

You are Invited to the 29th Annual Graduate Student Research Forum



Graduate Student Poster Exhibit

Thursday, November 13, 2008

9:00 am - 12:00 pm

4th Floor, MSB



Keynote Address:

"Immunity is a conversation, not a war"

1:30 - 3:00 pm

E351 Medical Sciences Building (MSB)

Polly Matzinger, Ph.D

Chief, T-Cell Tolerance and Memory Section

National Institute of Allergy and Infectious

Disease (NIAID), NIH



Awards Ceremony & Reception

3:00 pm - 4:00 pm

MSB Cafeteria (across from Starbucks)

Medical Sciences Building



2008 Graduate Student Research Forum



Trudy Aebig - Poster Number: 1


Title

PLECTIN 1b: A REAL CONNECTION BETWEEN MITOCHONDRIA AND INTERMEDIATE FILAMENTS

Authors

Aebig T. J., Giacalone N., and Parysek L. M.

Abstract



The cytoskeletal network, together with associated proteins, mediates attachments to mitochondria. Microtubules and microfilaments interact with outer mitochondrial membrane proteins via associated motor proteins, modulating mitochondrial morphology, division, and inheritance and coordinating mitochondrial motility and distribution. Mitochondria also interact with different types of intermediate filaments (IF), including desmin, keratins, and neurofilaments; the significance and mechanism of these associations, however, are poorly understood. Recently, a study in fibroblasts showed that the N-terminus of plectin 1b, a large cytolinker protein, spans the outer mitochondrial membrane, mediating a linkage to cytoplasmic IF. Our studies are aimed at analyzing the precise mechanism of mitochondria-plectin 1b interaction. We confirmed that the 1b exon-encoded amino acids, unique to the plectin 1b isoform, target plectin's association with mitochondria by demonstrating that removal of the 1b exon abolishes localization to mitochondria. Additionally, we found that 36% of 3T3 fibroblast cells transfected with truncated plectin 1b, which contains exons 1b-8 and does not contain the carboxy terminal IF-binding domain, expressed fusion plectin 1b protein that was localized to mitochondria, suggesting physiologic regulation of membrane insertion or retention of plectin 1b. Current studies focus on determining the role of a plectin 1b-interacting protein, which we have newly identified, in regulating the connection of plectin 1b to mitochondria. Investigating the molecular mechanism that regulates the connection of plectin 1b to mitochondria will aid in deciphering plectin 1b's role in mitochondrial function.



2008 Graduate Student Research Forum



Jane Allendorfer - Poster Number: 2


Title

fMRI brain activation in recreational ecstasy (MDMA) users during working memory.

Authors

Jane B. Allendorfer, Martine Lamy, James C. Eliassen

Abstract



Ecstasy is a widely abused street drug, with a lifetime prevalence of about 10-14% for college students. MDMA, or 3,4-methylenedioxy-methamphetamine, is the primary psychoactive substance in ecstasy, and has been shown in animal studies to damage serotonergic neurons and to impair memory performance. However, due to the variability of drug exposure in humans, the cognitive and functional consequences of recreational ecstasy (MDMA) use remain unclear. To investigate the effects of ecstasy use on memory, we studied three groups of subjects: current ecstasy users (n=14), non-ecstasy drug users (n=14) matched to the ecstasy users by cannabis use, and non-drug users (n=14). During fMRI, participants performed an N-back task (0-back, 1-back and 2-back) in which they pressed the button corresponding to the number either on the screen (0-back), one screen prior (1-back), or two screens prior (2-back). Since neurotoxic effects of MDMA are thought to underlie memory impairments in ecstasy users, we hypothesized that ecstasy users will exhibit abnormal activation during a working memory task in brain regions that receive dense serotonergic innervation. All three groups showed a consistent decline in accuracy as the task increased in difficulty. Compared to non-drug users, drug users overall exhibit a relative increase in active brain regions and in the degree of activation during the working memory task. Compared to the cannabis comparison subjects, ecstasy users showed increased activation in temporal and frontal brain regions during the working memory conditions (1- and 2-back). These regions receive heavy serotonergic innervation, and the differences in activation may be attributed to ecstasy use.



2008 Graduate Student Research Forum



Nabeel Almaomen - Poster Number: 3

Title


Heart failure acceleration and early death in (AE3^{-/-}::Tm180) double mutant mouse: An update.

Authors

Al Moamen NJ¹, Prasad V¹, Miller ML², Wieczorek DF¹, Lasko V³, Neuman M³, Lorenz JN³, Shull GE¹

¹Department of Molecular Genetics, Biochemistry and Microbiology, ²Department of Environmental Health, ³Department of Cellular and Molecular Physiology, University of Cincinnati College of Medicine, Cincinnati, Ohio.

Abstract



Ablation of anion exchanger isoform 3 (AE3^{-/-}) in a mouse model of hypertrophic cardiomyopathy, a transgenic mouse of mutant α -tropomyosin at codon 180 (Tm180), accelerates the onset of heart failure. Dramatic increase in death rate was observed in double mutant mice (AE3^{-/-}::Tm180) vs. transgenic mice with wild type AE3 (AE3^{+/+}::Tm180). At the time point of 5 months after birth, 80% of (AE3^{-/-}::Tm180) mice are dead, whereas only 10% of (AE3^{+/+}::Tm180) mice are dead at this stage of aging. Physiological evaluation of in vivo heart function showed a more severe phenotype of depressed heart contractility and relaxation in (AE3^{-/-}::Tm180) vs. (AE3^{+/+}::Tm180) mice. No differences were observed between the two genotypes in the heart-to-body (H/B) weight ratios and b-myosin heavy chain (b-MHC) levels in whole heart homogenates, two of the hallmarks of cardiac hypertrophy. However, both H/B ratios and b-MHC levels are significantly higher than wild type animals. Levels of phosphorylated troponin I (TnI-P) and phospholamban (PLN-P) were significantly reduced in both genotypes when compared with wild type animals. Both TnI and PLN are important components of the contractile apparatus and calcium handling system, respectively, in cardiac myocytes. Finally, levels of two major protein phosphatases, PP1 and PP2A, in cardiac myocytes were significantly increased in both genotypes vs. wild type mice. These findings highlight the importance of AE3 anion exchanger for heart function that would be severely compromised through the ablation of AE3 in a mouse model of hypertrophic cardiomyopathy.



2008 Graduate Student Research Forum



Jennifer Barger - Poster Number: 4


Title

The loss of S6K1 enhances the oncogenic potential of Bcr-Abl

Authors

Jennifer F Barger, Catherine A Gallo and David R Plas

Abstract



Chronic myelogenous leukemia (CML) is a clonal hematopoietic stem cell disorder characterized by the chromosomal translocation creating the Bcr-Abl oncogene. Bcr-Abl drives leukemia in part through an acquired resistance to apoptosis, which is linked to activation of the PI3K/Akt pathway. Rapamycin is a potential chemotherapeutic for cancers with activated Akt, inhibiting the activation of the downstream target S6K1. We tested Akt-dependent survival in response to rapamycin treatment or S6K1 knockdown in Bcr-Abl expressing hematopoietic progenitor cells. The loss of S6K1 through shRNA or rapamycin treatment prevented survival of cells expressing activated Akt, but did not prevent the survival of Bcr-Abl expressing cells. Rather, the loss of S6K1 enhanced the oncogenic potential of Bcr-Abl in a mouse model of leukemia. These results suggest that S6K1 is required for Akt-dependent survival, but Bcr-Abl compensates for the loss of S6K1 by activating another pathway.



2008 Graduate Student Research Forum



Matthew Benard - Poster Number: 5


Title

A GAIN OF FUNCTION APPROACH TO THE ROLE OF PLACENTAL GROWTH FACTOR IN THE ADULT HEART

Authors

Matthew Benard, Jeff Molkenin

Abstract



Angiogenesis is the process of generating new blood vessels from pre-existing ones. It is a critical regulator in the growth of new cells and tissue, controlling such cellular processes as proliferation, permeability, migration, and survival. Perhaps the most widely recognized protein family involved in angiogenesis is that of the vascular endothelial growth factor family (Vegf). Vegf proteins stimulate the angiogenic process by binding the Vegf receptors Flt1 (VegfR1) and KDR/Flk1 (VegfR2) on endothelial cell surfaces. One member of the Vegf family, placental growth factor (Pgf), shares a 53% homology with Vegf in their respective platelet-derived growth factor-like domains. These structural similarities allow Vegf and Pgf to form homo- or heterodimers with one another, however, dimers involving Pgf are incapable of binding VegfR2 and, thus, only act on VegfR1. Current dogma dictates that relevant angiogenic events are mediated solely through VegfR2 stimulation, suggesting that Pgf and VegfR1 function as decoys responsible for regulating the levels of soluble Vegf available to activate VegfR2. Some studies would suggest that Pgf serves more than a mere regulatory role. Carmeliet *et al.* generated a Pgf *-/-* mouse model that exhibited adult stage impaired angiogenesis during or after ischemia, inflammation, wound healing, and cancer, with no impact observed developmentally. Odoriso *et al.* overexpressed Pgf in skin using a keratin-14 promoter and demonstrated embryonic and adult stage increases in vessel number, size, and permeability. More recently, Iwama *et al.* have shown that elevated levels of Pgf can be detected in human plasma after reperfusion of infarcted arteries, indicating cardiac-specific production and release of the growth factor in response to stress. Building from the literature, we have developed a cardiac-specific, inducible, over-expressing placental growth factor transgenic mouse model to help us address the hypothesis that *placental growth factor is a novel, protective autocrine or paracrine angiogenic effector in the adult heart, which may function through Pgf-dependent VegfR1 signaling*. Preliminary data indicates that overexpression of Pgf does not appear to modulate cardiac, vascular vessel density. Interestingly, however, a significant cardiac hypertrophy is observed in these mice as early as four weeks of age. Putative signaling pathways responsible for this hypertrophy and whether this hypertrophy is a protective compensatory mechanism are currently being assessed.



2008 Graduate Student Research Forum



Dana Borcharding - Poster Number: 6


Title

REGULATION OF PROLACTIN EXPRESSION AND RELEASE IN HUMAN ADIPOCYTES BY CATECHOLAMINES

Authors

Dana Borcharding, Eric Hugo, Terry Brandebourg, and Nira Ben-Jonathan

Abstract



Prolactin (PRL) in humans is produced by multiple extrapituitary sites where it functions as a cytokine. Non-pituitary PRL is regulated by a superdistal promoter in a site-specific manner. In the pituitary, dopamine is the primary inhibitor of PRL expression and release, but has not been previously shown to regulate extrapituitary PRL. Our laboratory has discovered that human adipose tissue, preadipocytes and mature adipocytes produce PRL. Our objectives were to: a) use quantitative real-time PCR and Western blotting to examine whether human adipose tissue and adipocytes express dopamine receptors (DAR), b) determine whether catecholamines alter adipose PRL release, and c) examine the mechanism by which catecholamines regulate adipocyte PRL. All five DAR genes were expressed in visceral (vis), subcutaneous (sc), and breast adipose tissue and mature adipocytes, with the dopamine type-2 receptor (D2R) protein also detected by Western blot analysis. PRL release from adipose explants and differentiated primary preadipocytes was stimulated by isoproterenol, a β -adrenergic receptor agonist, and inhibited by both dopamine and bromocriptine, a D2R agonist. Bromocriptine also decreased PRL gene expression in a time-dependent manner. Dopamine can signal through D1R and D5R to increase cAMP levels, or through D2R, D3R, and D5R to decrease cAMP. Incubation of adipocytes with bromocriptine and dopamine lowered intracellular cAMP levels, while isoproterenol increased cAMP levels. In addition, dopamine sulfate, the primary form of dopamine in human serum, also reduced PRL secretion from adipose explants. Adipose tissue was found to have a high activity of Arylsulfatase A, the enzyme which converts dopamine sulfate to the biologically active parent compound. In conclusion, this is the first demonstration of functional dopamine receptors in human adipose tissue and adipocytes. Dopamine and bromocriptine inhibit adipose PRL production, and this is counteracted by the stimulatory effect of β -adrenergic agonists.



2008 Graduate Student Research Forum



Andrew Browne - Poster Number: 7


Title

RAPID POINT OF CARE (POC) BLOOD ANALYSIS USING INTEGRATED DYNAMIC BLOOD SEPARATION AND SANDWICH IMMUNOASSAY ON A POLYMER LAB CHIP

Authors

Andrew W. Browne, WooSeok Jung, Kang Kug Lee, SeHwan Lee, Jaephil Do, Chong H. Ahn

Abstract



Microfluidic biomedical chips will enable performance of clinical laboratory tests at the patients bedside. Practical microfluidic chips must be capable of integrating sample preparation and analysis. This work demonstrates a seamless integration of dynamic blood separation with sandwich immunoassay on a chip for clinical diagnostics of serum analytes in fewer than 7 minutes. Human blood samples are loaded into the device and propelled by pulsatile pressure down a long channel to affect serum separation from cellular components. Serum samples are further analyzed for target analytes in a small serpentine channel with a volume less than 200 nL. Ultimately this device can facilitate quantification of plasma protein concentrations at the patients bedside.



2008 Graduate Student Research Forum



Charles Burke - Poster Number: 8


Title

Targeted ablation of P5-Type ATPase *atp13a1* causes embryonic lethality in homozygous null mice.

Authors

Charles R. Burke, Marian L. Miller, Patrick J. Schultheis and Gary E. Shull.

Abstract



Gene members of the P5 subfamily of P-type ATPases are present in all known Eukaryotes, and are represented by five gene members, *ATP13a1-5* and *atp13a1-5*, in humans and mice, respectively. In mice, *atp13a1* appears to be expressed in all tissues, whereas the other members of this family exhibit tissue-specific expression patterns. Despite the ubiquitous occurrence of P5-atpases in all known Eukaryotes, and the presence of five separate genes encoding P5-ATPases in humans and mice, very little is known about the function of these proteins, including localization and substrate specificity. In yeast the ATP13a1 orthologue, Cod1p, is thought to be localized to the Endoplasmic Reticulum (ER) and is involved in ER homeostasis, processing and trafficking of proteins and regulated degradation of yeast Hmg-CoA reductase. We performed targeted mutagenesis on the gene encoding *atp13a1* in mice in hopes of better understanding the biology of this protein. Mice heterozygous for *atp13a1* are ostensibly normal with no overt phenotype being attributed to the loss of one copy of *atp13a1*. Homozygous null mutants die *in utero* between Embryonic Day (ED) 12.5 and birth. No living animals have been observed at birth. By ED 13.5 there is an approximate 20% decrease in the number of living *atp13a1*-null embryos. Approximately 40% of the surviving *atp13a1*-null embryos observed between ED 13.5 and 18.5 are significantly smaller than their wild type littermates. Light microscopy has not yet revealed significant differences in histology between knockout animals at ED 13.5 and ED15.5. However, in the knockouts at 13.5 and 15.5 ED there was a trend of increased numbers of reticuloendothelial cells in liver with red cell debris. Electron microscopy has not yet revealed differences at the cellular level between wild type and knockout embryos, either. Hemopoiesis and cardiovascular function are sufficiently well developed in the knockout embryos to permit survival of some embryos up to ED 18.5. Microarray analysis on total RNA taken from whole wild type and knockout ED 13.5 embryos shows significantly altered expression of many genes involved in translation, folding and trafficking of ER proteins, proteasomal-degradation and cholesterol homeostasis. These data indicate mammalian ATP13a1 may play a similar role to that which is performed by Cod1p in yeast, and also demonstrates for the first time at least one copy of ATP13a1 is critical for proper growth and development in mammals.



