. Topics that have been discussed thus far related to antibiotic resistance, autophagy, translation control, DNA repair, lipid metabolism, apoptosis, mechanisms of cancer, heart disease and neuropathy.

- April 4 — Appalachian Student — Research Forum (No meeting)
- April 11 — Mitch Robinson
- April 18 — Annie (Yan Wang)
- April 25 — Maya Breitman
- May 2 — Yue Zou

Any interested parties may contact Dr. Sharon Campbell if they are interested in giving a presentation.

Further details will be sent via email. Please put these dates on your schedule and plan to attend.

Microbiology Journal Club — Meets Alternating Thursdays at 9 a.m. — Year round except holidays.
Faculty Contact: Dr. Robert Schoborg.

Pharmacology Graduate Students Journal Club — Meets Mondays at 3:30 pm, Building 1, Room B06.
Faculty Contact: Dr. Don Hoover.

To schedule a seminar, contact Dr. Mike Kruppa, Seminar Committee Chair and Cindy Canter, Coordinator.

S.O.S. (Sharing of Science)
The monthly Research Roundup meetings will resume again in the Fall 2013. Anyone interested in being a presenter, please contact Dr. Mike Kruppa, Seminar Committee Chair, to schedule a date.
Altered XPA subcellular localization and deficient nucleotide excision repair in HGPS cells  
Nahid Mehraban, McKayla Johnson, Ben Hilton, Henry Gong, Zhengke Li, Yue Zou and Phillip R. Musich

Cells from Hutchinson-Gilford progeria syndrome (HGPS) patients exhibit enhanced sensitivity to various metabolic stresses and to DNA damaging agents. We have shown previously that genotoxin-induced DNA double-strand breaks (DSBs) are repaired while the age-accumulated endogenous DSBs persist through multiple cell cycles (Liu et al., 2006). Coincident with these endogenous DNA DSBs, marked as γ-H2AX foci, was the co-localization of XPA, a protein essential for nucleotide excision repair (NER). Even though XPA functions within the nucleus in the repair of bulky DNA adducts in the absence of such damage most of the cellular XPA protein normally is located within the cytoplasm of normal and cancer cells, and is transported to the nucleus as part of the DNA damage response. We hypothesized that proper functioning of XPA in NER related to its subcellular distribution and its re-distribution as part of the DNA damage response. To test this hypothesis HGPS cells were exposed to ultraviolet light (UV-C) and allowed to recover. Three cellular parameters were monitored over selected time scales to access the ability of HGPS cells to repair this DNA damage. These included the redistribution of cytosolic XPA to the nucleus (0–4 hr), the rate of removal of the cyclobutane pyrimidine dimer (CPD) and (6–4) photoproduct [(6–4) PP] DNA adducts (0–72 hr) and overall culture viability (0–15 days). The human adenocarcinomic alveolar basal epithelial cells A549 and the normal primary fibroblast cell line BJ were used as controls for comparison. We observed that in non-damaged HGPS cells the distribution of XPA differs with more of this NER protein localized throughout the nucleus in addition to that colocalized with γ-H2AX foci. Also, the normal cytoplasm-to-nuclear shift of XPA appears altered in HGPS cells. The NER repair of the (6–4) PPs appears normal; however, rate of repair of CPD adducts is greatly reduced in HGPS cells compared to the A549 cancer cells. Interestingly, the rate of CPD repair in the normal BJ fibroblasts also was less than in A549 cells though greater than in the HGPS fibroblasts. Culture viability after UV-C exposure followed a similar pattern with only a temporary delay in A549 growth and a significant delay in the BJ culture. The HGPS cells showed small but delayed recovery which was only temporary. Thus, the abnormal subcellular distribution of XPA appears to correlate with deficient nucleotide excision repair of CPD adducts. Further studies are in progress to elucidate the cause-vs.-effect nature of this relationship.

“Designer Genes and the Protein Factory”

Pichia pastoris, a methylotrophic yeast, has been used in our lab to express recombinant human proteins. The pPICz alpha vector (Invitrogen) contains Zeocin resistance and provides for secretion of the expressed protein by fusion with yeast alpha mating factor. The vector contains flanking sequences homologous to the alcohol oxidase 1 (AOX1) gene that results in the gene of interest being integrated under the control of the AOX1 gene promoter; consequently, growth on methanol as the primary carbon source induces expression. Transformation results in homologous recombination of the gene being expressed into the host genome. As the fusion protein traffics through the Golgi for secretion the Kex2 protease removes the alpha mating factor, so the mature active protein is secreted into the medium. Additionally, the secreted protein is properly folded and disulfides are correctly made. Zeocin resistance is used to select transformed colonies, which are then screened for protein expression. Gene synthesis costs have decreased to about 39¢ per base and the companies that synthesize the genes use algorithms to optimize the codons to more closely match the codon usage of Pichia. Our lab has successfully produced the following functional human proteins: mast cell tryptase, mast cell chymase, enteropeptidase, neutrophil cathepsin G, neutrophil elastase and C-reactive protein. Tryptase is a tetramer and CRP is a pentamer and both assumed their correct oligomeric functional structures in the media. Eliot Smith has developed a lot of expertise in fermentation to advance the expression of these proteins.
Background and Significance: Current theories suggest that chlamydiae persist at the site of infection via an alternative replicative form, the aberrant body (AB). Chlamydial persistence is thought to play a role in diseases like trachoma and atherosclerosis.

Objectives: We demonstrated that porcine epidemic diarrhea virus (PEDV) co-infection led to chlamydial persistence (Borel et al., 2010). We further hypothesized that the PEDV-induced persistence is reversible and does not require productive viral replication or de novo host protein synthesis.

