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Poster Abstracts, Sigma Xi Research Day 2008

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Field Topic:

Biochemistry/Molecular Pharmacology

- 2) Localization and Observation of Essential Enzyme: Arginyl-tRNA Protein Transferase in Zebrafish.** M. Brown, C Filoon, M Ramana, *E Wickstrom and *H Kaji, College of Health Professions and Department of Biochemistry and Molecular Biology, Kimmel Cancer Center, Thomas Jefferson University

Protein arginylation is a post-translational modification mediated by Arginyl-tRNA protein transferase (ATE1), which transfers arginine to the N-terminus of proteins ending with aspartic acid, glutamic acid or cysteine. ATE1 is one of many ubiquitin-protein ligase (E3) enzymes essential for the ubiquitin degradation pathway, as well as modification of existing proteins via arginylation. Arginylation has been shown to play key regulatory roles in a variety of cellular processes relevant to aging, stress management, transformation, and tissue distribution. ATE1 is an evolutionarily conserved enzyme that has shown to be necessary for embryonic development. The goal of our study is to determine how vital ATE1 is to embryogenesis of zebrafish (ZF), a model organism for human development. By means of anti-ATE1 antibodies, the quantification of ATE1 expression throughout early ZF development has been shown. Microinjection of ATE1 antisense oligonucleotides into ZF embryos showed phenotypic alteration and a decrease in survival rate. To localize ATE1 during ZF development, fluorescence in situ hybridization is being carried out. These studies might elucidate human disease states associated with up or down arginylation, regulated by ATE1.

- 1) The Sum1 Repressor of Meiosis is Actively Removed in an Ime2-dependent Pathway in *Saccharomyces cerevisiae*.** N T Ahmed, M Moore, M Shin and *E Winter. Department of Biochemistry and Molecular Biology, Thomas Jefferson University, Philadelphia, PA

Meiotic development in budding yeast is controlled by a transcriptional program that expresses three temporally distinct classes of genes – early, middle, and late. Middle genes activate nuclear segregation (MI and MII), after which cells are irreversibly committed to complete the program. This commitment point is governed by a transcriptional switch consisting of the Sum1 repressor and the Ndt80 activator which have been shown to compete for binding to elements in middle gene promoters (MSEs) in vitro. NDT80 is itself expressed as a middle meiosis-specific gene, and its transcription is regulated both by itself and Sum1 binding. Although it has been suggested that competition between Sum1 and Ndt80 regulate nuclear division in vivo, this idea has not been tested. In this study, we show that Sum1 repression is removed during meiosis in cells lacking Ndt80. Moreover, direct biochemical assays demonstrate that the Sum1 protein is removed from MSEs with indistinguishable kinetics in wild-type and *ndt80-Δ* cells. Ime2 is a meiosis-specific cyclin dependent kinase like kinase that regulates multiple events in meiosis. Previous in vitro data has shown that Sum1 is phosphorylated by Ime2. Here we show that Sum1 is phosphorylated on T306 in vivo. Phosphorylation of this residue is necessary for removal of Sum1 repression in the absence of Ndt80. This suggests that Sum1 removal is regulated by phosphorylation at T306 by Ime2. Our data define a new pathway for promoting the removal of the Sum1 brake to meiosis. This suggests that Sum1 removal is a necessary prerequisite for Ndt80 expression, and is a key trigger that promotes nuclear segregation.

- 3) Characterization of oxidative phosphorylation and alternate oxidase enzyme activities in *Neurospora crassa*.** Amber Cardani, Kyanna Ellerbee, Rashida Crawford, Matthew Scarnati and *Kelly Keenan Ph.D. Richard Stockton College

The goal of this project was to characterize the proteins of oxidative phosphorylation and alternate oxidase pathways in *Neurospora crassa*. The alternate oxidase pathway provides a back up method of oxidizing NADH if the oxidative pathway is blocked for some reason and it essentially allows a bypass of complexes III and IV. Two methods were used to decrease oxidative phosphorylation: one was to grow in presence of chloramphenicol which blocks synthesis of proteins encoded in the mitochondrial DNA and to use a knockout mutant that has been genetically constructed to lack a gene from complex IV. An enzyme assay was developed that could measure both total oxidation due to both pathways as well as that due to each pathway. Level of subunit 4 from complex IV was also measured by immunoprecipitation under conditions where protein should be decreased compared to normal or absent.

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- 4) Interactions of Cationic Porphyrin with G-Quadruplex DNA.** K E Colman, K A Longenecker, A Butterworth, L A Chirayath and *M Azam. Department of Chemistry, West Chester University of Pennsylvania.

The interaction of 5,10,15, 20-tetrakis-(1-methyl-4-pyridyl)-21H,23H-porphyrine (TMPyP4), with various forms of G-quadruplex DNA sequences, d(T4G4), d(T4G4T), and d(G2T2G2 TGTG2T2G2) was investigated in this study. TMPyP4 is a square planar structure and previous studies have shown this molecule to interact with parallel stranded duplex DNA structures. Although these interactions have been rigorously studied, the exact binding modes and binding affinities are still controversial. Using the different G-quadruplex sequences found in the telomeric portions of chromosomes, the number of bound molecules of porphyrin to the DNA were measured and the modes of binding were attempted to be determined. Continuous Variation Analysis was used to generate Jobs plots which yielded the bound ligands/quadruplex DNA. The binding stoichiometry for the binding of TMPyP4 to the sequences d(T4G4), d(T4G4T), and d(G2T2G2 TGTG2T2G2) were 2.33, 1.22, and 0.75, respectively. Currently studies are being completed to reproduce these results and also to ensure whether or not the concentration of the salt solution used has any effect on how well the porphyrin molecule binds to the quadruplex DNA structures.