2008 Graduate Student Research Forum



Jennifer Cash - Poster Number: 9


Title

THE STRUCTURE OF THE MUSCLE GROWTH INHIBITOR MYOSTATIN IN COMPLEX WITH FOLLISTATIN: INSIGHTS INTO ANTAGONIST AND RECEPTOR SPECIFICITY

Authors

Jennifer N. Cash and Thomas B. Thompson

Abstract



The transforming growth factor- β (TGF- β) family of secreted molecules consists of a large group of growth and differentiation factors that control a variety of cellular processes, both during embryogenesis and in mature tissues. One TGF- β ligand that functions as an important negative regulator of muscle growth is myostatin. Naturally occurring myostatin mutations have been shown to cause a hyper-muscling phenotype in cattle, dogs, and humans. *Myostatin* knockout mice exhibit muscles that are 2-3 times larger than those of wild-type mice, as do mice that overexpress inhibitors of myostatin, such as the antagonist follistatin. Furthermore, administration of myostatin neutralizing antibodies to adult mice also leads to increased muscle mass. Myostatin antagonism may be important for therapeutic treatment of muscle wasting diseases such as sarcopenia and muscular dystrophy. Our lab is interested in structural studies of myostatin antagonism, and we are currently investigating the follistatin family of extracellular antagonists. Using x-ray crystallography, we have solved the structure of the myostatin:follistatin288 complex and identified several differences between this structure and the previously determined structure of activin:follistatin288 which may contribute to antagonist specificity. We have also gained insight into unexpected features that allow myostatin to signal through a non-canonical receptor, Alk5. Furthermore, we have used these features to convert activin, which does not normally signal through Alk5, into a molecule that can utilize this receptor. Not only is the myostatin structure novel, but information gained from these studies may also assist the design of specific myostatin inhibitors that could be beneficial in the treatment muscle wasting disorders.



2008 Graduate Student Research Forum



Deborah Conrady - Poster Number: 10


Title

DIMERIZATION OF A G5 DOMAIN FROM THE *STAPHYLOCOCCUS EPIDERMIDIS* ACCUMULATION ASSOCIATED PROTEIN SUGGESTS A NEW MECHANISM FOR PROTEIN DEPENDENT INTERCELLULAR ADHESION IN STAPHYLOCOCCAL BIOFILMS

Authors

Deborah G. Conrady, Cristin C. Brescia and Andrew B. Herr

Abstract



The Accumulation Associated Protein (AAP) has been implicated in protein-dependent adhesion in the opportunistic pathogen *Staphylococcus epidermidis*. We hypothesize that the tandem repeated G5 domain "B-repeat" (Brpt) region of AAP mediates intercellular adhesion by binding to the Brpt of AAP on adjacent cells. To test this hypothesis, we expressed a single G5 domain plus a conserved C-terminal "half repeat" (Brpt1.5) from this region and tested its ability to dimerize. Brpt1.5 is a monomer at protein concentrations up to 70 mM. Some adhesive proteins require the presence of divalent cations for activity, and upon screening with divalent cations Brpt1.5 was found to dimerize in the presence of zinc. Circular dichroism indicates that zinc-dependent dimerization is not accompanied by any global structural changes. A 2.5 repeat-length construct was generated for comparison. Brpt1.5 requires two or three zinc ions per dimer, whereas Brpt2.5 requires four or five. This suggests a modular arrangement of G5 domains that is consistent with our hypothesis. Biofilm formation experiments were performed to test the relevance of zinc binding *in vivo*. Removal of zinc from the media via chelation prevented biofilm formation, while titration of biofilm-inhibited conditions with $ZnCl_2$ led to a recovery of biofilm formation. This evidence supports the ability of the Brpt region of AAP to mediate interactions between neighboring cells. The conservation of tandem repeated G5 domains in the protein SasG from the pathogen *Staphylococcus aureus* led us to examine the effects of zinc chelation on biofilm formation in the strain USA300. Zinc chelation with DTPA (Diethylenetriaminepentaacetic acid) prevents biofilm formation by *S. aureus* under the conditions tested. This suggests that intercellular adhesion via self-association of tandem repeated G5 domain proteins may be a general mechanism for Staphylococcal adhesion.



2008 Graduate Student Research Forum



Xiaodi Deng - Poster Number: 11


Title

Mechanism of BAMBI Inhibition


Authors

Xiaodi Andy Deng and Thomas Thompson

Abstract



The Transforming Growth Factor Beta (TGF- β) superfamily is a large class of regulatory proteins, which make up a key biological pathway that controls cellular proliferation and differentiation. The important biological role of the TGF- β pathway is further emphasized as it is conserved from invertebrates to mammals. The uniqueness of TGF- β pathway is its ability to promote or inhibit cell proliferation depending on the cell's genetic profile. Due of this duality, the TGF- β pathway is highly regulated for proper biological function and misregulation is linked to various types of cancer and multiple facets of heart disease. To combat these pathological states, TGF- β research has focused on targeting the source of the pathway activation, the ligands. TGF- β ligands are divided into three classes: TGF- β , Bone morphogenetic protein (BMP) and Activins. Inhibiting specific ligands show therapeutic promise due to its specificity, but ligands are global signals. Inhibiting a global signal that has multiple effects in a range of tissues can result in severe side effects. To inhibit TGF- β signaling and minimize the side effects, a localized inhibition is best. We believe BAMBI (BMP and Activin Membrane Bound Inhibitor) has the unique features to be a local and a broad range inhibitor of TGF- β pathway signaling. BAMBI was first believed to be specific for BMP2 and Activin, but later research has indicated BAMBI can also inhibit TGF- β 1 signaling, which makes BAMBI unique in that it can inhibit all three classes of TGF- β ligand. The current TGF- β field lacks thorough understanding of how BAMBI inhibition occurs. BAMBI is thought as a pseudo-receptor, where it is recruited into ligand-receptor complex but lack a kinase domain for phosphorylation. This hypothesis requires BAMBI to interact with all three classes of ligand, which is unprecedented. This gap in our understanding of how BAMBI inhibits signaling is best filled with atomic resolution structures, biophysical analysis and functional characterization. Furthermore, understanding the mechanism of BAMBI inhibition will support the design of treatments that target TGF- β family related cancers and heart disease.



Our long-term goal is to understand how BAMBI fits into the complex regulation scheme of TGF- β signaling and how it can be targeted therapeutically. The objective of this proposal is to determine the mechanism at the root of BAMBI inhibition. *Our hypothesis is that BAMBI inhibition of ligand signaling is the result of the extra-cellular domain (ECD) and inter-cellular domain (ICD).* We believe that ECD is able to compete with ligand for receptor binding and ICD is able to conformationally alter receptor kinase domain to prevent signaling. Our rationale behind this hypothesis stems from structural alignment and preliminary data. BAMBI ECD has around 30% homology to various ECD of TGF- β receptors and can be structurally modeled to various structurally solved receptors. Due to structure similarity and previous research indicating receptors can dimerize we believe BAMBI ECD can interact with various receptors. Preliminary data, further supporting our hypothesis, show BAMBI extra-cellular domain (ECD) inhibits a variety of TGF- β ligands. To test our hypothesis, we propose structural and biophysical experiments in which our laboratory has extensive expertise.



2008 Graduate Student Research Forum



Thomas Doerdelmann - Poster Number: 12


Title

A STRUCTURAL AND BIOPHYSICAL CHARACTERIZATION OF THE DNA BINDING PROPERTIES OF THE K-50 CLASS OF HOMEODOMAINS.

Authors

Thomas Doerdelmann, Jamie Baird-Titus, Douglas Kojetin and Mark Rance.

Abstract



Homeodomain proteins play critical roles in diverse biological processes, including cell differentiation and cell pattern formation. The human Pitx2 homeodomain and the Drosophila Bicoid homeodomain both bind several different DNA sequences. As the recognition of specific DNA sequences represents an essential biochemical function of all DNA-binding proteins, we have chosen the K-50 class of homeodomain proteins to investigate the mechanisms that convey biological specificity in these protein-DNA interactions. We have employed Nuclear Magnetic Resonance Spectroscopy, Circular Dichroism Spectroscopy and Isothermal Titration Calorimetry to investigate the binding properties of both homeodomains. While having a conserved fold, distinct differences in structure, chemical shifts, and energetics of binding are observed when comparing free protein to multiple DNA-protein complexes.



2008 Graduate Student Research Forum



Michael Flagler - Poster Number: 13

Title


Mutational Analysis of the Receptor Binding Sites of Shiga Toxins 1 and 2

Authors

Michael J. Flagler¹, Colleen M. McGannon¹, David R. Friedmann¹, Deborah G. Conrady¹, Sujit S. Mahajan³, Claudia L. Chalk², Andrew B. Herr¹, Rhett A. Koval¹, Jane E. Strasser², Suri S. Iyer³ and Alison A. Weiss¹

¹ Department of Molecular Genetics, Biochemistry and Microbiology, University of Cincinnati College of Medicine; ² Division of Infectious Diseases, Cincinnati Children's Hospital Medical Center; ³ Chemical and Biosensors Group, Department of Chemistry, University of Cincinnati

Abstract



Shiga toxin (Stx) is a major virulence factor of *Escherichia coli* O157:H7 and is essential to the ability of the pathogen to cause severe disease. Stx is a member of the AB₅ family of bacterial toxins. The A-subunit is enzymatically-active, leading to protein synthesis inhibition in host cells. A pentamer of five identical B-subunits is responsible for interacting with the glycolipid receptor globotriaosylceramide (Gb3) on the surface of host cells and delivering the A-subunit to the cytoplasm. There are two principal variants of Stx, Stx1 and Stx2, which share 55% amino acid homology. The majority of fatal disease cases in humans result from Stx2- (and not Stx1-) producing strains of *E. coli*, and Stx2 has been shown to be far more potent than Stx1 in animal models of disease. However, the basis for the difference in toxicity is unknown. To determine the contribution of Stx subunits to toxicity, we created chimeras containing the A-subunit of Stx1 with the B-subunit of Stx2 (Stx1A-Stx2B), and the A-subunit of Stx2 with the B-subunit of Stx1 (Stx2A-Stx1B). Preliminary studies measuring protein synthesis inhibition in Vero cells and toxicity in mice suggest that the A- and B-subunits have a modest influence on toxicity in vitro, and that the B-subunit of Stx2 contributes heavily to toxicity in vivo. We hypothesize that receptor binding differences between Stx1 and Stx2 play an important role in mediating toxicity in vivo. We have synthesized analogues of the Stx receptor, Gb3, that bind selectively to Stx1 or Stx2. To determine the structural basis for the differential binding of Stx1 and Stx2 to Gb3 analogues, amino acids that are not conserved in the three receptor binding sites of the B-subunits were 'swapped' by site-directed mutagenesis (residues from Stx1 were replaced with the corresponding residues from Stx2, and vice versa). Binding studies using Surface Plasmon Resonance indicate that amino acids in receptor binding site 2 contribute more to specificity than those in site 1. X-ray crystallography studies are underway to determine the structure of the Stx2 B-subunit in complex with Stx2-specific receptor analogues.



2008 Graduate Student Research Forum



David Friedmann - Poster Number: 14


Title

CSL - DNA interactions: from thermodynamics to structure

Authors

David R. Friedmann and Rhett A. Kovall

Abstract



Canonical Notch signaling ultimately results in changes in gene expression. The transcription factor CSL (CBF1, Su(H), Lag-1) is central to the regulation of transcription from all Notch responsive genes. CSL is a site-specific DNA binding transcription factor that serves as a scaffold upon which both transcriptional repression and activator complexes are assembled. Additional regulation arises from complex CSL binding elements that contain a pair of CSL binding sites arranged in a head-to-head manner, as typified by the HES-1 proximal promoter region. While the DNA consensus binding sequence has been identified for CSL (-CGTGGGAA-) and high-resolution crystal structures of CSL bound to this site have been determined, there is a considerable gap in our understanding of the thermodynamics that define CSL-DNA complexes. In particular, a quantitative description of the energetics that underlies CSL-DNA interactions with both consensus and nonconsensus binding sites is not available. The overall goal of this study is to biochemically, thermodynamically, and structurally characterize the interaction of CSL with DNA, including binding to consensus and nonconsensus sites, as well as its interaction with more complex DNA elements, such as the HES-1 paired binding site. Our preliminary calorimetry studies suggest that CSL binding to DNA is an entropically driven reaction with a rather moderate affinity (~ 200 nM K_d), which challenges current models in the field that hypothesize CSL is statically bound to DNA. Completion of these studies will provide for a more comprehensive understanding of the factors that govern DNA-recognition by CSL and potentially provide insights into the molecular mechanism for assembly of CSL complexes on paired binding sites.



2008 Graduate Student Research Forum



Michelle Gomes - Poster Number: 15

Title

STRUCTURAL AND BIOPHYSICAL INSIGHTS INTO THE IgA1 HINGE AS AN ANTIGEN OF IgA NEPHROPATHY


Authors

Michelle M Gomes¹, Hitoshi Suzuki², Jan Novak² and Andrew B Herr¹

¹ Department of Molecular Genetics, Biochemistry, and Microbiology, University of Cincinnati, Cincinnati, OH 45267.

² Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL 35294.

Abstract



Alterations in IgA1 glycosylation have been linked to the pathogenesis of IgA nephropathy (IgAN), a kidney disease characterized by deposits of IgA1 immune complexes in the glomerular mesangium. IgA1 antibodies from IgAN patients typically exhibit abnormal O-glycosylation in the hinge that connects the Fab and Fc regions. In particular, galactose (Gal)-deficient O-glycans are commonly found; these can act as epitopes for naturally occurring anti-glycan IgG or IgA1 antibodies. The resulting circulating immune complexes are then trapped in the glomerular mesangium of the kidney. In order to understand how the Gal-deficient hinge acts as an antigen for naturally occurring IgG antibodies, we have begun growing and optimizing crystals of the Fab fragment of a murine anti-glycan IgG, alone and in complex with a synthetic Gal-deficient IgA1 hinge glycopeptide (called HR-GalNAc). In parallel, we are studying the recognition of undergalactosylated IgA1 by two lectins that are specific for the IgAN glycan epitope (*i.e.*, the GalNAc group). These lectins, *Helix aspersa* agglutinin (HAA) and *Helix pomatia* agglutinin (HPA), are being tested for diagnostic use given that the underglycosylated IgA1 hinge is a marker for the disease. We have characterized the binding of HAA and HPA to HR-GalNAc as well as various underglycosylated myeloma IgA1 samples (k, l Ale and Mce) by surface plasmon resonance spectroscopy. Both HAA and HPA bind HR-GalNAc similarly, although HPA has a slightly higher affinity. A comparison of the binding of four different IgA1 samples to the lectins indicated that for both HAA and HPA, the apparent affinity is proportional to the number of exposed GalNAc groups. In order to understand how HAA recognizes GalNAc, we have crystallized HAA alone and in complex with GalNAc. Our preliminary data suggests that the spacegroup is trigonal or hexagonal, consistent with analytical ultracentrifugation experiments revealing that HAA forms a hexamer in solution. These studies will lead to a better understanding of how (1) immune complexes are formed in IgAN and (2) how lectins can be used in the diagnosis of the disease.