Methods: C. pecorum persistence induction was evaluated using titer assays, immunofluorescence (IF) and transmission electron microscopy (TEM). To determine the reversibility of persistence, time course experiments were performed in which Vero cells were C. pecorum/PEDV infected in the presence of cycloheximide (Cx) and samples were collected at 24, 48, 72 and 96 hours post-PEDV addition. Cx inhibits host and viral protein synthesis but not that of chlamydiae. Co-infection experiments were additionally performed using UV-inactivated PEDV. Binding of PEDV to the host cell surface in the presence of Cx was confirmed by IF analysis.

Results: Persistence was observed at 24 hours post PEDV-addition. Persistence started to reverse at 48 hours post-PEDV infection and productive C. pecorum replication ensued at later times. Cx-exposure completely inhibited PEDV replication, but did not inhibit C. pecorum persistence as indicated by production of ABs and reduced production of infectious EBs in sub-passage titer assays. Co-infection with UV-inactivated PEDV also induced C. pecorum persistence, confirming these results.

Conclusions: Our data showed that (i) PEDV-co-infection-induced chlamydial persistence is reversible; (ii) does not require productive viral replication; (iii) does not require de novo host or viral protein synthesis; and (iv) does require viral binding and/or entry. We predict that PEDV induces persistence directly by interacting with the host cell receptor CD 13.
IN Volvement of HOST CELL SIGNAL TRANSDUCTION PATHWAYS IN HSV-CO-INFECTION-INDUCED CHLAMYDIA TRACHOMATIS PERSISTENCE

Jennifer Vanover Hall, J. Kintner, J. Sun, M. Bambino, M. Novak and R.V. Schoborg,
Department of Biomedical Sciences, Quillen College of Medicine, East Tennessee State University

Background and Significance: Herpes Simplex Virus (HSV) co-infection induces C. trachomatis serovar E (CtE) to enter into a viable but non-infectious (persistent/stressed) state by a mechanism that requires HSV glycoprotein D/host nectin-1 interaction. Recently, human herpes virus 6 (HHV6) and HSV-2 have been shown to stimulate C. trachomatis L2 persistence by oxidative stress induction. HSV/host cell binding also activates JAK, JNK, PI3K and AKT, suggesting these signaling pathways may be involved in HSV-mediated, persistence induction.

Objectives: To test the hypothesis that JAK, JNK, PI3K and/or AKT inhibition will rescue CtE from HSV-induced persistence.

Methods: HeLa monolayers were mock- or CtE-infected for 21-23 hours (h) before addition of AKT, JAK, JNK or PI3K inhibitors. One to 3h later, replicate monolayers were mock- or HSV-2-infected. At 20h post-viral infection, cultures were evaluated for viral entry, progeny virus production, kinase activity and infectious EB production.

Results: None of the inhibitors tested diminished viral entry. Interestingly, JAK, JNK and PI3K inhibition reduced EB production in CtE singly-infected cells compared to DMSO-exposed controls. AKT inhibition, in contrast, did not alter EB progeny production in CtE singly-infected cultures. Finally, JAK, JNK, PI3K and AKT inhibitors did not reverse the drop in EB production induced by HSV-co-infection.

Conclusions: These data indicate that neither JAK, JNK, PI3K nor AKT inhibition is sufficient to rescue CtE from HSV-2-induced persistence, suggesting that viral activation of these pathways does not alter chlamydial development in co-infected cells. Alternatively, simultaneous activation of multiple pathways may be required – a possibility we are currently evaluating.

IMMUNOSUPPRESSION INCREASES POST-AMOXICILLIN SHEDDING IN C. MURIDARUM-INFECTED MICE.

Regenia Phillips Campbell, Jennifer Kintner, Robert V. Schoborg,
Department of Biomedical Sciences, Quillen College of Medicine, East Tennessee State University

Background and Significance: In culture, exposure to stressors induce chlamydiae to reversibly detour from normal development, entering a non-infectious, viable state alternately termed persistence or the chlamydial stress response. Recently, we demonstrated that a similar transition occurs in amoxicillin (amox)-treated, Chlamydia muridarum-infected BALB/c mice. Amox treatment decreased vaginal shedding by >99%, while chlamydial pre-rRNA accumulation remained unchanged. Shedding of infectious EB resumed within 1 week after treatment cessation. Additionally, post-amox shedding was significantly higher than that observed at the same day post-infection (dpi) in water-gavaged mice. Interestingly, earlier amox treatment altered the timing and magnitude of post-amox shedding. These data suggested that host immunity might limit chlamydia’s exit from persistence in our model.

Objectives: To test the hypothesis that cyclophosphamide (CTX) ablation of the immune response will increase the magnitude of chlamydial shedding observed after amox-treatment termination.

Methods: Nine-week-old mice were vaginally infected with C. muridarum and swab samples collected for titer every third day. Amox was administered 5-11 dpi and >99% decreased shedding was observed, as previously noted. A loading dose of CTX was given at 12 dpi and lower doses continued for 10 days.

Results: All amox-treated mice resumed shedding, but maximal post-amox shedding observed in CTX-treated was 3.74 times higher (1.2x10^7 IFU/mouse) than the peak observed before amox-treatment (3.2x10^6 IFU/mouse). CTX-treatment increased post-amox shedding by more than 10-fold compared to amox-only controls.

Conclusions: These data suggest that the immune response strongly restricts recovery of persistent chlamydiae. Ongoing studies seek to determine the in vivo effects of persistence on the effectiveness of anti-chlamydial antibiotic therapy.
INDUCTION OF CHLAMYDIAL PERSISTENCE/STRESS RESPONSE INCREASES AZITHROMYCIN TREATMENT FAILURE IN A MOUSE GENITAL INFECTION MODEL

Regenia Phillips Campbell, Jennifer Kintner, Robert V. Schoborg

*Chlamydia trachomatis* is the most common sexually transmitted bacterial disease agent worldwide. Though frequently asymptomatic, chlamydial infection can cause extreme pathology including ectopic pregnancy and infertility. Chlamydial species exhibit a unique biphasic developmental cycle, interchanging between the elementary body (EB), a form that is able to infect a cell but that does not replicate, and the reticulate body (RB), which is able to replicate but is not infectious. Once attached to an epithelial cell surface, EB are internalized by receptor-mediated endocytosis and form vacuoles. These fuse to form an inclusion, the membrane-bound structure in which EB transform to RB. After multiple rounds of division by binary fission, RB condense to form intermediate bodies (IB) and then infectious EB, which are released and can infect new host cells. In culture, exposure to stressors induce chlamydiae to reversibly detour from this normal developmental cycle, entering a non-infectious, viable state alternately termed persistence or the chlamydial stress response. Data from cell culture model systems suggests that these persistent forms are resistant to eradication by exposure to azithromycin and doxycycline, two first-line antibiotics. Recently, we demonstrated that the viable but non-infectious state occurs in amoxicillin (amox)-treated, *Chlamydia muridarum*-infected BALB/c mice. In our model, amox-treatment decreased vaginal shedding of infectious chlamydiae by >99%, while viability of the organisms remained unchanged. Shedding of infectious EB resumed within 1 week after amox-cessation. Additionally, post-amox shedding was significantly higher than that observed at the same day post-infection (dpi) in water-gavaged mice, suggesting that host immunity might limit chlamydia's exit from persistence in our model. Thus, we hypothesized that cyclophosphamide (CTX) ablation of the immune response would increase the magnitude of chlamydial shedding observed after amox-treatment termination. To test this hypothesis, mice were vaginally infected with *C. muridarum* and swab samples collected for titer every third day. Amox was administered 5-11 dpi and >99% decreased shedding was observed, as previously noted. A loading dose of CTX was given at 12 dpi and lower doses continued for six days. Interestingly, CTX-treatment increased post-amox shedding by more than 10-fold compared to amox-only controls. These data indicate that host immunity does indeed reduce the efficiency with which persistent chlamydiae re-enter normal development in our system. To determine whether persistent chlamydiae are resistant to antibiotic eradication *in vivo*, we induced persistence by administering amox as before and treated mice with either a single high dose or repeated moderate doses of azithromycin (azith) beginning 6 dpi. Mice with productive chlamydial infections were completely cured following either dose of azithromycin. However, persistently-infected mice demonstrated 25% treatment failure following high-dose azith therapy. These data suggest that persistent chlamydiae are refractory to treatment *in vivo* and provide a cogent explanation for the observation that azith therapy fails in some patients. In addition, these experiments provide clinically important evidence that persistent/stressed chlamydial forms may serve as a long-term reservoir of infectious organisms *in vivo*. 

2013 APPALACHIAN STUDENT RESEARCH FORUM
APRIL 4, 2013
Millennium Centre, Johnson City, TN

*The Appalachian Student Research Forum is a regional competition in which participants present their research in a formal setting. The Forum is open to undergraduate, graduate, medical, and pharmacy students, as well as post-docs, medical residents, and fellows from Universities and Colleges across the Appalachian region.*
ELEVATED GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) PROTEIN EXPRESSION IN WHITE MATTER FROM AUTISM SPECTRUM DISORDER SUBJECTS

Jessica D. Crawford, Michelle J. Chandley, Brandon L. Waters, Jason L. Crawford, and Gregory A. Ordway

Current theories of the pathophysiology in autism spectrum disorders (ASD) suggest that dysfunction of the brain’s neurons contribute to the abnormal behaviors observed in ASD patients. Neurons depend on brain glial cells to provide nutrients and growth factors as well as to propagate action potentials between cells. Additionally, glial cells support neuronal axons that project through white matter regions to connect brain regions. Hence, glial cells are as important as neuronal cells in the biology of neurotransmission in the brain. A popular theory is that ASD behaviors result from a disruption in neuronal connectivity between brains regions in autism. We considered that dysfunctional connectivity in autism may be a result, at least in part, of glial pathology. In this study, we examined potential glial pathology in autism using postmortem brain tissues from ASD patients and normal control subjects matched by gender and age. Several glial cell proteins and mRNAs were measured quantitatively in cingulate cortex (Brodmann’s Area 24) white matter (CCWM). The cingulate cortex is a brain area that mediates social interaction, emotion, and learning; behaviors that are typically and often severely disrupted in ASD patients. Two types of glial cells highly expressed in CCWM, astrocytes and oligodendrocytes, were studied. We used the cell-specific marker genes glial fibrillary acidic protein (GFAP) for astrocytes and myelin oligodendrocyte glycoprotein (MOG) for oligodendrocytes. Any changes in either of these marker genes would indicate possible damage to the myelin sheath surrounding axons. Using Western blot (N=13) analyses, we found a significant increase (p=0.005) in GFAP protein expression in CCWM, but MOG protein expression was unaltered. This change in GFAP protein expression was not found in the neocortical gray matter tissue from the same brain area. To determine if the change in GFAP may be mediated transcriptionally, we performed polymerase chain reaction (PCR) analyses of gene expression on both CCWM samples (N=13) and GFAP positive astrocytes (N=8) captured from the same region by laser microdissection. GFAP gene expression was unchanged between ASD and control subjects showing pathology was present only at a translational or protein level. These findings demonstrate the need to further explore the overall health of white matter astrocytes to determine their possible contributory role in ASD pathology. Within this population of white matter glia, other markers of glial health as well as major cellular pathways could be affected. The present study moves the field of ASD research forward by discovering an additional cell type that could contribute to disease pathology.