2008 Graduate Student Research Forum



Serena Heyse - Poster Number: 16


Title

Functional divergence between *Tetrahymena* telomere proteins: potential role for Pot1b in new telomere synthesis

Authors

Serena Heyse and Carolyn Price

Abstract



Telomeres are the nucleoprotein complexes at the ends of eukaryotic chromosomes. They protect the chromosome from degradation and end-to-end fusions and serve as a substrate for the enzyme telomerase. Telomeric DNA consists of a duplex of repeated sequence followed by a short terminal stretch of single-stranded DNA. This short overhang is bound by a specialized telomere protein called Pot1. Only one Pot1 gene has been identified in human and chicken cells; however, *Tetrahymena thermophila* has two Pot1 homologs, Pot1a and Pot1b. Pot1a is essential because it is required to prevent a telomeric DNA damage response. It also has a role in telomere length regulation. The function of Pot1b is unclear. Disruption of the Pot1b gene does not affect telomere length, overhang structure, or growth rate in vegetative cells. However, recent results have demonstrated that the gene is developmentally regulated. RT-PCR analysis of RNA obtained from mating *Tetrahymena* revealed that Pot1b is upregulated during formation of the new macronucleus (macronuclear development), just prior to the addition of new telomeres. Immunofluorescence data also indicates that Pot1b is localized to the developing macronucleus at the time of new telomere synthesis. These results indicate that Pot1b may play a role in the production of new telomeres. To determine whether Pot1a or Pot1b can bind directly to telomeric DNA, we expressed the DNA-binding domain (DBD) and tested their ability to bind telomeric DNA in a mobility shift gel. Surprisingly, only the DBD of Pot1a was able to bind. Chromatin immunoprecipitation (ChIP) experiments also indicated that Pot1b does not bind telomeric sequence or the sequence to which telomeres are added *de novo*. This suggests that Pot1b may interact with telomerase RNA instead of sites of new telomere addition. Pot1 proteins usually associate with telomeric DNA or telomerase; thus, Pot1b in *Tetrahymena* could be a telomerase processivity factor or be necessary for the recruitment of telomerase to sites of new telomere addition.



2008 Graduate Student Research Forum



Mary Horn - Poster Number: 17


Title

TRANSCRIPTIONAL REGULATION OF MESENCHYMAL GENE EXPRESSION IN ENDOCARDIAL CUSHION CELLS

Authors

Mary P. Horn and Katherine E. Yutzey

Abstract



Congenital heart defects (CHD) are among the most common birth defects in the USA. CHDs include structural defects of the valvuloseptal region, which may result in valve malformations. Valve development begins with endocardial cushions of the atrial ventricular canal (AVC) and outflow tract (OFT) composed of proliferative, undifferentiated, and migratory mesenchymal cells. These endocardial cushions elongate and differentiate expressing an organized extracellular matrix. Factors regulating proliferation, migration, and differentiation in endocardial cushions have been identified, however, transcriptional regulatory hierarchies have yet to be established. Tbx20 and Twist1 transcription factors promote proliferation, migration, and maintain the undifferentiated state of cultured endocardial cushion cells. Furthermore, AVC and OFT endocardial cushions express high levels of Msx1 and Msx2, but their functions in endocardial cushion mesenchyme and differentiation are unknown. Cell adhesion molecule, Cadherin-11 is highly expressed in endocardial cushions and Twist1 affects its expression. The goal of these studies is to further identify regulatory hierarchies within mesenchymal cells of the endocardial cushions. Through gain and loss of function studies Twist1 affects expression of Tbx20. rVista genomic analysis was performed and a highly conserved regulatory element upstream of chicken Tbx20 (tbx20boxA) containing an Ebox which potentially interacts with Twist1, and two Hox sites which potentially interact with Msx1 and Msx2. We hypothesize that Twist1 regulates Tbx20 transcription through activation at the tbx20boxA element. However, luciferase assays showed that Msx1 and Twist1 do not transactivate tbx20boxA, and site directed mutagenesis of the Ebox sequence had no effect upon transactivation. Future studies include a loss of function approach investigating Msx1 and Msx2 function in cultured endocardial cushion cells. Additional potential downstream targets such as Cadherin-11, which contains a region with conserved Eboxes, will be studied. These experiments will further elucidate transcriptional hierarchies controlling proliferation, migration, and differentiation of endocardial cushion valve progenitor cells.



2008 Graduate Student Research Forum



Amanda Huber - Poster Number: 18


Title

Dissecting the Role of CD40 in Graves' Disease


Authors

Amanda K. Huber and Yaron Tomer M.D.

Abstract



Graves' disease (GD), an organ specific autoimmune disease targeting the thyroid gland, is one of the most common autoimmune diseases in the United States with a prevalence of about 1% of the population. GD is characterized by the production of high levels of thyroid stimulating hormone receptor (TSHR) auto-antibodies. These auto-antibodies stimulate the TSHR resulting in proliferation of thyroid follicular cells (TFCs) as well as secretion of thyroid hormone, leading to GD hallmarks of goiter and thyrotoxicosis. Genetic screens done in our lab and others of patients with GD, have shown that the CD40 gene locus is linked and associated with GD. Fine mapping and sequencing of this locus led to the discovery of a C/T single nucleotide polymorphism (SNP) at the -1 position in the Kozak sequence of CD40. The CC genotype of this SNP has been shown to confer susceptibility to GD, and is significantly associated with high levels of auto-antibodies in GD patients. Our lab has reported that functionally this CC genotype leads to increased cell surface expression of CD40, suggesting that individuals with the CC genotype could have increased cell surface expression of CD40 on TFCs. CD40, a member of the tumor necrosis factor-family of receptors (TNFR), is normally found on the surface of antigen presenting cells such as B-cells. CD40 is a particularly attractive molecule to study in relation to GD as it is an immunoregulatory gene responsible for many aspects of antibody mediated immunity including: co-stimulation needed for B-cell proliferation, Ig class switching, antibody secretion, as well as cytokine production. Our hypothesis is that increased expression of CD40 on TFCs could lead to thyroidal inflammation triggering more severe GD. To test this hypothesis, we created a transgenic mouse which over-expresses CD40 in the thyroid. We then used the currently accepted GD model and induced disease in these mice. Looking at both frequency and severity of disease, we have shown that there is an increase of severity of disease in those mice which over-express CD40 in their thyroids. In addition to these results, experiments in primary human thyroid cultures have shown that stimulation of CD40, with a stimulating antibody, leads to the secretion of pro-inflammatory cytokines. This suggests that over-expression and subsequent activation of CD40 on the surface of TFCs could lead to the secretion of pro-inflammatory cytokines, causing inflammation in the thyroid, resulting in a more severe disease.





2008 Graduate Student Research Forum



Robert Hufnagel - Poster Number: 19


Title

Basic helix-loop-helix (bHLH) transcription factors direct distinct retinal neuron fates: implications for future gene therapies

Authors

Hufnagel, Robert B; Quinn, Malgorzata; Le, Tien T; Riesenber, Amy N; Brown, Nadean L

Abstract



Neural encoding of visual information begins in the retina, composed of seven major classes of neurons and glia. Light is first received by photoreceptors and then transmitted through bipolar neurons to retinal ganglion cells (RGCs), which convey this information directly to the brain. Retinal neurons are particularly susceptible to primary or secondary insult in a variety of diseases, including macular degeneration, optic nerve hypoplasia, retinitis pigmentosa, and glaucoma. During mouse retinal development, bHLH transcription factors, including *Atoh7*, *Neurog2*, and *Ascl1*, direct the differentiation of progenitors and specification of distinct retinal neuron classes. *Atoh7* and *Neurog2* are both expressed at the initiation of retinal neurogenesis. *Atoh7* is required for normal RGC differentiation, and adult mutants (*Atoh7^{-/-}*) mostly lack RGCs. *Neurog2*-expressing progenitors differentiate into multiple retinal fates, including RGCs and cone photoreceptors. Interestingly, *Atoh7^{-/-}* mice have increased cone photoreceptors and *Neurog2* expression, suggesting that *Atoh7* represses cone differentiation through inhibition of *Neurog2*. Another bHLH factor, *Ascl1*, is expressed three days later and directs the development of bipolar neurons. To test the hypothesis that bHLH factors are not functionally interchangeable during retinal neurogenesis, we generated an *Atoh7^{Ascl1}* targeted replacement allele, which expresses *Ascl1* within the endogenous *Atoh7* locus. Homozygous (*Atoh7^{Ascl1/Ascl1}*) mice, like *Atoh7* mutants, have a failure in RGC development and an increase in cone photoreceptors. However, bipolar neurons are specifically increased in *Atoh7^{Ascl1/Ascl1}* compared to *Atoh7^{-/-}* mice. Therefore, *Ascl1* function cannot replace *Atoh7* in RGC development or cone repression, but potentially redirects these progenitors to adopt bipolar fates. In the future, genes such as *Atoh7*, *Neurog2*, and *Ascl1* are interesting gene candidates for directing the differentiation of embryonic or endogenous stem cells into mature retinal neurons for cellular replacement therapies.



2008 Graduate Student Research Forum



Nicholas Jury - Poster Number: 20

Title


Alterations in peripheral and central serotonin physiology during lactation

Authors

N. J. Jury¹, B. A. McCormick³, N. D. Horseman⁴, and K. A. Gregerson^{2,4}

¹Neuroscience Graduate Program, ²College of Pharmacy, ³Obstetrics and Gynecology, and ⁴Molecular and Cellular Physiology; University of Cincinnati, Cincinnati, OH

Abstract



The pathophysiology of postpartum depression (PPD) remains obscure although evidence is accruing that serotonergic (5-HT) dysfunction may play a major role. Even the potential correlation between PPD and breastfeeding remains elusive, with numerous studies reporting a positive correlation and many others reporting a negative correlation. We have recently reported that 5-HT biosynthesis occurs in mammary epithelial cells where it is highly up regulated during lactation. This has led us to investigate if lactation results in changes in 5-HT physiology that may be correlated with changes in mood. In the current study, the forced swim test, a standard in assessing depressive behavior and antidepressant efficacy, was administered to age-matched lactating (day 10 postpartum) and non-lactating female C57/Bl6 mice. Some mice received five daily i.p. injections of a low dose of Citalopram (5mg/kg), a selective serotonin reuptake inhibitor (SSRI). Control mice received saline and all swim tests were conducted 30 minutes after the fifth injection. In vehicle-treated controls, lactating dams spent a greater percent time swimming compared to non-lactating mice ($30.3\% \pm 9.5$ vs. $17.1\% \pm 3.0$, respectively). Lactating mice also displayed a greater sensitivity to the SSRI. At this low dose, Citalopram had no effect on swim time in the non-lactating mice ($17.1\% \pm 3.0$ vs. $14.4\% \pm 3.4$; vehicle vs. SSRI), but it increased swim time in lactating mice ($30.3\% \pm 9.5$ vs. $50.3\% \pm 7.4$; vehicle vs. SSRI). In addition, serum concentrations of 5-HT were measured by RIA. In lactating dams (day 10 postpartum) serum [5-HT] was approximately 25% higher than in non-lactating females (3131 ± 203 versus 2511 ± 188 ng/ml, $p < 0.05$). We have previously shown that immunocytochemical staining for 5-HT in the dorsal raphe is significantly reduced in lactating mice. These and the present results demonstrate significant alterations in both peripheral and central 5-HT physiology during lactation in female mice and indicates a significant interaction between the two systems.



2008 Graduate Student Research Forum



Gina Kavanaugh - Poster Number: 21


Title

DEK Proto-Oncogene Depletion Results in ATM inhibition, S Phase Progression Defects and DNA Damage

Authors

Gina M. Kavanaugh, Trisha M. Wise-Draper, Lu Lu, Paul R. Andreassen, John J. Bissler, James M. Wells, Gerard C. Grosveld, and Susanne I. Wells

Abstract



The human DEK proto-oncogene, originally identified as a fusion protein with CAN nucleoporin in a subset of acute myeloid leukemia patients, is frequently overexpressed in its wild type form in human cancer. DEK nucleic acid binding properties have been extensively characterized in vitro, and have been related to possible roles for DEK in the regulation of chromatin architecture, transcription, and RNA splicing. How such DEK activities might contribute to human carcinogenesis remains poorly understood. We recently reported that DEK is a senescence and apoptosis inhibitor, and that the depletion of DEK in primary and HPV-positive cancer cells resulted in apoptosis through p53 stabilization. In the absence of any detectable physical interactions between DEK and p53, we investigated whether DEK plays a role in cellular DNA damage responses, a possibility that could account for the observed apoptosis phenotype. We found that DEK depletion in human cancer cells was sufficient for the detection of DNA damage markers such as γ H2AX and FANCD2 in a manner that was independent of p53 status or apoptosis. DEK-deficient cells were unable to activate the ATM pathway upon etoposide treatment, or to recover from aphidicolin-induced S phase arrest. Additionally, DEK-depleted human cancer cells and DEK-deficient mouse embryo fibroblasts were hypersensitive to genotoxic agents. Finally, in vivo targeting of DEK in human tumor xenografts resulted in the appearance of DNA damage markers and apoptosis. Interestingly, DEK protein levels were rapidly induced upon hypotonic buffer treatment, which induces aberrant chromosome remodeling. This supports a role for DEK as a chromatin stabilizer and/or DNA damage sensor. Taken together, our findings suggest that DEK is required for ATM kinase activation and signaling, and that DEK inhibition – by itself or in combination with conventional chemotherapeutics – might be exploited for the treatment of human cancer.



2008 Graduate Student Research Forum



Shikha Khatri - Poster Number: 22


Title

FoxO3a Regulates Cellular Glycolysis via Indirect Control of mTORC1

Authors

Shikha Khatri, Hasmik Yepiskoposyan, Preeti Tandon and David R. Plas

Abstract



PI3K/Akt signaling contributes to inappropriate apoptosis resistance in a broad spectrum of cancers. We have previously shown that elevated cellular glycolysis is a key requirement for Akt-induced cell survival. Akt-induced glycolysis correlates with a decline in the expression of evolutionarily conserved regulators of cellular metabolism: the FOXO family of transcription factors and the Tsc2 tumor suppressor protein. However, it is not known if Akt mediates increased glycolysis by suppressing FOXO or Tsc2 function. We tested whether FoxO3a knockdown was sufficient to activate the Akt metabolic program in growth factor-dependent cells. Like activated Akt, FoxO3a knockdown triggered sustained glycolysis despite growth factor withdrawal, suggesting an important role of FoxO3a in regulating metabolism. Unexpectedly, FoxO3a knockdown also triggered a partial decline in protein levels of Tsc2. Promoter analysis revealed conserved FoxO consensus binding sites in the promoter of Tsc1, a molecular chaperone that is required to stabilize Tsc2. Further investigation confirmed Tsc1 as a direct transcriptional target of FoxO3a. In parallel with decreased Tsc1, FoxO3a knockdown relieved repression of rate limiting glycolytic enzyme Hexokinase.

Therefore, FoxO3a inactivation by Akt appears to be key to the successful launch of its metabolic program; making re-activation of FoxO3a an attractive therapeutic goal to treat cancers that thrive on inappropriate Akt signaling.



2008 Graduate Student Research Forum



Tammy Kindel - Poster Number: 23


Title

THE HINDGUT IMPROVES GLUCOSE HOMEOSTASIS AFTER METABOLIC SURGERY INDEPENDENT OF WEIGHT LOSS AND INSULIN SECRETION IN THE GOTO-KAKIZAKI RAT.

Authors

Kindel TL, Yoder SM, Tso P.

Abstract



Introduction. Roux-en-y gastric bypass (RYGB) results in the rapid resolution of type 2 diabetes in 80-90% of morbidly obese patients. RYGB involves gastric restriction, duodenal exclusion and early hindgut stimulation by nutrients. Definitely separating metabolic and hormonal changes due to the surgery from weight loss has proven difficult in animal and human studies. We hypothesized that in Goto-Kakizaki (GK) rats, a lean model of type 2 diabetes, hindgut stimulation, and not duodenal exclusion, accounts for the early improvement in glucose homeostasis, independent of weight loss. To test this hypothesis, we compared 2 metabolic surgeries, duodenal-jejunal exclusion (DJE) and ileal transposition (IT) in GK rats. **Methodology.** DJE bypasses the duodenum and first 10 cm of the jejunum with a 15 cm jejunal roux limb. IT involves transposing a 10 cm segment of ileum into the jejunum, 10 cm distal to the ligament of Trietz. We performed a DJE, IT, DJE Sham, and IT Sham on male, 14 week old GK rats (n=9 per group). Rats were observed for 30 days post-operatively. **Results.** There was no difference in body weight or food intake at the end of the 30 day study period. At only 2 weeks after surgery, DJE and IT rats showed an improvement in glucose homeostasis after an oral glucose tolerance test (OGTT) at 120 minutes. A statistically significant improvement at 120 minutes in glucose concentration was again noted at the 4 week OGTT in DJE and IT rats (DJE 198.4 ± 10.6 vs DJE Sham 241.2 ± 15.0 , $p=0.03$; IT 191.7 ± 17.4 vs IT Sham 242.4 ± 8.2 , $p=0.02$). Surprisingly, there was no difference in insulin concentrations during the OGTT at any time point between the DJE/IT and Sham rats, nor a change in insulin sensitivity as measured by an insulin tolerance test. A mixed meal study at 30 days revealed an extended elevation in plasma GLP-1 at 30 minutes for both DJE and IT rats (DJE: 4.51 ± 0.36 vs DJE Sham 2.75 ± 0.22 , $p=0.001$; IT 4.38 ± 0.52 vs IT Sham 3.06 ± 0.25 , $p=0.005$). **Conclusions.** This study found that in GK rats, DJE and IT result in the same improvement in glucose homeostasis at 120 minutes, suggesting that hindgut stimulation is the responsible factor. This is supported by the similar increase in GLP-1, the incretin hormone secreted from the hindgut, by both metabolic surgeries. Interestingly, the increase in GLP-1 did not result in a statistically significant increase in insulin secretion, leading us to postulate that the primary mechanism in this model is in an extra-pancreatic peripheral or central action of GLP-1.



2008 Graduate Student Research Forum



Kori Klustaitis - Poster Number: 24


Title

IDENTIFICATION OF THE GLUCAGON-LIKE PEPTIDE-1 RECEPTOR IN THE RODENT INTESTINE


Authors

Klustaitis KM, Yoder SM, Bitner RD, Herman JP, D'Alessio DA.

Abstract



Introduction: Glucagon-like Peptide 1 (GLP-1) is a protein secreted from intestinal L-cells that is necessary for normal glucose tolerance. It is widely accepted that GLP-1 stimulates insulin secretion by binding directly to its receptors on islet β cells. However, recent studies have identified an interaction between GLP-1 and the nervous system, and previous data from us and others have suggested a portal vein site of action for this to occur. Recent emerging data is leading us to look at a site located more pre-hepatically for this interaction to take place. Hypothesis: We hypothesize that GLP-1 is secreted from intestinal L-cells and acts directly on GLP-1r located on sensory vagal nerves present in the wall of the intestines to initiate neural responses to changes in blood glucose in response to incoming nutrients. Methods: To identify the presence of GLP-1r in the sections of the intestines, we utilized 2 methods, QRT-PCR and Immunohistochemistry, on rat and mouse duodenum, ileum, and jejunum sections, as well as lung samples for the QRT-PCR positive control. Results: Immunohistochemistry showed cells positive for the GLP-1r in all 3 sections tested in the mouse samples. QRT-PCR gave a positive mean normalized expression for the rat lung, rat jejunum, and rat ileum. In the mouse, a positive mean normalized expression was found for the lung and the jejunum, but not for the ileum. We are currently running IHC for the rat intestinal sections and running neuronal tracing experiments to look for co-localization of the GLP-1r on vagal afferents in the intestine. Conclusion: We have identified, via two methods, the presence of the GLP-1r in the intestine of the rodent. This supports the hypothesis that GLP-1 could signal to GLP-1r on vagal afferent nerves in the intestine to stimulate insulin secretion.





2008 Graduate Student Research Forum



James Klyza, Jr, MSPH - Poster Number: 25

Title

The Use of Hypertonic Saline in the Reduction of Elevated Intracranial Pressure in Closed-Head Trauma Meta Analysis

Authors

James P. Klyza, Jr, MSPH, MCEH Training Grant Doctoral Candidate

Abstract

Abstract

Objectives: All of the existing methods of treatment for elevated ICP have limitations. In the past ten years, hypertonic saline administration has shown promise as an alternative treatment. The goal of this paper is to conduct a meta analysis and summarize the results of hypertonic saline trials.

Data sources: A broad literature search of PubMed/Medline, a forward search of these articles references, and personal contact.

Major inclusion/exclusion: Hypertonic saline treatment of closed head injuries with elevated ICP.

Stat methods: Bayesian Analysis of mean difference in ICP (pre vrs post-treatment) with standard error and a 95% confidence interval.

Results: All four of the models used, two fixed and two random, give a significant mean effect of hypertonic saline in reducing ICP in closed head trauma. Most notably, the random effects model with unknown variances gives a mean effect of -11.3 mmHg (-17.34, -5.692).

Conclusion: The implication is that hypertonic saline provides another possible treatment for a condition with few options for success.





2008 Graduate Student Research Forum



Elizabeth Kopras - Poster Number: 26


Title

Environmental Health Sciences Research: Role of Scientists; Roles and Rights of Community Participants

Authors

Elizabeth J. Kopras, Erin Haynes, Kim Dietrich, Susan Pinney, Kathy McCann, Amber Twitty, Victoria Straughn, John Schlep, Joyce Martin, James Krabacher¹, and Shuk-mei Ho

Abstract



The University of Cincinnati Center for Environmental Genetics and the National Institute of Environmental Health Sciences co-sponsored a Town Meeting, "Your Home, Your Health, Your Voice," on September 15, 2008, followed by an Issues and Ethics workshop on September 16th.

Goals for the town meeting included:

- Convey information to community members on the ways that their homes affect their health
- Give community members the opportunity to voice their concerns about environmental health issues
- Make connections and build bridges with communities that are impacted by environmental health issues.

Eighty-five attendees, comprised of presenters, NIEHS staff, regional university staff and faculty, local health departments, community activist groups, and community members, participated in the town hall meeting. Fourteen posters or activities were on display, illustrating NIEHS-sponsored research projects, community activists groups, and local health departments. Evaluations showed that the conference was rated well overall. However, attendees expressed a desire to have an increased amount of time in discussion groups. The Issues and Ethics workshop had 57 people attend, in addition to the 18 speakers or workshop leaders. On-going research programs were used as illustrations to discuss the issues and ethics of measures.



2008 Graduate Student Research Forum



Leah Kottyan - Poster Number: 27

Title

Eosinophils respond to acidic environments with cAMP production, decreased apoptosis, and a decrease in the expression of pro-apoptotic Bcl-2 family members

Authors

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Abstract

RATIONALE: A recently appreciated aspect of asthma is the decreased pH of patient's exhaled breath condensate. The effect of acidic pH on eosinophils, hallmark cells in asthma, has not been carefully studied.

METHODS: Mice were challenged with ovalbumin and bronchoalveolar lavage fluid (BALF) pH was measured with a microelectrode. Eosinophils were incubated in buffered solutions of pH 7.5 and 6.0 (modeling acute asthma); cAMP levels, apoptosis and expression of Bcl-2 family proteins were measured by ELISA, flow cytometry and qRT-PCR, respectively.

RESULTS: We found a mean of 0.23 ± 0.01 decrease in the pH of the BALF of allergen-challenged mice compared to saline-challenged mice. To assess the effect of acidic pH on eosinophils, we measured the production of the second messenger cAMP. Indeed, eosinophils respond to protons in a dose-dependent manner by increasing cAMP production: cAMP level was 546.6 ± 153 and 2713.8 ± 138 fmol/ 10^7 cells at pH 5.5 and 7.5 respectively. Forskolin and other cAMP-elevating agents decreased murine eosinophil apoptosis. Indeed, acid exposure decreased eosinophil apoptosis *in vitro*; specifically, at pH 6 there were 2.6 ± 0.7 -fold less eosinophils in early apoptosis compared to eosinophils incubated at pH 7.5. Furthermore, we found a unique Bcl-2 family expression profile in eosinophils incubated at an acidic pH. Specifically, while several pro-apoptotic Bcl-2 family members (e.g. Bok and Bcl-Rambo) were expressed at a higher level with *in vitro* incubation at physiological pH, the expression was suppressed in eosinophils incubated in acidic pH.

CONCLUSIONS: Our data demonstrate that acidic microenvironments induce intracellular signaling and decrease apoptosis in eosinophils.



2008 Graduate Student Research Forum



Danielle Kroetz - Poster Number: 28


Title

CCR5-DEPENDENT RECRUITMENT OF REGULATORY T CELLS FAVORS PATHOGEN PERSISTENCE DURING PRIMARY INFECTION WITH *HISTOPLASMA CAPSULATUM*

Authors

Danielle N. Kroetz and George S. Deepe Jr., *Department of Molecular Genetics, Biochemistry, and Microbiology & Division of Infectious Diseases, University of Cincinnati College of Medicine, Cincinnati, OH*

Abstract



Protective immunity against the dimorphic fungus *H. capsulatum* (*Hc*) requires the development of a Th1 immune response. Although the roles of several proinflammatory cytokines have been characterized, the involvement of chemokines remains unclear. Microarray analysis has shown that CCR5 and its ligands are up-regulated during *Hc* infection and thus, we hypothesized that signaling via CCR5 was involved with inflammation and resolution of infection. Despite having impaired inflammatory cell recruitment into the lungs, CCR5^{-/-} mice controlled infection more efficiently than wild-type when given a sub-lethal inoculum of *Hc* yeasts. Resolution of infection in CCR5^{-/-} mice was associated with a reduced proportion of CD4⁺ CD25⁺ cells and number of Foxp3⁺ T regulatory cells. Accordingly, greater than 70% of wild-type T regulatory cells in the lungs expressed CCR5 day 14 post-infection. In contrast, the number of Foxp3⁺ T regulatory cells in the thymus of CCR5^{-/-} mice was significantly higher than in wild-type day 14 post-infection. Similar results were observed when wild-type mice were treated with a monoclonal antibody to CCL4, a chemokine that exclusively signals via CCR5. Transfer of wild-type or CCR5^{-/-} CD4⁺ CD25⁺ cells intraperitoneally or intranasally into CCR5^{-/-} mice elevated fungal burden comparable to wild-type. It has previously been shown that inhibition of endogenous TNF- α alters protective immunity during *Hc* infection by stimulating the emergence of a population of antigen-specific CD4⁺ CD25⁺ cells. Neutralization of TNF- α increased the proportion and number of CD4⁺ CD25⁺ T cells in the lungs of wild-type, but not CCR5^{-/-} mice. Consistently, CCR5^{-/-} mice treated with a monoclonal antibody to TNF- α displayed prolonged survival. Enhanced clearance of *Hc* as a result of impaired T regulatory cell recruitment was specific to primary infection, as CCR5 expression was associated with more efficient resolution during secondary infection. These results suggest that CCR5 mediates the emigration of natural T regulatory cells out of the thymus, as well as into the lungs, which in turn, regulate the adaptive immune response to favor fungal replication during primary infection with *Hc*.



2008 Graduate Student Research Forum



Rishikesh Kulkarni - Poster Number: 29


Title

NFATc1 regulates lymphatic endothelial development


Authors

Rishikesh M. Kulkarni, James M. Greenberg and Ann L. Akeson

Abstract



NFATc1 transcription factor is critical for lineage selection in cardiac valve morphogenesis and osteoclastogenesis. We identified a role for calcineurin-NFAT signaling in lymphatic development and patterning. NFATc1 was colocalized with lymphatic markers Prox-1, VEGFR-3 and podoplanin on cardinal vein as lymphatic endothelial cells (LEC) are specified and as they segregate into lymph sacs and mature lymphatics. In *NFATc1* null mice, Prox-1, VEGFR-3 and podoplanin positive endothelial cells sprouted from the cardinal vein at E11.5, but failed to coalesce into lymph sacs. NFAT activation requires the phosphatase calcineurin. Embryos treated *in utero* with the calcineurin inhibitor cyclosporine-A showed cytoplasmic NFATc1, diminished podoplanin expression by the lymphatics and irregular patterning of the LEC sprouts coming off the jugular lymph sac, which suggests a role for calcineurin-NFAT signaling in lymphatic patterning. In a murine model of injury-induced lymphangiogenesis, NFATc1 was expressed on the neolymphatics induced by lung-specific overexpression of VEGF-A. Mice lacking the calcineurin A β regulatory subunit, with diminished nuclear NFAT, failed to respond to VEGF-A with increased lymphangiogenesis. *In vitro*, endogenous and VEGF-A-induced VEGFR-3 and podoplanin expression by human microvascular endothelial cells was reduced by siRNA to NFATc1, to levels comparable to reductions seen with siRNA to Prox-1. In reporter assays, NFATc1 modestly activated lymphatic specific gene promoters. These results demonstrate the role of calcineurin-NFAT pathway in lymphangiogenesis and suggest that NFATc1 is the principle NFAT involved.