HUMAN ENTEROPEPTIDASE LIGHT CHAIN: BIOENGINEERING OF RECOMBINANTS AND KINETIC INVESTIGATIONS OF STRUCTURE AND FUNCTION

Eliot T. Smith and David A. Johnson

The serine protease enteropeptidase exhibits a high level of substrate specificity for the cleavage sequence DDDDK−X, making this enzyme a useful tool for the separation of recombinant protein fusion domains. In an effort to improve the utility of enteropeptidase for processing fusion proteins and to better understand its structure and function, two substitution variants of human enteropeptidase, designated R96Q and Y174R, were created and produced as active (>92%) enzymes secreted by P. pastoris with yields in excess of 1.7 mg/Liter. The Y174R variant showed improved specificities for substrates containing the sequences DDDDK (k_{cat}/K_M = 6.83x10^6 M^{-1} sec^{-1}) and DDDDR (k_{cat}/K_M = 1.89x10^7 M^{-1} sec^{-1}) relative to all other enteropeptidase variants reported to date. BPTI inhibition of Y174R was significantly decreased. Kinetic data demonstrate the important contribution of the positively charged residue 96 to extended substrate specificity in human enteropeptidase. Modeling shows the importance of the charge-charge interactions in the extended substrate binding pocket.
2013 APPALACHIAN STUDENT RESEARCH FORUM—Cont’d.

EXOGENOUS UBIQUITIN PLAYS A PROTECTIVE ROLE IN THE HEART AGAINST ISCHEMIA/REPERFUSION INJURY: ROLE OF CXCR-4 IN FIBROBLAST FUNCTION

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We have shown that exogenous ubiquitin (UB) plays an important role in modulation of β-adrenergic-receptor stimulated remodeling with effects on left ventricular function, fibrosis, and myocyte apoptosis. In isolated cardiac fibroblast, UB enhanced expression of matrix metalloproteinase-2 and tissue inhibitor of MMP-2. The objectives of this study were to 1) investigate the role of exogenous UB in structural and functional remodeling of the heart following ischemia/reperfusion (I/R) injury; and 2) examine the activation of signaling pathways (ERK1/2 and Akt) and wound healing in cardiac fibroblasts in response to UB. Methods and Results: C57BL/6 mice (25-30g) were infused with vehicle (I/R group) or UB (UB+I/R group; 1μg/g/h) using mini-osmotic pumps one day prior to surgery. Hearts were then subjected to ischemia (45 min) followed by reperfusion (3 days). Mice in the sham group underwent the same procedure except for I/R. All the mice survived in I/R group, while only 50% survived in I/R group. Analysis of heart function using echocardiography exhibited decreased percent fractional shortening and ejection fraction in I/R group when compared to SHAM and UB+I/R groups (N=6; p<0.05 vs SHAM and UB+I/R). Interestingly, heart function was preserved in UB+I/R group. Quantitative analyses of fibrosis using Masson’s trichrome stained sections and myocyte cross sectional area using immunohistochemistry demonstrated no change among the groups (SHAM, I/R and UB+I/R). Apoptosis was significantly lower in UB+I/R when compared to I/R group. In isolated cardiac fibroblasts, UB treatment (10 μg/ml) for 15 min activated ERK1/2, not Akt, as analyzed by western blots. Activation of ERK1/2 was inhibited by pretreatment with PD 98059 (15 μM; ERK1/2 inhibitor). Pretreatment with AMD3100 (10 μM; inhibitor of CXCR-4 receptor) failed to inhibit UB-mediated activation of ERK1/2. Wound healing assay showed that UB inhibits migration of fibroblasts in the wound 24 and 48 hours after introduction of the wound when compared to controls (N=4; p<0.05). AMD3100 enhanced the migration of fibroblasts in the wound in the presence of UB at both time points. Conclusion: Exogenous UB plays a cardioprotective role in I/R injury with decreased cardiac cell apoptosis. Fibroblast migration appears to involve CXCR-4 receptor-dependent mechanism. However, further investigations are needed to identify the receptor/s involved in activation of ERK1/2 in response to extracellular UB.

AN EXAMINATION OF THE INHIBITORY EFFECTS OF ANTIBIOTIC COMBINATIONS ON MOLECULAR TARGETS IN STAPHYLOCOCCUS AUREUS

Justin Beach and Scott Champney

Bacteremia initiated by Staphylococcus aureus infections can be a serious medical problem. Although a number of antibiotics are used to combat staphylococcal infections, resistance has continued to develop. Combination therapy for certain infections has been used to reduce the emergence of resistance when a single agent has become ineffective. We hypothesize that the use of rifampicin and ciprofloxacin combinations with azithromycin, known for its inhibitory effects on the bacterial ribosome, can create potential synergistic effects resulting from indirect effects on ribosomal subunit synthesis.

Antibiotics in combination were tested for relative inhibition of CFUs. Rates of ³H thymidine, uridine and alanine incorporation were measured with each drug combination. ³H uridine pulse and chase labeling measured subunit synthesis rates RNA. RNA turnover was assayed by Northern blotting.

Combination drug treatments gave a sharp decrease in numbers for cells grown in the presence of azithromycin and rifampicin (28% of control) when compared to those grown with azithromycin or rifampicin alone (90% and 68% of control). A further decrease was observed when ciprofloxacin was added along with azithromycin and rifampicin (to 10% of control). DNA, RNA, and protein synthesis rates were reduced with single antibiotic treatments, and showed further decreases when different combinations were used (10% of control for triple antibiotic treated samples). Ribosomal subunit synthesis for 30S and 50S subunits was complete at 30 minutes in the absence of drug. In the presence of antibiotics the time to completion was 60-90 minutes. The largest decrease in synthesis rates was observed in cells treated with azithromycin and rifampicin (78% of control with one antibiotic). Turnover of 16S and 23S rRNA was observed in each case and was stimulated by antibiotic combinations.