2008 Graduate Student Research Forum



Meggan Laskowski - Poster Number: 30


Title

HISTOPLASMA CAPSULATUM MAP KINASE, *HMK1*, MEDIATES FILAMENTATION IN RESPONSE TO EXTRACELLULAR CUES

Authors

Meggan Laskowski and George Smulian

Abstract



Mitogen-activate protein (MAP) kinase pathways have been shown to play a role in fungal virulence. Homologs of *FUS3* and *KSS1*, the pheromone sensing and filamentation/invasion MAP kinases in the model organism *Saccharomyces cerevisiae*, have been characterized in several fungal pathogens. In plant pathogens such as *Ustilago maydis*, the *FUS3/KSS1* homolog is required for mating and virulence, and in the human pathogen *Candida albicans*, the *FUS3/KSS1* homolog is required for mating, filamentation, invasion, and full virulence. In addition to mating, the *FUS3/KSS1* homolog of *Aspergillus nidulans* is implicated in regulation of secondary metabolism. Homology searches of the *Histoplasma capsulatum* genome database reveal three putative MAP kinases in the genome of this fungal pathogen. However, the functions of these MAP kinases have not been characterized in *H. capsulatum*. In this study, we used RNA hairpin silencing to determine the function of a putative *FUS3/KSS1* homolog, *HMK1*, in *H. capsulatum*. The silencing vector was integrated randomly into the genome of a clinical *H. capsulatum* strain, UH1, using *Agrobacterium tumefaciens*-mediated transformation. Resulting colonies were screened for silencing of *HMK1* by qRT-PCR. Silenced strains were grown in the presence of cAMP, which is known to stimulate filamentation. Two silenced strains, MK1#2 and MK1#3, showed filamentation defects compared to empty vector control strains. These strains also displayed agarose invasion defects when exposed to activators of the cell wall stress MAP kinase pathway, with simultaneous nutritional stress. This invasion defect was not observed when silenced strains were exposed to activators of the high osmolarity growth MAP kinase pathway with simultaneous nutritional stress. These results indicate that *HMK1* of *H. capsulatum* is involved in the filamentation and invasion response to certain stresses, including cell wall and nutritional stress, but not osmotic stress. This suggests cross-talk between the filamentation, cell wall stress, and nutrient sensing pathways of *H. capsulatum*.



2008 Graduate Student Research Forum



Robert Littleton - Poster Number: 31


Title

A Flow Dynamic Hypothesis to Explain the Comorbidity of Hydrocephalus and Spina-Bifida

Authors

Robert M. Littleton, Jay R. Hove


Abstract



The idea that the balance between bodily fluids is a major determiner in health has been intuitive since long before the existence of modern medicine. Hippocrates (ca 460 BC to ca 370 BC) wrote that the balance of humours, which consisted of blood, phlegm, black bile and yellow bile determined health and disease. If flow between the bodily systems is aberrant, malady results. Likewise, in healthy individuals, flow is equilibrant.

Today, we know that fluid flow directly contributes to numerous diseases, including polycystic kidney disease, pancreatitis, acute and chronic renal failure, congestive heart failure, atherosclerosis, hydrocephalus, spina bifida, alzheimer's, and cerebral aneurysm disease.

Hydrocephalus is the consequence of a blockage in cerebrospinal fluid flow. Spina bifida results from the failed closure of the neural tube during development. Eighty percent of patients with spina bifida present with hydrocephalus. Given the high comorbidity, it seems there is some fundamental link between the two diseases. This poster will attempt to answer the question: could the aberrant flow conditions caused by blockage of cerebrospinal fluid in hydrocephalus lead to the failure of the neural tube to fuse properly seen in spina bifida?





2008 Graduate Student Research Forum



Karunyakanth Mandapaka - Poster Number: 32


Title

Identification of the Molecular Mechanisms of SP-C^{I73T} Quality Control

Authors

Karunyakanth Mandapaka 1, 2, Timothy E. Weaver 1, 2 1Department of Molecular and Developmental Biology, UC, Cincinnati, OH; 2Division of Pulmonary Biology, CCHMC, Cincinnati OH

Abstract



Mutations in *SFTPC*, encoding misfolded SP-C, are associated with Interstitial Lung Disease (ILD). SP-C^{I73T} is the most prevalent mutation among ILD patients. While other mutations like SP-C^{7exon4} and SP-C^{L188Q} are shown to be degraded by ER associated degradation (ERAD), our preliminary studies suggested that SP-C^{I73T} escapes ERAD and is modified by addition of an O-linked moiety. This led to our initial hypothesis that "SP-C^{I73T} is modified by mucin-like O-glycosylation and fails quality control in the Golgi". However, our subsequent studies to test that hypothesis showed that SP-C^{I73T} is not modified by mucin-like O-glycosylation and is not trafficked to Golgi. These findings were not consistent with our initial hypothesis and lead to our current hypothesis that "SP-C^{I73T} is subject to an autophagy-associated quality control mechanism called ERAD (II)", where the misfolded protein in the ER is engulfed by autophagosomes and routed to the lysosome for degradation. Our preliminary studies with 3-methyladenine (3MA), a specific inhibitor of autophagy-associated lysosome degradation, suggest that SP-C^{I73T} is degraded by ERAD (II). This will further be tested by studying the quality control of SP-C^{I73T} in cells deficient for *ATG5* (an upstream regulator of autophagy) and in the presence of lysosomotropic reagents. The modification on SP-C^{I73T} will be identified using 2D electrophoresis, HPLC and mass-spectrometry.



2008 Graduate Student Research Forum



Timmy Mani - Poster Number: 33


Title

MERLIN FERM DOMAIN PHOSPHOINOSITIDE BINDING REGULATES ITS MEMBRANE RAFT ASSOCIATION, SUBCELLULAR LOCALIZATION AND SUBCELLULAR DYNAMICS

Authors

Timmy Mani, Wallace Ip

Abstract



Loss of the functional *NF2* gene product, merlin, gives rise to neurofibromatosis type 2 (NF2). The NF2 tumor suppressor protein, merlin, is related to the ERM (ezrin, radixin, and moesin) family of plasma membrane-actin cytoskeleton linkers. For ezrin, phosphatidylinositol 4,5-bisphosphate (PIP₂) binding to the amino-terminal FERM domain is required for its conformational activation, proper subcellular localization and function, but less is known about the role of phosphoinositide binding for merlin. A significant proportion of cellular merlin resides in membrane rafts, plasma membrane microdomains in which many signaling events occur, but how merlin attaches to rafts is unknown. Here we report that merlin binds phosphoinositides including PIP₂, via a conserved binding motif in its FERM domain. FERM domain-mediated phosphoinositide binding is necessary for membrane raft association of merlin and regulates its subcellular localization. FERM domain phosphoinositide binding also regulates merlin dynamics in living cells as analyzed by fluorescence recovery after photobleaching. Unlike in ERM proteins, the carboxy-terminal half of merlin also binds phosphorylated phosphoinositides via sites that are as yet uncharacterized. However, this carboxy-terminal half mediated interaction with phosphoinositides is neither necessary nor sufficient for membrane raft localization of merlin. Our findings highlights the importance of FERM domain mediated phosphoinositide binding in merlin function.



2008 Graduate Student Research Forum



Aaron Marshall - Poster Number: 34


Title

Role of the Serotonin Transporter in Mammary Epithelial Homeostasis During Lactation

Authors

Aaron M. Marshall, Erin L. Pangallo, Nelson D. Horseman

Abstract



Recently it was been discovered that the mammary epithelium synthesizes the monoamine serotonin. Serotonin acts as a feedback inhibitor of lactation through binding to the 5-HT7 receptor located on the mammary epithelial cells. The synthesis of serotonin by mammary epithelium provided cause for investigating other serotonin regulating proteins such as the serotonin transporter (SERT). Utilizing a polarized human mammary epithelial cell model, we were able to demonstrate SERT is expressed in the apical membrane. This localization is specific to barrier-forming cells and does not overlap with 5-HT7 specific staining in the basolateral membranes. Other studies investigating SSRI use and lactation were entirely focused on passage of SSRI to the infant. The results of these studies indicate that certain SSRIs do leak into milk, and therefore would be able to affect SERT function in the apical membrane of epithelial cells. Here we show that SERT is expressed in the apical membrane of both mouse virgin and lactating mammary epithelial cells. Furthermore we characterize lactation and involution parameters, in mice treated with a selective serotonin reuptake inhibitor (SSRI), with the hypothesis that SSRI will cause precocious inhibition of lactation and involution.



2008 Graduate Student Research Forum



Jennifer McGuire - Poster Number: 35


Title

Neuropeptide Y (NPY) and Posttraumatic Stress Disorder (PTSD): Studies In A Rodent Model of Chronic Variable Stress

Authors

*J. L. MCGUIRE¹, J. P. HERMAN², F. R. SALLEE³, R. SAH³; ¹Molecular and Developmental Biology, ²Neurosci., ³Psychiatry, Univ. of Cincinnati, Cincinnati, OH

Abstract



The development of PTSD has been associated with abnormalities in the major stress response systems of the body, the hypothalamic-pituitary-adrenal (HPA) axis, the CNS noradrenergic system, and the sympathetic nervous system. Pathological anxiety in PTSD is indicative of impairment in anxiety regulating mechanisms in the CNS. A major transmitter that is linked to the regulation of stress and anxiety is neuropeptide Y (NPY), which is increasingly suspected to be a potential "stress resilience factor". Currently, pathophysiological relevance of NPY in PTSD is not known. The objective of this study was to a) develop a chronic variable stress (CVS)-recovery model that elicits "PTSD-like" behavior and b) investigate whether NPY expression is dysregulated in the CVS-recovery model. Adult male Long Evans rats were subjected to variable stressors for 7 days followed by 7 d recovery period. Animals were tested for PTSD-pertinent behavior (contextual fear conditioning, extinction and re-activation), NPY mRNA / protein expression and neuronal cfos activation. Rats exposed to CVS showed significant potentiation of freezing response following fear-reactivation ($p=.0247$, $t=2.540$, $df=13$), as well as delayed impairment of fear extinction ($p=.008$, $t=3.177$, $df=12$). Early and delayed changes in NPY expression were observed dependent on brain region. NPY expression was attenuated in the hippocampus at 16 hours post CVS (35.29% of controls, $p=.0251$, $t=2.632$, $df=10$). Significant reduction in amygdalar NPY was evident after 7 days (38.4% of controls, $p=.045$, $t=2.258$, $df=10$). These are regions relevant to contextual fear-conditioning. Current studies are investigating cfos and NPY expression in fear re-activated animals at a cellular level. In conclusion, our studies indicate that persistent NPY dysregulation in limbic regions of CVS animals may contribute to enhanced fear responses upon re-exposure as well as impaired extinction. NPY may be relevant to the pathophysiology of PTSD.



2008 Graduate Student Research Forum



Timothy Mead - Poster Number: 36

Title

THE ROLE OF NOTCH1 IN ENDOCHONDRAL OSSIFICATION

Authors

Timothy J. Mead and Katherine E. Yutzey, Ph.D.



Abstract

The Notch pathway has been implicated in bone development and associated diseases, including spondylocostal dysostosis. Notch1, a signaling and transcriptional regulator, is associated with expansion of undifferentiated progenitor populations in a variety of cell types. In developing cartilage, Notch1 is expressed in the early chondrocyte lineage and we hypothesize Notch1 is a negative regulator of chondrocyte differentiation in endochondral ossification, the process of bone formation from cartilage precursors. A Cre-responsive Notch1 intracellular domain over-expressing mouse (NICD) was used in gain of function experiments *in vivo*. We crossed NICD with Col2a1Cre mice to express NICD in all chondrocytes of the developing embryo. The NICD;Col2a1Cre mice do not survive beyond birth, most likely due to respiratory defects, and present with gross skeletal malformations of the vertebrae, limbs, cranium, and ribs. At E18.5, the double transgenic mice present with a malformed or non-existent vertebral column, but segmentation and patterning of somites are normal at E10.5. The limbs of the mutant mice are stunted and have a decreased hypertrophic chondrocyte zone, as marked by Col10a1 and Runx2 expression. These observations are consistent with an increase in the reserve/proliferative chondrocytes and an accompanied decrease in hypertrophic chondrocytes in the mutant embryos causing truncated limbs with an overall decrease in bone mass. To determine the accompanied loss of Notch function *in vivo*, Cre-responsive RBPJk^{fl/fl} and Notch1^{fl/fl} mice were obtained and crossed with Col2a1Cre mice. RBPJk^{fl/fl};Col2a1Cre mice are lethal at birth and preliminary data suggests vertebral anomalies as well as an increase in hypertrophic chondrocytes of the developing limbs in Notch1^{fl/fl};Col2a1Cre mice. Together these studies provide evidence for the role of Notch1 in cartilage and bone development and disease.

Research support is provided by the NIH Teratology Training Grant.



2008 Graduate Student Research Forum



Sara Meyer - Poster Number: 37


Title

Ron receptor signaling regulates mammary gland development in mice

Authors

Sara E. Meyer, Glendon M. Zinser and Susan E. Waltz

Abstract



The receptor tyrosine kinase Ron is overexpressed and tyrosine phosphorylated in a large fraction of human breast cancers, and mammary-specific overexpression of Ron is sufficient to induce mammary tumorigenesis in mice with 100% incidence. While numerous studies have analyzed the role of Ron in breast tumorigenesis, virtually nothing is known about the function of Ron in the normal breast. Previous studies demonstrate Ron expression is progressively increased in the mouse mammary gland during postnatal development. To define the significance of Ron in mammary gland development, we utilized mice with a targeted ablation of the tyrosine kinase domain of Ron (RonTK^{-/-}). Analysis of pubertal mammary gland development in RonTK^{-/-} mice revealed increases in ductal extension and the number of branch points compared to glands from wild-type mice. In addition, primary RonTK^{-/-} mammary epithelial organoids cultured in Matrigel also exhibited increased branching morphogenesis compared to wild-type. Serum estrogen and progesterone levels, as well as receptor protein levels, were not different between RonTK^{+/+} and RonTK^{-/-} mice. Moreover, RonTK^{-/-} ovariectomized mice maintain an increased branching phenotype compared to ovariectomized wild-type controls suggesting that the effect of Ron signaling on mammary branching morphogenesis may be independent of ovarian hormone stimulation. Increased branching morphogenesis in RonTK^{-/-} mammary glands was found to be associated with increased levels of phosphorylated Akt and Erk. The upregulation of pAkt and pErk was found predominantly in the mammary epithelium. Addition of the Mapk inhibitor PD98059, significantly reduced branching of the RonTK^{-/-} organoids in Matrigel. In total, these studies are the first to identify the Ron receptor as a negative regulator of mammary gland branching morphogenesis, one of only a few molecules known to have a similar function. The goal of future studies are to further understand an unknown receptor in mammary gland biology in hopes of ultimately impacting the treatment of human breast cancer.



2008 Graduate Student Research Forum



Scott Millen - Poster Number: 38


Title

The Pertussis Toxin B-Pentamer INDUCES THE INTERCELLULAR EXCHANGE OF PLASMA MEMBRANE IN JURKAT CELLS.

Authors

Scott H. Millen and Alison A. Weiss

Abstract



Bordetella pertussis, the causative agent in Whooping cough, is an obligate human pathogen, with no animal reservoir. Persistence of pertussis in the human is due to the ability of the bacterium to reinfect hosts. Unlike other pathogens that achieve reinfection by antigenic variation, *B. pertussis* reinfect hosts by altering immune cell function with toxins, specifically pertussis toxin. Pertussis toxin, often considered the major virulence factor of *B. pertussis*, is included as a component in all acellular pertussis vaccines. Pertussis toxin is an AB₅ toxin which is comprised of the enzymatically active A-subunit, S1, and the binding B-pentamer (PTx-B), composed of subunits S2, S3, S4, and S5, in a 1:1:2:1 ratio. The B-pentamer was once thought only to deliver S1 into target cells; recent studies indicate that the B-pentamer has toxic effects independent of S1 including antigen-independent T-cell activation and mitogenicity. PTx-B aggregates Jurkat cells, a human T-cell lymphoma line. Further analysis of Jurkat aggregation by flow cytometry revealed that treatment with PTx-B promotes membrane exchange, as the lipophylic dye DiI (red) appeared to be transferred from DiI (red) stained cells to DiO (green) stained cells, and vice versa. Microscopic analysis of sorted cell populations confirmed the transfer of lipophylic dye. The intercellular transfer of lipophylic dye did not occur in the absence of PTx-B. T-cells are known to take up plasma membrane and integral membrane proteins from antigen presenting cells during immunological synapse contact, a process called trogocytosis in literature. Pretreatment of Jurkat cells with Cytochalasin D to inhibit actin polymerization or performing the toxin treatment at low temperatures inhibited the transfer of lipophylic dye, which is consistent with trogocytosis in T-cells. PTx-B, which has 4 or more carbohydrate binding sites, may act by facilitating the inappropriate formation of an immunological synapse by cross linking both intracellular and intercellular surface receptors. Widespread and improper immune activation by PTx-B may aid the ability of *B. pertussis* to reinfect immune hosts.



2008 Graduate Student Research Forum



Monique Morrison - Poster Number: 39


Title

HPV E2 induced senescence stimulates migration in HPV positive cervical cancer cells

Authors

Monique A. Morrison and Susanne I. Wells, PhD *advisor*

Abstract



Cellular senescence is defined as the finite proliferative lifespan of cells and widely considered a tumor suppressive process and a hallmark of the premalignant state. In addition to replicative exhaustion, DNA damage and oncogene expression, a well established model for senescence induction involves the re-introduction of HPV E2 into HPV positive cervical cancer cell lines. The E2 protein is usually lost in most cervical cancers due to its disruption upon integration into the genome. Its re-introduction results in the suppression of the two critical HPV oncogenes E6 and E7 and subsequent upregulation of the tumor suppressors p53 and pRb. It has previously been reported that senescence induction in a proliferating cell colony led to cell dispersement over time. To test the hypothesis that E2 induced senescence could result in migration, we conducted two-dimensional video microscopy and transwell migration assays. Interestingly, E2 induced senescence led to increased motility which was not a mere consequence of cell cycle arrest or a direct effect of E2 expression but was specific to its ability to induce senescence. Increased migration was not a general feature of senescence as human foreskin keratinocytes (HFKs) passaged to replicative senescence did not show the same trend. Migration was dependent upon high cell density, and associated with increased cellular invasion. While Rho GTPases are critical for migration in many other systems, our data showed that at least Rac1, Cdc42 and RhoA activities were not upregulated during senescence. Moreover, soluble Rac1 and Cdc42 inhibitors did not affect E2 migration. Using microarray data from previous studies, we are currently probing several genes found to be induced by E2. Additionally we are examining the role of the individual oncogenes (E6 and E7) that are suppressed during E2 expression in order to determine which, if any, is responsible for the migration phenotype. Our data suggests novel properties for senescent cancer cells in vitro, that may have implications for cancer cell migration and metastases.



2008 Graduate Student Research Forum



Gregory Motz - Poster Number: 40


Title

Persistence of Lung CD8 T Cell Oligoclonal Expansions upon Smoking Cessation in a Mouse Model of Cigarette Smoke-Induced Emphysema

Authors

Gregory T. Motz, Bryan L. Eppert, Guanyun Sun, Scott C. Wesselkamper, Michael J. Linke, Ranjan Deka, and Michael T. Borchers

Abstract



The role of adaptive immunity in the development or progression of chronic obstructive pulmonary disease (COPD) remains undefined. Recently, the presence of autoantibodies and autoreactive T cells has been demonstrated in COPD patients. In addition, oligoclonal expansions of lung T cells have been observed in COPD patients, but the overlapping incidence of infections, tumors, and cigarette smoke exposure obscures the antigenic stimulus. We analyzed the TCR V β repertoire of CD4 and CD8 T cells purified from the lungs and spleens of mice chronically exposed to cigarette smoke. In a mouse model of COPD, we demonstrate that chronic cigarette smoke exposure causes oligoclonal expansions of T cells isolated from the lungs, but not spleens. TCR V β repertoire analyses revealed oligoclonal expansions predominantly occurred in lung CD8 T cells, with preferential usage of V β 7, V β 9, V β 13, and V β 14. Using nucleotide sequence analysis based on J β analyses, we demonstrate selection of complementarity-determining region 3 (CDR3) amino acid motifs, which strongly suggests antigen-driven oligoclonal T cell expansion. Analysis of the lung TCR V β repertoire of mice with cigarette smoke-induced emphysema which had undergone smoking cessation for 6 months revealed that oligoclonal expansions persisted. This study formally demonstrates that chronic cigarette smoke exposure, alone, causes a persistent adaptive T cell immune response. These findings have important implications for therapeutic approaches in the treatment of COPD, and provide insight into potential mechanisms involved in disease pathogenesis.



2008 Graduate Student Research Forum



Sunil Nair - Poster Number: 41

Title


Bilirubin Oxidation Products (BOXes) induce stress fiber formation in primary vascular smooth muscle cells through Rho but not PKC activation.

Authors

Nair SG¹, Clark JF^{1,2}, Wurster WL², Pyne-Geithman GJ²

(¹: Systems Biology and Physiology, University of Cincinnati College of Medicine, ²: Department of Neurology, University of Cincinnati College of Medicine)

Abstract



Bilirubin Oxidation Products (a.k.a. BOXes) have emerged as a putative molecule contributing to the development of Cerebral Vasospasm (CV), an often fatal complication of Subarachnoid Hemorrhage (SAH). We have previously reported BOXes to be present in significant concentrations in the cerebrospinal fluid (CSF) of SAH patients who suffer from CV. Chemically prepared BOXes have been shown to produce intense and sustained vasospasm of rat cerebral vessels *in vivo*. *In vitro*, they have been shown to enhance phenylephrine (PE)-induced contraction of porcine carotid artery (PCA). Hence, we hypothesized that BOXes increased the Ca²⁺ sensitivity of vascular smooth muscle by acting on Rho and/or Protein Kinase C (PKC), the two main proteins involved in the Ca²⁺ sensitization of vascular smooth muscle. Here, using qualitative and quantitative methods, we have demonstrated the effect of BOXes on Rho and PKC activity in cultured vascular smooth muscle cells (VSMCs). Model: Rho and/or PKC have been shown to alter the formation of stress fibers in cultured mammalian cells. We studied the effect of BOXes on stress fiber assembly in primary porcine VSMCs in the absence and presence of C3 transferase (CT04, a Rho inhibitor) or Bisindolylmaleimide (BI, a PKC inhibitor). Furthermore, quantitative assays were performed to measure Rho and PKC activity in VSMCs after BOXes treatment. Finally, we investigated the potentiation of PE-induced contraction in PCA smooth muscle rings over a range of concentrations of BOXes similar to those seen with CV. Results: 30-minute BOXes-treatment induced a profound increase and redistribution of stress fibers in VSMCs. The development of stress fibers was completely abolished when VSMCs were pretreated with CT04. On the other hand, BI pretreatment did not affect BOXes stimulated stress fiber assembly. Intermediate doses of BOXes significantly increased Rho activity in 30 minutes, without affecting PKC activity in VSMCs. In addition, intermediate doses of BOXes significantly enhanced PE-induced force generation that correlated with increased Rho activity in VSMCs. Conclusions: BOXes-stimulated stress fiber assembly in VSMCs is a result of Rho and not PKC activation. The potentiation of PE-induced contraction by BOXes is possibly due to increased Rho activation. Thus, BOXes may increase the Ca²⁺ sensitization of vascular smooth muscle cells through activation of Rho but not PKC.



2008 Graduate Student Research Forum



Sumeda Nandadasa - Poster Number: 42


Title

N- and E-cadherins in *Xenopus* are specifically required in the neural and non-neural ectoderm, respectively, for cortical actin assembly and morphogenetic movements.

Authors

Sumeda Nandadasa, Qinghua Tao, Nikhil Menon, Pierre McCrea, Janet Heasman and Christopher Wylie

Abstract



Transmembrane cadherins are calcium-dependent intercellular adhesion molecules. Recently, they have also been shown to be sites of actin assembly during adhesive contact formation. Recent studies from our lab using the *Xenopus* blastula identified that the cell adhesion molecule C-cadherin plays a central role in regulating the actin cytoskeleton during early stages of development. We demonstrated that membrane presentation of C-cadherin is the rate-limiting step to organize actin and the cadherin juxta membrane domain binding protein p120 catenin is also necessary for this activity (Nandadasa, Tao et al., 2007). However, the roles of actin assembly on transmembrane cadherins during development are not fully understood. We show here, using the developing ectoderm of the *Xenopus* embryo as a model, that cortical actin assembly is a primary function of both N-cadherin in the neural ectoderm, and E-cadherin in the non-neural (epidermal) ectoderm, and is essential for the characteristic morphogenetic movements of these two tissues. However, depletion of N-cadherin and E-cadherin did not cause dissociation in these tissues at the neurula stage. Furthermore, depletion of each of these cadherins is not rescued by the other, nor by the expression of C-cadherin, which is expressed in both tissues (Nandadasa et al., 2008, manuscript in review). These data indicate the combinatorial nature of cadherin function, the fact that N- and E-cadherin play primary roles in cortical actin assembly, in addition to roles in cell adhesion, and that this function is specific to individual cadherins. They also show how cell adhesion and motility can be combined in morphogenetic tissue movements that generate form and shape of the embryonic organs.



2008 Graduate Student Research Forum



Nicholas Olshavsky - Poster Number: 43


Title

Increased expression of ASF/SF2 promotes alterations in cyclin D1 levels

Authors

Nicholas A. Olshavsky, Clay E. Comstock, Jinsong Zhang, Linda M. Parysek and Karen E. Knudsen

Abstract



Prostate cancers (PCa) are dependent upon androgens for growth, survival and secretion. In prostatic epithelia, androgens are converted to a more potent androgen, dihydrotestosterone, which exerts their biological effect through AR, a ligand-dependent transcription factor of the nuclear receptor superfamily. Previous work has demonstrated that AR governs the cyclin D-RB axis in prostate cancer (PCa) cells, wherein androgens act through AR to promote the accumulation of D-cyclins in an mTOR-dependent fashion. D-cyclin accumulation promotes cell cycle progression by facilitating RB phosphorylation and G1 to S-phase progression. However, a function of cyclin D1 unique to PCa cells is the ability of cyclin D1 to act in a negative feedback loop to block subsequent AR activity. Recently, a polymorphism (G/A870) was identified within the cyclin D1 locus and has been associated with increased risk and poor prognosis in a variety of malignancies, including PCa. The polymorphism is a silent mutation that occurs at the exon 4-intron 4 boundary and is hypothesized to alter constitutive splicing. The variant cyclin D transcript, deemed transcript b, is encoded for by the first four exons, and a portion of intron 4 sequences due to a failure to properly splice at the exon 4-intron 4 boundary. Prior work from several labs established that the cyclin D1b splice variant harbored increased oncogenic capacity compared to its full-length counterpart. Recently, our lab demonstrated that cyclin D1b is compromised in its ability to negatively regulate AR activity compared to full-length cyclin D1 and that the cyclin D1b transcript is upregulated in tumor tissue compared to matched normal tissues and is maintained in lymph node metastases. Based upon this evidence, we hypothesized that the cyclin D1b splice variant may promote PCa initiation or progression. However, the factors that regulate cyclin D1b production remain poorly understood. Our studies demonstrate that the polymorphism influences cyclin D1b production and that the well-characterized SR protein, ASF/SF2, appears to have altered binding abilities that modulates the alternative splicing event of cyclin D1.



2008 Graduate Student Research Forum



Meghan Rojas - Poster Number: 44

Title


Ajuba functions as an HDAC-dependent co-repressor for autoregulation of the growth factor independent-1 transcription factor.

Authors

Meghan EB Rojas

Montoya-Durango DE, Velu CS, Kazanjian A, Jay CM, Longmore GD, Grimes HL.

Abstract



Growth factor independent-1 (Gfi1) is a zinc finger protein with a SNAG-transcriptional repressor domain. Ajuba is a LIM-domain protein that shuttles between the cytoplasm and the nucleus. Ajuba functions as a co-repressor for synthetic Gfi1 SNAG-repressor-domain containing constructs, but a role for Ajuba co-repression of the cognate DNA bound Gfi1 protein has not been defined. Coimmunoprecipitation of synthetic and endogenous proteins, and co-elution with gel filtration suggest that an endogenous Ajuba-Gfi1-HDAC multiprotein complex is possible. Active histone deacetylase activity co-immunoprecipitates with Ajuba or Gfi1, and both proteins depend upon histone deacetylases for full transcriptional repression activity. Ajuba LIM domains directly bind to Gfi1, but the association is not SNAG domain dependent. ChIP analysis and reciprocal knock-down experiments suggest that Ajuba selectively functions as a co-repressor for Gfi1 autoregulation. The data suggest that Ajuba is utilized as a corepressor selectively on Gfi1 target genes.



2008 Graduate Student Research Forum



Olivia Schneider - Poster Number: 45


Title

THE B SUBUNIT OF PERTUSSIS TOXIN SIGNALS THROUGH THE T CELL RECEPTOR TO DESENSITIZE CXCR4

Authors

Olivia D. Schneider, Alison A. Weiss, William E. Miller

Abstract



Pertussis toxin (PTx) is an AB₅ toxin made and secreted by the pathogenic bacteria *Bordetella pertussis*. PTx is known to inactivate G α_i proteins through its ribosyltransferase activity encoded in the A subunit. Due to its ability to inactivate G α_i proteins, PTx is used in signaling assays to assess the contribution of G α_i to various GPCR signaling pathways. Although the B subunit (PTxB) has been thought to mainly be responsible for the binding and delivery of the A subunit, studies have suggested that PTxB has a multitude of signaling activities, including the ability to modulate GPCR signaling pathways. The mechanism in which PTxB alters GPCR signaling is unknown. Recently, work from our lab has shown that PTxB activates a T cell receptor (TcR) signaling pathway in Jurkat T cells very similar to canonical TcR signaling activated in response to anti-CD3 mAbs. We hypothesize that PTx, via its B subunit, utilizes TcR signaling to heterologously desensitize CXCR4. Using WT and TcR deficient Jurkat cell lines, we found that TcR signaling, activated by both PTxB and anti-CD3, rapidly causes phosphorylation, internalization, and inhibits signaling of CXCR4. Together these data suggest that TcR crosstalk with CXCR4 is likely a normal cellular process exploited by *B. pertussis* through secretion of PTx.