These studies have revealed synergistic interaction occurring with combination antibiotic treatments with ribosomal subunit assembly as a target. This research will provide information needed for the design of more effective antibiotic combinations.
ROLE OF ATM IN STRUCTURAL AND FUNCTIONAL REMODELING OF THE HEART FOLLOWING MYOCARDIAL INFARCTION
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Background: ATM (Ataxia Telangiectasia Mutated Kinase) recognizes DNA double stranded breaks and phosphorylates several proteins including p-53 (ser15) in order to initiate repair. Previously, our laboratory has shown that β-adrenergic receptor (b-AR) stimulation increases ATM expression in the heart, and ATM plays a significant role in myocardial remodeling following b-AR stimulation with effects on heart function, fibrosis and apoptosis. The objective of this study was to investigate the role of ATM in myocardial remodeling one day following acute myocardial infarction (MI). Methods: MI was performed in ATM heterozygous knockout (hKO) and wild type (WT) mice by ligation of the left anterior descending artery. Cardiac function was measured using M-mode echocardiography. Fibrosis, apoptosis, and levels of α-smooth muscle actin (α-SMA) were examined using histochemical techniques. Phosphorylation of p53 and protein levels of MMP-2 and MMP-9 were examined using western blots. In-gel zymography was performed to examine the activity of MMP-2 and MMP-9. Results: Two dimensional M-mode echocardiography revealed that MI causes a decrease in percent fractional shortening (%FS) and ejection fraction (EF) in WT mice when compared to WT-sham. However, %FS and EF were preserved in hKO-MI group when compared to hKO-sham. In addition %FS and EF were significantly higher in hKO-MI when compared to WT-MI (p<0.05; n=8-9). Quantitative analysis of fibrosis using Masson’s trichrome stained sections and quantitative analysis of α-SMA using immunohistochemical staining showed no differences among the groups. Measurements of cardiac cell apoptosis, using TUNEL assay, showed significantly higher apoptosis in hKO-MI when compared to WT-MI (hKO 27.32% vs WT 17.21%; p<0.05; n=5-7). Phosphorylation of p53 was increased in both MI groups when compared to sham groups. However, it was not found to be significantly different between the two MI groups. Increased MMP-2 protein levels and activity were not different between the two MI groups. However, increased MMP-9 activity (84 kDa band) was significantly higher in WT-MI group when compared to hKO-MI (P<0.05; n=3). Summary: Deficiency of ATM preserves heart function and is associated with an increase in cardiac cell apoptosis and a decrease MMP-9 activity.

THE PURINE NUCLEOTIDE CYCLE: A NOVEL PATHWAY INDUCED BY HIF1α
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Hypoxia inducible factor 1α (HIF1α) is a transcription factor that promotes cellular adaption when oxygen availability is insufficient. While oxidative phosphorylation supplies ATP during normoxia, this energy generating mechanism is abolished in the absence of oxygen. Therefore, it should not be surprising that HIF1α is well-established to induce compensatory glycolytic activity that promotes ATP generation in the absence of O2. Here we have identified the purine nucleotide cycle (PNC) as another metabolic pathway that is upregulated by HIF1α. The PNC may compensate for hypoxia via several mechanisms. We find that HIF1α stabilization leads to increased mRNA, protein, and activity of the enzyme AMP deaminase in mice hearts. This enzyme represents the committed step of the PNC and catalyzes the reaction that converts AMP to IMP. The conversion of AMP to IMP has been proposed as a key biochemical reaction that supports cell survival. In subsequent steps of the PNC, IMP is converted to adenylosuccinate by adenylosuccinate synthetase. The PNC is completed when adenylosuccinate is converted back to AMP by adenylosuccinate lyase while generating fumarate as a side product. We have previously shown that HIF1α expression allows cardiac myocytes to utilize fumarate to maintain mitochondrial membrane potential. Here mitochondrial complex I can initiate electron flow by oxidizing NADH. This electron is then passed onto complex II, where fumarate can act as an alternative terminal electron acceptor during anoxia. This process allows for continued electron flow that is coupled to pumping of H+ into the mitochondrial intermembrane space, thus allowing mitochondrial membrane potential to be sustained during anoxia. Therefore, the PNC may protect mitochondrial integrity by providing fumarate for anaerobic respiration. In support of this, recent experiments show that PNC inhibitors diminish the ability of HIF1α expressing cells to maintain mitochondrial membrane potential. In addition, the PNC conserves cellular resources by salvaging nucleotides broken down during ischemia rather than relying on de novo pathways for their re-synthesis upon reperfusion. Taken together, it is likely that the upregulation of the PNC by HIF1α is a critical mechanism that provides cardioprotective effects.
Major depressive disorder (MDD) is the third leading human disease worldwide and it is associated with a high rate of suicides. Inflammatory responses are accompanied by the induction of oxidative and nitrosative stress pathways and are key mediators in the pathophysiology of MDD. Increased levels of reactive oxygen species (ROS) and shortened telomere lengths are a focus of research in the field, and both are hypothesized to contribute to accelerated cellular aging in patients with MDD. The defense system protecting the cell against ROS consists of key antioxidant enzymes. Glial cells (oligodendrocytes and astrocytes) express pathology in MDD as well as in other behavioral disorders. The purpose of this study was to evaluate enzymes in the ROS defense system within glial cells in MDD patients who have committed suicide, and to evaluate the relationship between the ROS defense system and accelerated cellular agingmarked by shortened telomere lengths observed in these same cell populations.