2008 Graduate Student Research Forum



Emily Schulz - Poster Number: 46


Title

Expression of Non-Phosphorylatable α -Tropomyosin *in vivo* Causes Hypertrophic and Dilated Cardiomyopathy

Authors

Emily M. Schulz and David F. Wieczorek

Abstract



α -Tropomyosin (α -TM) is a dimeric alpha-helical coiled-coil protein which stabilizes actin and is involved in calcium regulation of the sarcomeric thin filament in striated muscle. Single point mutations in α -TM have been shown to cause familial hypertrophic cardiomyopathy (FHC) and dilated cardiomyopathy (DCM). Recent work has indicated that phosphorylation levels of α -TM decrease in a disease model of DCM. As α -TM is phosphorylated at approximately 70% in the developing heart, decreasing after birth to 30%, it is important to understand the effect of phosphorylation or loss of phosphorylation on α -TM function in the heart. In order to do this, transgenic mice were generated in which the endogenous phosphorylation site, a serine at the penultimate amino acid 283 of α -TM, was mutated to a non-phosphorylatable alanine residue. Eight transgenic mouse lines have been generated with varying levels of transgene expression. High expression in one line resulted in death of the mice by 21 days post-natally. These hearts are extremely dilated with no attendant myocyte disarray or fibrosis. Histological analysis and heart weight to body weight ratios indicate that moderate expression lines exhibit a hypertrophic phenotype with mild to moderate hypertrophy of the left ventricle. Additional studies to be conducted include working heart studies, echocardiography, biochemical studies, and measurement of contractile protein expression and phosphorylation status. These findings demonstrate a novel role for phosphorylation of α -TM in the heart.



2008 Graduate Student Research Forum



Hajer Sheikh - Poster Number: 47


Title

Tropomyosin Phosphorylation in Familial Hypertrophic Cardiomyopathy

Authors

Hajer N Sheikh, David F Wiczorek

Abstract



Tropomyosin (TM) is an alpha-helical coiled-coil dimer wrapped around actin in eukaryote cells. In muscle, it is also a component of thin filament of the sarcomere. TM regulates muscle contraction by blocking the binding of myosin heads to actin. Mammalian heart expresses many TM isoforms, with alpha being the predominant isoform in adult heart. TM is phosphorylated at amino acid Ser-283. This phosphorylation has been suggested to play a role during myofibrillogenesis as fetal cardiac and skeletal muscle TM have higher phosphorylation levels than adult tissue levels. The precise role of TM phosphorylation in vivo is not clear yet; however, in vitro phosphorylation of TM increases the head to tail polymerization of adjacent TM molecules and increases the ATPase rate. To examine this role, TM phosphorylation was studied in each cardiac chamber separately in wild type hearts to address whether pressure gradients generated in different cardiac chambers are associated with altered phosphorylation levels.

Hypertrophic cardiomyopathy (HCM), the most common inherited cardiac disease, is an autosomal dominant disease characterized by left ventricular hypertrophy. Its prevalence is 1:500. Many mutations in different sarcomeric proteins are associated with causing HCM. Mutations in alpha-TM are also responsible for HCM. Two transgenic mouse models expressing mutations in alpha-TM (Glu 180 Gly, and Asp 175 Asn) were studied to determine the effect of this disease on TM phosphorylation. The current study will address if phosphorylation has a role in mediating the hypertrophy caused by these mutations. The future clinical implications of this study would be to modulate phosphorylation levels in HCM patients as a potential therapy.



2008 Graduate Student Research Forum



Nisha Sipes - Poster Number: 48

Title

Role of Cdc42 in primary cell morphogenesis and extracellular matrix remodeling in a three dimensional matrix

Authors

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Jonathan Cheng‡, and Yi Zheng*†


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Children's Hospital Medical Center;

†Department of Cell and Cancer Biology, University of Cincinnati;

‡Department of Medical Oncology, Fox Chase Cancer Center

Abstract



Extracellular matrix (ECM) actively participates in normal cell regulation and in the process of tumor progression. The Rho GTPase Cdc42 has been shown to regulate cell-ECM interaction in 2D culture. These previous studies suffer inherent limitations in experimental design without consideration of the 3D environment cells experience under physiologic conditions and most Rho GTPase studies have been extensively studied using immortalized cell lines and dominant mutant overexpression approaches. The goal of our present studies is to define the role of Cdc42 in cell morphogenesis, ECM interaction, and matrix remodeling of primary cells in 3D culture. Using Cdc42^{loxP/loxP} mouse embryonic fibroblasts and our established 3D culture conditions, we found that Cdc42 deficiency leads to defects in global cell-matrix interactions, as measured by a decrease in collagen gel contraction. This defect is associated with an altered mechanical interaction with the matrix, as observed by morphologic changes and altered focal adhesion patterning. It is also associated with an altered synthesis/secretion of ECM components and/or proteases, as observed by altered fibronectin deposition patterning and a decrease in MMP activity the Cdc42^{-/-} cell medium. Experiments to reconstitute Cdc42 effector binding mutants into Cdc42^{-/-} cells to dissect the signaling pathways involved in matrix remodeling are underway. We expect that these studies will not only provide novel insights into the cellular role of Cdc42 in 3D, but may help implicate new avenues in cancer cell biology and tissue engineering.



2008 Graduate Student Research Forum



Kristy Stengel - Poster Number: 49


Title

Loss of the Cdc42 GTPase Results in Critical Reductions of the Inflammatory Cytokine, IL-6, in Both Immune Cell and Tumorigenesis Models

Authors

Kristy R. Stengel and Yi Zheng

Abstract



The Rho family of GTPases represent a class of mitogenic signaling molecules commonly deregulated in cancer. Rho family proteins are members of the Ras superfamily of GTPases, which switch from a GDP-bound, inactive state to a GTP-bound, active state in response to growth factor signaling. In their active form, these proteins bind to a number of effector molecules, activating signaling cascades which regulate a variety of cellular processes including cytoskeletal events, cell cycle progression and transcription. One member of the Rho family, Cdc42, has recently been implicated in the modulation of the activity of several transcription factors including serum response factor (SRF), NF- κ B, and STAT3. However, the mechanism by which Cdc42 signals to modify transcriptional programs and how these changes in gene expression contribute to Cdc42-associated oncogenicity have not been sufficiently explored. Here we have used a conditional *cdc42* knockout to examine the implication of Cdc42 loss on NF- κ B-mediated transcription. Loss of Cdc42 resulted in a target gene-specific deregulation in which the inflammatory cytokine, IL-6, was identified as a gene significantly deregulated upon Cdc42 loss. Given the expanding knowledge implicating IL-6 in not only immune function, but also tumorigenesis, we further examined how loss of Cdc42 could impact IL-6 production in a macrophage model, as well as a model of Ras-induced transformation. Both models identified significant IL-6 reduction upon Cdc42 loss, further implicating Cdc42 in the regulation of IL-6 expression in two physiologically relevant models in addition to primary fibroblast models. Ongoing studies seek to determine the mechanism of IL-6 regulation by Cdc42 and to additionally determine the impact of IL-6 reduction upon Cdc42 loss in *in vivo* models of tumorigenesis.



2008 Graduate Student Research Forum



Wendy Szymczak - Poster Number: 50


Title

Phagocyte Derived IL-4, Not Defective Inflammatory Cell Recruitment, Impairs Immunity to *Histoplasma capsulatum* Infection in CCR2^{-/-} Mice

Authors

Wendy A. Szymczak and George S. Deepe Jr.

Abstract



In several infection models, the chemokine receptor CCR2 is necessary for leukocyte recruitment and a T_H1 immune response. Its absence can be either detrimental or beneficial for infection resolution. We sought to determine if signaling through CCR2 contributes to inflammation and immunity to infection by the fungus *Histoplasma capsulatum*. Mice lacking CCR2 or its ligand, CCL2, were infected with a sub-lethal number of yeasts. One week post infection, CCR2^{-/-} mice exhibited increased fungal burden in lungs, and all CCR2^{-/-} mice succumbed by day 26. CCL2^{-/-} mice exhibited a transient increase in burden but resolved infection. Lungs of CCR2^{-/-} and CCL2^{-/-} mice manifested similar decrements in lung leukocytes in comparison to WT, but only CCR2^{-/-} mice displayed elevated levels of IL-4. Neutralization of IL-4 in CCR2^{-/-} mice decreased burden and prevented mortality without restoring leukocyte recruitment. IL-4 was produced in a CCL7-independent manner primarily by CD11c⁺ phagocytic cells of which ~50% were Mac3⁺. Phagocytic cells in CCR2^{-/-} mice exhibited increased arginase transcription and markers of alternative activation which are induced by IL-4 and promote yeast intracellular growth. Thus, CCL2, but not CCR2, is dispensable for protective immunity to *H. capsulatum* infection. Furthermore, the primary defect in CCR2^{-/-} mice is increased generation of IL-4, not perturbation of inflammatory cell recruitment.



2008 Graduate Student Research Forum



Preeti Tandon - Poster Number: 51

Title


AKT dependent cell survival and metabolism require S6K1

Authors

Preeti Tandon, Shikha Khatri, Jennifer F. Barger, Catherine A. Gallo, David R. Plas.

Department of Cancer and Cell biology, University of Cincinnati, Cincinnati, OH, USA.

Abstract



Constitutive Akt activation is frequently associated with apoptosis resistance in cancer. The protein kinase S6K1, a downstream substrate of Akt, has been recently suggested as a negative feedback regulator of the Akt pathway. In the S6K1 feedback loop, Akt activation induces S6K1 activity which in turn feeds back to suppress Akt activation. To test whether S6K1 feedback signaling opposes Akt induced apoptosis resistance, we investigated apoptosis control in IL3 dependent hematopoietic cells expressing S6K1 shRNA. S6K1 deficiency resulted in increased Akt activation, as predicted by the feedback loop. However, this increase in Akt activation did not correlate with increased apoptosis resistance. Instead, decreased cell survival was observed upon loss of S6K1. To determine why S6K1 is required for Akt dependent cell survival we analyzed key features of Akt apoptosis control: Bax translocation and cellular metabolism. Loss of S6K1 prevented Akt-induced glycolysis triggering Bax translocation and commitment to apoptosis. These results demonstrate that S6K1 opposes Akt activation through negative feedback, while simultaneously activating Akt-induced glycolytic metabolism.



2008 Graduate Student Research Forum



Megan Thobe - Poster Number: 52


Title

THE RON RECEPTOR TYROSINE KINASE REGULATES PRODUCTION OF ANGIOGENIC CHEMOKINES IN PROSTATE CANCER CELLS

Authors

Megan N. Thobe and Susan E. Waltz

Abstract



The Ron receptor tyrosine kinase is over-expressed in several human cancers including cancer of the prostate. The nuclear factor-kappaB (NF- κ B) transcription factor is also highly active in prostate cancer, and has been shown to regulate transcription of a select set of angiogenic chemokines in prostate cancer cell lines. NF- κ B dependent chemokine production is important for prostate tumor angiogenesis, growth, and metastasis. Given that the Ron receptor is upregulated in a significant fraction of prostate cancer we wanted to test the hypothesis that Ron receptor signaling promotes angiogenic chemokine production through a mechanism dependent on NF- κ B regulation. Interestingly, our data demonstrate that prostate cancer cells which exhibit high levels of Ron also produce high levels of angiogenic chemokines. In contrast, prostate cancer cell lines which demonstrate low Ron expression produce little to no angiogenic chemokines. Furthermore, a knockdown of Ron in PC-3 or DU145 prostate cancer cells leads to a significant decrease in angiogenic chemokine production that correlates with a decrease in NF- κ B activation. Exogenous overexpression of Ron in either the prostate cancer cell line LNCaP, or in the non-transformed prostate cell line PZ-HPV-7, results in a significant increase in angiogenic chemokine production. Angiogenic chemokines have been shown to be important in the recruitment of endothelial cells and in the development of new blood vessels during tumorigenesis. To examine the functional significance of the Ron-dependent chemokine induction in prostate cancer cell lines, we examined the ability of Ron to modulate endothelial migration. Prostate cancer cell supernatants from control cells were able to efficiently induce the migration of endothelial cells. However, following Ron inhibition, a significant decrease in migration was observed. To assess the impact of Ron on prostate tumor growth in vivo, we utilized the TRAMP mouse model of prostate cancer. TRAMP mice crossed with mice deficient in Ron signaling have delayed prostate tumor onset, and a smaller tumor burden when compared to TRAMP mice with functional Ron. Taken together, these data suggest that Ron is not only important in modulating the production of angiogenic chemokines in prostate cancer cells and the subsequent migration of endothelial cells, but is also critical in regulating prostate tumor growth in vivo.



2008 Graduate Student Research Forum



Elisia Tichy - Poster Number: 53


Title

PATHWAYS MAINTAINING EMBRYONIC STEM CELL GENOMIC INTEGRITY


Authors

Elisia D Tichy and Peter J Stambrook; University of Cincinnati, Cincinnati, OH

Abstract



Maintaining genomic integrity is crucial in embryonic stem cells (ESCs) due to their role in early development. Mechanisms are in place to prevent an undue mutational burden, as ESCs display a two order of magnitude lower mutation frequency when compared to their differentiated counterparts, mouse embryo fibroblasts (MEFs). A plausible explanation of this is a higher DNA repair capacity. Using a neutral comet assay to observe DNA double-strand break (DSB) repair, it is clear that ESCs have a remarkable DNA repair capacity, with DSBs being repaired within 1 hour after low-dose etoposide treatment. MEFs lag for up to 3 hours to repair DSBs under the same conditions. There are two major DSB repair pathways: homologous recombination (HR), which uses a template for high fidelity repair, and non-homologous end joining (NHEJ), which processes damaged ends prior to religation. The importance of ES cells using HR versus NHEJ for DSB repair would constitute another layer of protection for the genome. Consistent with this hypothesis, all HR proteins examined were highly elevated in ESCs compared with MEFs and could not be induced from basal levels upon etoposide treatment. NHEJ protein expression was variable in ESCs compared to MEFs, with DNA Ligase IV displaying several previously undescribed modifications. Functional assays to observe actual DNA repair abilities by these cells showed that ES cells are highly HR proficient and NHEJ deficient, whereas the reverse trend is true for MEFs. Current directions include deciphering the mechanism(s) of this NHEJ suppression in ESCs.