Oligodendrocytes and astrocytes were identified with 2',3'-cyclic-nucleotide 3'-phosphodiesterase (CNP) and glial fibrillary acidic protein (GFAP) immunostaining, respectively. Glia were captured by laser microdissection from uncinate fasciculus (UF, connecting prefrontal cortex to temporal lobe) and prefrontal cortex BA10 white matter (PCWM) from MDD and control subjects (11-12 pairs) matched for age, sex, postmortem interval and smoking history, and having no psychoactive drugs in blood. Total RNA was isolated, cDNA was synthesized by reverse transcription and gene specific primers were designed for target genes within the ROS defense system, which included cytosolic superoxide dismutase (SOD1), mitochondrial superoxide dismutase (SOD2), catalase (CAT) and glutathione peroxidase 1 (GPx1). Quantitative end-point PCR was used to measure the expression of target genes (SOD1, SOD2, CAT, GPx) in each of the samples, and the PCR products were quantified using an Agilent Bioanalyzer. In BA10, the expression of SOD1, SOD2, CAT and GPx1 were down-regulated in oligodendrocytes of patients with MDD (significant @ p = 0.0015, <0.0001, 0.0011, 0.0011). Similarly in the uncinate fasciculus, the expression of SOD1, SOD2, CAT and GPx1 were down-regulated in the oligodendrocytes of patients with MDD (significant @ p = 0.0012, <0.0001, 0.0015, 0.0021). In contrast to oligodendrocytes, gene expression of ROS defense enzymes was normal in astrocytes collected from the same brain regions. These novel findings describe a cell type specific ROS defense compensation that correlates directly to telomere shortening previously reported in oligodendrocytes from these MDD subjects. These data provide evidence that telomere shortening in glia of patients with MDD is accompanied by oxidative stress damage. Additionally, the absence of astrocyte pathologies suggest that oligodendrocytes in the deep white matter may be particularly susceptible to oxidative stress damage and accelerated cellular aging, which may be due to the high level of lipid production and associated oxidative load that is lower in astrocytes. Understanding the system of ROS defense and its influence on cellular aging marked by telomere shortening in glial cells is essential in understanding the biological underpinnings of the disease, and additionally serve as a potential target for MDD patients resistant to current pharmacological treatments.

EXPRESSON OF RECOMBINANT HUMAN NEUTROPHIL CATHEPSIN G

Evan Perry, Eliot Smith, David Johnson

Cathespin G (CatG), a serine protease found in the azurophil granules of neutrophils, participates in killing engulfed microorganisms. CatG has dual specificity for chymotrypsin-like and tryspin-like substrates. CatG is a poorly understood enzyme and is currently only commercially available as purified enzyme purified from human sources. The yeast *Pichia pastoris* is being used to express CatG to study its dual specificity and its C-terminal processing. The full length (C-terminus present) human CatG amino acid sequence was modified to remove one glycosylation site and eight dibasic sites to avoid potential cleavage by yeast kexin protease. The construct was engineered to have an N-terminal 6-His-cytochrome B5 (CytB5) heme binding fusion domain linked to the modified human CatG by an enterokinase cleavage site for activation. The amino acid sequence was used to generate a codon-optimized gene that was placed in the pPICZa secretion vector. After transforming *Pichia pastoris* strain X-33, 48 Zeocin-resistant clones were screened for relative levels of CatG activity. Recombinant CatG has been partially purified from fermentation media by nickel affinity chromatography and its activity has been confirmed by assays using synthetic substrates. Supported by a Student Faculty Collaborative Grant from the ETSU Honors College and ETSU Office of Research and Sponsored Programs and by NHLB grant R15HL091770.
THE ROLE OF TYPE-2 CANNABINOID RECEPTOR (CB2) IN CALCIFICATION OF ATHEROSCLEROTIC LESIONS
Kaitlyn Hinshaw, Zachary Lahr and Douglas Thewke, Department of Biomedical Sciences, Quillen College of Medicine, East Tennessee State University, Johnson City, TN

Introduction: Atherosclerosis is a chronic inflammatory disease characterized by lesions containing cholesterol, fat and other cellular debris within arterial walls. The cellular composition of early atherosclerotic lesions in mice is modulated by the type-2 cannabinoid receptor (CB2). As atherosclerotic lesions advance they undergo a cell-mediated calcification similar to bone remodeling. However, the role of CB2 in lesion calcification is not well understood. During bone remodeling, CB2 is known to alter differentiation of osteogenic precursor cells. Thus, we hypothesize that CB2 may modulate lesion calcification by affecting osteogenic differentiation of cells within lesions. To test this hypothesis, we examined the effect of CB2 signaling on osteoclastogenesis from murine monocyte/macrophages and osteoblastogenesis from vascular smooth muscle cells in vitro. Methods: RAW264.7 cells, a murine monocye/macrophage cell line, which undergoes osteoclastogenesis in response to receptor activator of nuclear factor kappa B ligand (RANKL), were cultured in media containing RANKL and various CB2 agonists or antagonists. Osteoclastogenesis was evaluated by measuring tartrate-resistant acid phosphatase (TRAP) activity and further verified by visual quantitation of multinucleated, TRAP-positive cells. MOVAS-1 cells, a murine vascular aortic smooth muscle cell line capable of differentiating into osteoblasts when cultured in osteogenic media, were cultured in osteogenic media supplemented with CB2 agonists or antagonists. Osteoblastogenesis was evaluated by measuring alkaline phosphatase (ALP) activity and confirmed by alizarin red staining for calcium deposition. Results: RAW264.7 cells treated with Win55,212-2, a nonselective CB agonist, or HU-308, a selective CB2 agonist, displayed a dose-dependent decrease in RANKL-induced TRAP activity. Co-administration of a CB2-selective antagonist (SR144528), but not a CB1-selective antagonist (AM251), blocked this effect. Visual quantitation of multinucleated TRAP-positive cells confirmed Win55,212-2 treatment reduced osteoclastogenesis in RANKL-treated RAW264.7 cells. Induction of osteoblastic differentiation of MOVAS-1 cells, as determined by ALP activity, was enhanced by supplementation with Win55, 212-2 or 2-archidonyl glycerol (2-AG), an endocannabinoid. Co-administration of SR144528, but not AM251, reduced the activation of ALP by Win55,212-2 and 2-AG in MOVAS-1 cells. Alizarin red staining revealed increased calcium deposition in cultures of MOVAS-1 cells grown in osteogenic medium supplemented with Win55,212-2 compared to those cultured in osteogenic medium alone.

Conclusions: These results demonstrate that CB2 activation alters osteogenic differentiation in cell lines representative of two prominent cell types found within lesions. This supports the hypothesis that CB2 promotes lesion calcification by stimulating osteoclastogenesis and inhibiting osteoblastogenesis.

MOLECULAR ANALYSIS OF GFAP POSITIVE ASTROCYTES FROM NEOCORTICAL GRAY MATTER OF THE ANTERIOR CINGULATE CORTEX IN AUTISM SPECTRUM DISORDER
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Brodmann’s area 24 (BA24) is part of the cingulate cortex within the human brain. It occupies most of the anterior cingulate cortex around the genu of the corpus callosum. This brain area plays a significant role in a variety of cognitive function including decision-making, social interaction, and reward anticipation. In autism spectrum disorder (ASD), there is distinct pyramidal neuronal pathology in BA24 from male subjects. Astrocytes are glia cells that support neurons by providing growth factors, maintaining ion concentrations, and participation in neurotransmission. Astrocyte dysfunction may contribute to neuronal pathology in this area. The present study examines the expression of genes in astrocytes that participate in glutamate regulation, diffusion, and synaptic plasticity. Immunohistochemically-guided laser capture microdissection was used to obtain astrocytes from BA24 neocortical gray matter brain tissue of autistic subjects and psychiatrically normal control brain tissue (N=8). End-point PCR was used to determine gene expression changes in four genes in astrocytes: an astrocyte-specific water channel, two glutamate transporters, and one marker gene for reactive astrocytes. Astrocyte specific marker genes and genes involved in glutamate transport did not demonstrate expression differences in cells collected from the autism brain tissue when compared to control tissue. The initial molecular assessment of reactive astrocytes reveal normal amounts of messenger RNA involved in essential astrocyte functions. However, this does not exclude astrocytes as a contributing factor to the observed neuronal pathology. Further examination of distinct signaling pathways is necessary to conclusively determine the condition of astrocytes in autism.
**9-PHENANTHROL INHIBITS CALCIUM OSCILLATIONS IN HL-1 MOUSE CARDIOMYOCYTES**

Rees Burt1, Bridget M. Graves2, Chaunfu Li2, David L. Williams2, Santiago P. Fregoso1, Donald B. Hoover1, Robert Wondergem1. 1Biomedical Sciences, 2Surgery, East Tennessee State University, Johnson City, TN

Transient Receptor Potential Melastatin 4 (TRPM4) is functionally expressed throughout the heart and has been implicated as a calcium-activated nonselective cation channel that mediates membrane depolarization. The functional significance of TRPM4 in regards to Ca\(^{2+}\) signaling and its effects on cellular excitability and pacemaker function remains inconclusive. We show by Fura2 Ca-imaging that pharmacological inhibition of TRPM4 in HL-1 mouse cardiac myocytes by 9-phenanthrol (10 \(\mu\)M) decreases Ca\(^{2+}\) oscillations followed by an overall increase in \([\text{Ca}^{2+}]_i\). The latter occurs also in HL-1 cells in Ca-free solution and after depletion of sarcoplasmic reticulum Ca\(^{2+}\) with thapsigargin (10 \(\mu\)M). Furthermore, by whole-cell voltage clamp we show that 9-phenanthrol reversibly inhibits TRP-like membrane current; by fluorescence immunohistochemistry we demonstrate that HL-1 cells display punctate surface labeling with TRPM4 antibody. We conclude that 9-phenanthrol inhibits TRPM4 ion channels in HL-1 cells, which in turn decreases Ca\(^{2+}\) oscillations followed by a compensatory increase in \([\text{Ca}^{2+}]_i\) from an intracellular store other than the sarcoplasmic reticulum. We speculate that the most likely source is the mitochondrion.