2008 Graduate Student Research Forum



Traci Tuttle - Poster Number: 54


Title

DOPAMINE AND APOMORPHINE INCREASE APOPTOSIS AND DECREASE VIABILITY IN BREAST CANCER CELLS

Authors

Traci Tuttle and Nira Ben-Jonathan

Abstract



Background: A recent study found that dopamine acts synergistically with anti-cancer drugs in an in vivo model of breast cancer, but a direct effect of dopamine on the cancer cells themselves was not observed. Our lab has recently discovered that the breast cancer cell lines T47D, MDA-MB-468 (468) and MDA-MB-231 (231) express four of the five dopamine receptors (DAR). DAR fall into two categories: D1 type (D1 and D5) which increase, and D2 type (D2, D3 and D4) which decrease intracellular cAMP. We have found that dopamine and the pan DAR agonist apomorphine decrease the viability of breast cancer cells, as measured by MTT assay.

Objectives: Using cultured breast cancer cells, our objectives were a) to use selective DAR agonists and antagonists to determine which receptors mediate the decrease in cell viability, b) use annexin V/propidium iodide staining and flow cytometry to determine whether dopaminergic agents cause apoptosis, and c) to determine whether dopamine, apomorphine or specific DAR agonists potentiate the effects of chemotherapeutic drugs.

Results: Of the DAR agonists tested only the D1R agonist decreased 231 and 468 cell viability. Flow cytometry indicated that apomorphine caused apoptosis, but this was not as profound as the corresponding decrease in cell viability. In 231 cells, which are relatively resistant to cisplatin, the combination of apomorphine and cisplatin caused increased cell death and decreased cell viability as compared to cisplatin alone. Specific antagonists of D1R and D4R did not reverse the effects of apomorphine.

Conclusions and future directions: Dopamine and apomorphine directly decrease cell viability and cause apoptosis in breast cancer cells, and these effects may be mediated by the D1 receptor. We will use siRNA knockdown to identify the receptors which mediate these effects of dopamine and apomorphine. We will also use inhibitors of signaling downstream of the receptors to attempt to reverse the effect of apomorphine.



2008 Graduate Student Research Forum



Purnima Wagh - Poster Number: 55


Title

Ron-Dependent Beta-Catenin Activation is Associated with Human and Murine Breast Tumorigenesis

Authors

Purnima Wagh and Susan Waltz

Abstract



The Ron receptor tyrosine kinase, a member of the Met receptor family, is overexpressed in variety human cancers including breast cancer. To delineate the role of Ron in breast tumorigenesis, our laboratory generated transgenic mice that overexpressed Ron selectively in the mammary epithelium. These transgenic mice developed mammary tumors with 100% penetrance, short latency and exhibited lung and liver metastasis. Microarray analysis comparing tumors from Ron overexpressing mammary glands and wild type mammary glands identified beta-catenin as one of the genes upregulated following Ron overexpression. Mammary tumors from these mice also demonstrated an elevation in total and phosphorylated beta-catenin. Analysis of human breast cancer specimens and cell lines demonstrated elevated levels of both Ron and beta-catenin coordinately. Further, treatment of Ron overexpressing breast cancer cell lines with an inhibitor of beta-catenin lead to decreased cell viability. Based on this data, we propose to test the hypothesis that beta-catenin nuclear translocation and activation are critical downstream signaling events during Ron-induced mammary tumorigenesis. To test this hypothesis, we show that ligand (hepatocyte growth factor like protein/HGFL)-induced Ron activation leads to the nuclear localization of beta-catenin which is tyrosine phosphorylated. To investigate the tyrosine residues in beta-catenin in which tyrosine phosphorylation may play an important role downstream of Ron activation, analyses of different tyrosine mutants of beta-catenin are being studied. Our preliminary data suggests that tyrosines Y654 and Y670 in beta-catenin are important, as mutation of these residues abolishes beta-catenin nuclear localization following Ron activation. Co-immunoprecipitation studies have also demonstrated that Ron physically associates with beta-catenin and that Ron associated beta-catenin is also tyrosine phosphorylated. In total, these studies suggest that beta-catenin may be a critical mediator of Ron-induced mammary tumorigenesis. Future studies will examine the significance of Ron-dependent beta-catenin tyrosine phosphorylation and nuclear localization in specific aspects of breast tumorigenesis.

2008 Graduate Student Research Forum



Michael Wilhide - Poster Number: 56

Title

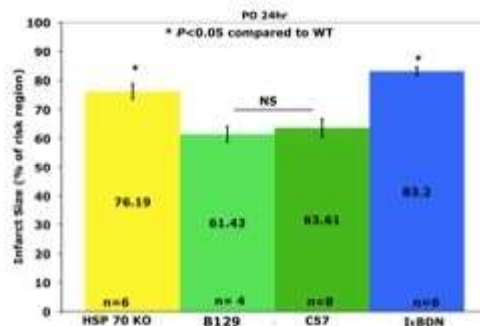
Heat Shock Protein 70 Contributes to NF- κ B-dependent Cardioprotection after Permanent Coronary Artery Occlusion.

Authors

Michael E. Wilhide, Xiaoping Ren, Michael Tranter, W.Keith Jones

Abstract

NF- κ B has been shown to contribute to myocardial infarction (MI) after ischemia/reperfusion (I/R) and yet mediates pro-survival signaling after specific manipulations, including after permanent coronary artery occlusion (PO). The aim of this study was to discover the mechanism by which NF- κ B regulates cardioprotection after PO compared to cell injury seen after I/R. The hypothesis is that NF- κ B conducts the orchestration of gene expression associated with cardioprotection after PO. We subjected transgenic mice that block NF- κ B (I κ BDN) in a cardiac-specific manner to PO and microarrays (n=4) were used to delineate genes regulated acutely by NF- κ B. We found that PO significantly regulated 196 genes ($P \leq 0.001$; PO vs. Sham) and NF- κ B significantly regulated 25 of these ($P \leq 0.001$). One of these was the Hsp70.1/70.3 locus. Both Hsp70s were upregulated in an NF- κ B-specific manner after PO. Infarct studies using HSP70.1/70.3 knockout mice support, that HSP70 is part of the pro-survival NF- κ B-dependent network after PO (Figure), as both NF- κ B blockade and Hsp70 ablation increase infarct size to about the same extent. Understanding the role of NF- κ B in the regulation of HSP70 and other genes will aid in the discovery of novel therapeutic targets for the treatment of heart disease. This work was supported by NIH grant R01 HL63034 (WKJ) and 5F31 HL081923 (MW).





2008 Graduate Student Research Forum



Tara Willson - Poster Number: 57

Title

The Role of STAT3 Activation in Acute and Chronic Intestinal Inflammation

Authors

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Lee Denson

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University of Cincinnati, College of Medicine

Abstract

The Inflammatory Bowel Diseases (IBD) are chronic relapsing and remitting disorders of the gastrointestinal tract which affect one million individuals in the US, including approximately 50,000 children. The pathogenesis involves a complex interplay between genetic predisposition, environmental factors, and the enteric flora which leads to induction of a maladaptive intestinal immune response. Genetic studies link IBD susceptibility to defects in innate immune recognition, antigen presentation and epithelial barrier function thereby subjecting the intestinal immune system to non-physiological amounts of luminal antigens. Thus the mucosa of IBD patients exhibits a T-cell rich infiltrate and abnormal cytokine balance with IL-6, a pro-inflammatory cytokine, being up regulated. IL-6 activates the signal transducer and activator of transcription (STAT) 3 shown to be involved in cell survival, proliferation, differentiation and malignancies. In normal tissues STAT3 activation is transient, is under stringent regulatory control, and likely promotes homeostatic epithelial responses to injury. However, in the mucosa of chronic intestinal inflammatory disorders including IBD, STAT3 activation persists in T-cells suggesting that it is an important signaling molecule during gut inflammation. Prior studies have in fact shown that IL-6:STAT3 activation in T cells may contribute to chronic mucosal inflammation by promoting T cell survival, and is required for differentiation of the Th17 subset of effector cells. However, a recent clinical trial of a soluble IL-6 receptor antibody did not result in a significant reduction in mucosal inflammation in CD, suggesting that the IL-6:STAT3 pathway may in fact mediate both homeostatic and pro-inflammatory responses in the gut. In our lab, microarray and Ingenuity Systems bioinformatics analysis identified IL-6:STAT3-dependent biological networks up-regulated in the mucosa of IBD patients which control leukocyte recruitment, HLA expression, angiogenesis, and tissue remodeling. We also showed that mucosal samples from IBD patients consistently exhibited an increased frequency of pSTAT3-positive epithelial and immune cells that was associated with the histological severity of disease including epithelial damage, architectural distortion and erosion/ulcers. Taken together, these data indicate that STAT3 is likely an important mediator of intestinal inflammation in both T-cells and intestinal epithelial cells. We hypothesize that STAT3 is necessary within gut-epithelium for homeostasis in acute injury, while in T-cells STAT3 promotes chronic inflammation. Utilizing a murine model of T-cell and intestinal epithelial cell specific deletion of STAT3, we will evaluate T-cell homing, survival, proliferation and effector function as well as disease activity in piroxicam and TNBS induced murine ileitis and colitis, respectively. Furthermore, functional deletion of STAT3 in either T-cells or epithelial cells during the pathogenesis of colitis will allow greater clarity into the impact of STAT3 targeting for therapeutic intervention in human IBD.



2008 Graduate Student Research Forum



Chang Xiao - Poster Number: 58

Title

PARIETAL CELL-EXPRESSED SONIC HEDGEHOG MAINTAINS A FUNCTIONAL AND DIFFERENTIATED GASTRIC EPITHELIUM

Authors


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Abstract



Background and Aims: It is well established that Sonic Hedgehog (Shh) is expressed and secreted from the gastric parietal cell, but the function of this gastric morphogen is still unclear. The objective of the current study was to identify the mechanism by which Shh acts as a regulator of gastric epithelial cell differentiation and function. **Methods:** A mouse model expressing a parietal cell-specific deletion of Shh was developed. Gastric morphology and function was studied in control and homozygous knockout (HKCre/Shh^{KO}) mice at 4 months of age. **Results:** In contrast to the control animals, HKCre/Shh^{KO} mice developed gastric hypochlorhydria, hyperplasia, cystic glands and dysplasia. Ultrastructural analysis of the parietal cell revealed architectural disorganization with an unclear boundary between tubulovesicles and canaliculi in the HKCre/Shh^{KO} mice. A well-interdigitated basolateral membrane with numerous infoldings between the parietal and neighboring cells was lacking in the HKCre/Shh^{KO} mouse parietal cells. Immunofluorescence revealed altered expression of E-cadherin and β catenin, and Western blot analysis confirmed a significant increase in nuclear β catenin in the gastric mucosa of HKCre/Shh^{KO} mice, accompanied by a significant increase in Wnt5A and Cyclin D1 mRNA expression. **Conclusion:** In the adult stomach, parietal cell-expressed Shh is crucial for maintenance of epithelial cell function and differentiation. Furthermore, the Hedgehog signaling pathway plays a role as a regulator of cell adhesion.



2008 Graduate Student Research Forum



Stephanie Yoder - Poster Number: 59

Title


THE INCRETIN HORMONES GIP AND GLP-1 RESPOND DIFFERENTLY TO INCREASING DOSES OF INGESTED LIPID

Authors

Yoder, SM, Qing, Y, Kindel, TL, Tso, P

Department of Pathology and Laboratory Medicine

Abstract



Background: Following the ingestion of nutrients, the incretin hormones glucose-dependent insulintropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) are secreted from the enteroendocrine cells; it has been documented that the incretin response is altered in type 2 diabetic and obese individuals. To gain a better understanding of the etiology and thus treatment of the defects in GIP and GLP-1 secretion, it is imperative to first understand the physiological mechanisms regulating the release of the incretin hormones in a non-pathological state. Previous studies have shown that oral ingestion of fat stimulates secretion of both incretins; however, it is unclear if there is a dose-dependent response between the amount of lipid ingested and the secretion of the hormones *in vivo*. We hypothesized that a direct dose-dependent relationship exists between the amount of lipid ingested and the secretion of GIP and GLP-1.

Methods: To test this hypothesis, we used our newly established lymph fistula rat model. The major mesenteric lymphatic duct of male Sprague-Dawley rats was cannulated under isoflurane anesthesia. Each animal received a single, intraduodenal bolus of saline or the fat emulsion Liposyn II (0.275, 0.55, 1.1, 2.2, 4.4 kcal lipid). Lymph was continuously collected for 3 h and analyzed for GIP and GLP-1 content.

Results: In response to increasing infused lipid calories, the secretion of GLP-1 increased dose-dependently over a 3 h time period. On the other hand, a threshold amount of 1.1 kcal lipid was required to elicit a sustained GIP response greater than that produced by the saline control over 3 h. Moreover, the caloric amounts above 1.1 kcal did not further augment the secretion of GIP, thus indicating an 'all-or-nothing' pattern to ingested lipid.

Conclusions: Our data suggest that the mechanisms underlying lipid-induced GIP and GLP-1 secretion differ. Furthermore, we speculate that GIP and GLP-1 play different physiological roles when challenged with lipid. As the first incretin hormone to be influenced by nutrients, the primary function of GIP is to prepare for incoming calories; in contrast, GLP-1 secreting cells contact the lipid more distally and therefore provide a final, more sensitive control signal.



2008 Graduate Student Research Forum



Monica Brooks - Poster Number: 60


Title

ANALYSIS OF THE INTERACTION BETWEEN IMMUNOGLOBULIN A AND FcαRI

Authors

Monica T. Brooks, George M. Ibrahim, Bryan W. Poulsen, and Andrew B. Herr

Abstract



Immunoglobulin A (IgA) performs an important role in preventing and countering pathogenic challenge to the immune system. In the absence of antigen, an interaction between IgA and the IgA-specific receptor FcαRI activates an anti-inflammatory pathway. When immune-complexed (antigen-bound) IgA interacts with the IgA-specific receptor FcαRI on the surface of immune effector cells, pro-inflammatory responses such as phagocytosis are triggered. Based on previous crystallographic studies of the complex between the FcαRI ectodomain and a core IgA1-Fc fragment (Fcα), we have identified the IgA1 residues that contact FcαRI. Using site-directed mutagenesis, we have created Fcα mutants with single amino acid substitutions at these residues. Binding studies using surface plasmon resonance spectroscopy will be carried out to quantitate changes in affinity observed upon binding of mutants to the receptor. Initial equilibrium binding studies have shown that mutation of hydrophobic residues within the binding site results in a pronounced decrease in the affinity of the Fcα:FcαRI interaction. Further studies with the other Fcα mutants will allow us to determine the relative contribution of individual IgA1 residues to the Fcα:FcαRI interaction. Using a truncated Fcα construct, we have also demonstrated that both FcαRI-binding domains on IgA are necessary for complex formation. These results will lay the groundwork for engineering recombinant IgA molecules for therapeutic applications, such as more effective anti-tumor immunotherapy.