**OPTIMUM CONDITIONS FOR ENTEROPEPTIDASE EXPRESSION IN PICHIA PASTORIS**

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The yeast *Pichia pastoris* has been used extensively for the expression of recombinant proteins. DNA coding for human enteropeptidase light chain was cloned into the pPICz\(\alpha\)A vector which contains flanking sequences homologous with the alcohol oxidase (AOX1) gene promoter of *Pichia*, and a sequence coding for alpha mating factor for foreign protein secretion. AOX1 is up-regulated in the presence of methanol, resulting in the expression of foreign proteins coded by DNA under control of the AOX1 gene promoter. The X-33 strain of *Pichia* was transformed with the vector containing the coding region of human enteropeptidase light chain (rhEPL), which is a serine protease with specificity for cleaving proteins with DDDDK-X sequences. Transformation results in homologous recombination of the foreign protein coding DNA into the *Pichia* genome. In the presence of methanol, *Pichia* secrete active rhEPL, which can be measured in the media using Z-Lys-thiobenzyl ester substrate. Published protocols usually involve growing *Pichia* in media with glycerol as the carbon source and then switching to methanol. Glycerol has been reported to suppress AOX1, whereas the carbon source sorbitol does not. This study examined co-feed conditions using varying amounts of glycerol or sorbitol, coupled with methanol. Baffled shake flasks were fed daily and monitored throughout log phase for cell growth and rhEPL activity. Daily 1 mL samples were taken from each culture over a five day period and the OD600 was measured. Assays reporting the activity of rhEPL for each sample were conducted using Z-Lys-thiobenzyl ester substrate once log phase was completed and the results were analyzed. The conclusions of this study confirm that glycerol severely inhibits rhEPL expression in a concentration dependent manner. Sorbitol, while being slightly inhibitory, increases cell growth such that total rhEPL production is improved with sorbitol/methanol co-feeds. Supported by NIH grant R15HL091770.
PROXIMITY-LABELING OF MOUSE FIBROBLASTS PROTEINS THAT ARE NEAR-NEIGHBORS OF LAMIN A AND LAMIN A-D50 (PROGERIN)

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Abstract: Mutations in the gene encoding nuclear lamin A cause the premature aging disease Hutchinson-Gilford Progeria Syndrome. One of these mutations results in the expression of a mutant lamin A, with a 50-aa in frame deletion within its C terminus. Our laboratory previously demonstrated that this deletion leads to a stable farnesylation and carboxymethylation of the mutant lamin A. These modifications cause an abnormal association of progerin during mitosis; induce changes in the cell cycle progression and may cause DNA damage, among many other effects. In an attempt to isolate and identify proteins that differentially interact with or locate near Lamin A and progerin, we used a previously described method named BioID (proximity-dependent biotin identification). This method is based on fusion of a promiscuous E. coli biotin-protein ligase (BL) to a targeting protein (in this study, lamin A-GFP and progerin-GFP). The biotin ligase biotinylates amino residues in proteins that are near-neighbors of the fusion protein. To create the fusion proteins, BL was sub-cloned from a pCDNA3.1 MCS-BirA(R118G)-HA plasmid donated by Kyle Roux from University of South Dakota. The BL fragment was ligated into a pNEBR-X1-Lamin A-GFP and pNEBR-X1-progerin-GFP which indubitably express the fusion proteins in mammalian cells, under control of pNEBR-R1 Rheoswitch regulator plasmid. Two stable cell lines expressing the GFP-BL-lamin A and GFP-BL-progerin chimeras were created. The expression of the chimeras was induced by incubation with 500nM of Genostat for 24h in the presence of 50nM Biotin. Biotinylated proteins were isolated from cell lysates by incubation with streptavidin magnetic beads. Proteins were separated by SDS-PAGE and sent for identification by mass spectrometry. In conclusion, we isolated multiple proteins that differentially associate with and/or are proximate to lamin A and progerin in vivo. The identification of such proteins may shed light into the mechanism by which progerin causes its deleterious effects.

COMPENSATED OXIDATIVE STRESS DEFENSE IN MAJOR DEPRESSIVE DISORDER

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Major depressive disorder (MDD) is the third most prevalent disease worldwide. Inflammation and the induction of reactive oxygen species (ROS) are hypothesized to be key mediators in the disease. The purpose of this study was to evaluate the ROS defense system and telomere shortening in glia of patients with MDD.

THE ROLE OF TYPE-2 CANNABINOID RECEPTOR (CB2) IN CALCIFICATION OF ATHEROSCLEROTIC LESIONS

Kaitlyn Hinshaw, Zachary Lahr and Douglas Thewke. Kaitlyn Hinshaw, Zachary Lahr and Douglas Thewke, Department of Biomedical Sciences, Quillen College of Medicine, East Tennessee State University, Johnson City, Tennessee.

The mechanisms controlling calcification of atherosclerotic lesions may be similar to those of bone remodeling. The type-2 cannabinoid receptor (CB2) regulates bone remodeling; however its role in lesion calcification is unclear. This project examines the potential role of CB2 in altering osteogenic processes of vascular cells in vitro.

-PHENANTHROL INHIBITS CALCIUM OSCILLATIONS IN HL-1 MOUSE CARDIOMYOCYTES

Rees Burt1, Bridget M. Graves2, Chaunfu Li2, David L. Williams2, Santiago P. Fregoso1, Donald B. Hoover1, Robert Wondergem1. 1Biomedical Sciences, 2Surgery, East Tennessee State University, Johnson City, TN.

Transient Receptor Potential Melastatin 4 (TRPM4) is functionally expressed throughout the heart and has been implicated as a calcium-activated nonselective cation channel that mediates membrane depolarization. The functional significance of TRPM4 in regards to Ca2+ signaling and its effects on cellular excitability and pacemaker function remains inconclusive. We show by Fura2 Ca-imaging that pharmacological inhibition of TRPM4 in HL-1 mouse cardiac myocytes by 9-phenanthrol (10 μM) decreases Ca2+ oscillations followed by an overall increase in [Ca2+]i. The latter occurs also in HL-1 cells in Ca-free solution and after depletion of sarcoplasmic reticulum Ca2+ with thapsigargin (10 μM). Furthermore, by whole-cell voltage clamp we show that 9-phenanthrol reversibly inhibits TRP-like membrane current; by fluorescence immunohistochemistry we demonstrate that HL-1 cells display punctate surface labeling with TRPM4 antibody. We conclude that 9-phenanthrol inhibits TRPM4 ion channels in HL-1 cells, which in turn decreases Ca2+ oscillations followed by a compensatory increase in [Ca2+]i from an intracellular store other than the sarcoplasmic reticulum. We speculate that the most likely source is the mitochondrion.