- 6) Enhanced EGFR Inhibition and distinct epitope recognition by EGFR antagonistic mAbs C225 and 425.** JM Donaldson, V Kamat, C Kari, MRD Quadros, E Papazoglou, U Rodeck, JC Williams* Depts. of Biochem & Mol Biol, Dermatol & Cut Biol, Radiation Oncology, TJU and Drexel University, Phila., PA

Monoclonal antibodies (mAbs) that inhibit activation of the epidermal growth factor receptor (EGFR) have shown therapeutic potential in select malignancies including breast cancer. Here, we describe that combined use of two such mAbs, C225 (Cetuximab) and 425 (EMD55900), reduced growth and survival of MDA-MB-468 breast cancer cells overexpressing EGFR more effectively than either antibody alone. Similarly, the C225/425 antibody combination more effectively inhibited AKT and MAPK phosphorylation in these cells. Size exclusion chromatography and analytical ultracentrifugation demonstrated that mAbs C225 and 425 simultaneously bind to distinct antigenic epitopes on domain III of the soluble wild-type EGFR. Furthermore, neither mAb competed with the other for binding to cells expressing either wild-type EGFR or a mutant EGFR (EGFRvIII). Mutagenesis experiments revealed that residues S460/G461 in EGFR domain III are essential components of the 425 epitope and clearly distinguish it from the EGF/TGF- β binding site and the C225 interaction interface. Collectively, these results support the conclusion that therapeutic EGFR blockade in cancer patients by combined use of mAbs C225 and 425 could provide advantages over the use of the two antibodies as single agents.

- 5) Structural determinants of binding between cyclophilin A (CypA) and HIV-1 capsid (CA).** A Conde and *CP Scott, Department of Biochemistry and Molecular Biology, TJU

Cyclophilin A (CypA) is an isomerase that binds to HIV-1 capsid (CA) and is recruited into virions. This interaction results in increased infectivity, therefore antagonists may be useful as antiviral agents. Our approach is to define the structural elements involved in binding, which may be used as the basis for developing small molecule inhibitors of HIV. Analysis of CA sequences from HIV isolates reveals several conserved residues, including Arg97 and Glu113. These residues are not in direct contact with CypA in the co-crystal structure, but their position suggests a role in stabilizing the extended loop that is necessary for CypA-CA interaction. To assess the importance of these residues in the CypA-CA interaction, we generated charge-reversal mutants and used Isothermal Titration Calorimetry (ITC) to determine their affinity for CypA relative to wild-type CA. Mutation of Glu113 to Arg had no effect on binding, but mutation of Arg97 to Asp resulted in a 7-fold decrease in affinity. However, the R97D mutation can be rescued by addition of magnesium (Mg²⁺), which can coordinate carboxylic acids. In the presence of Mg²⁺, only a 3-fold decrease in affinity was observed. Addition of Mg²⁺ to wild-type CA had no effect on its binding to CypA, suggesting that the effect is specific to R97D. A compensatory mutation (R97D-E113R) had the same effect as Mg²⁺. These results suggest that these residues are important for CypA-CA binding and that this interaction site may be amenable to disruption by small molecule inhibitors.

- 7) Cisplatin induces a Chk1-dependent DNA damage checkpoint response in *Schizosaccharomyces pombe*.** Dane Fletcher, Karen Piwowar, Kimberly Baldino, and *Stephen Dunaway. Department of Biology, Drew University, Madison, New Jersey

DNA damage checkpoints exist to insure that the integrity of genomic DNA is faithfully maintained through the eukaryotic cell cycle. In the presence of damaged DNA, checkpoints are triggered to delay cell cycle progression to allow for DNA repair. This process is particularly well studied in the fission yeast *Schizosaccharomyces pombe*. In fission yeast, the kinase Chk1 is a main component in the DNA damage checkpoint pathway. Cisplatin is a drug that has the ability to interact with DNA and is capable of causing both inter and intra strand DNA cross-linking. When cisplatin interacts with DNA, it can induce damage in a number of ways, which can lead to single and double-stranded DNA breaks. Our study attempted to determine whether or not treatment with cisplatin in fission yeast causes a Chk1-dependent DNA damage signal. We have shown sensitivity to cisplatin treatment of chk1- fission yeast strains and that Chk1 is phosphorylated in response to cisplatin treatment. We have also shown that a Chk1-dependent DNA damage checkpoint pathway is activated to stall the cell cycle progression after treatment with cisplatin, presumably to allow the cell time to fix the cisplatin mediated damage. We have also shown that rescue of the chk1- strain through insertion of a high copy plasmid encoding the chk1 gene. Taken together, our studies show that chk1- fission yeast strains are sensitive to cisplatin treatment, and that cisplatin does induce a Chk1-dependent DNA damage signal in fission yeast.

- 8) The Tumor Suppressor Guanylyl Cyclase C Directs Epithelial-Mesenchymal Interactions Opposing Stromal Reactivity through TGF- β 1 Mediated Signaling.** AV Gibbons, A Snook, P Li, J Lin, S Schulz, GM Pitari, *SA Waldman. TJU

The epithelium of the intestine undergoes continuous homeostatic regeneration mediated by proliferation, migration, and differentiation, an imbalance in which may be one mechanism contributing to colorectal carcinogenesis. Guanylyl cyclase C, the receptor for the endogenous paracrine hormones guanylin and uroguanylin and the exogenous bacterial diarrheagenic heat-stable enterotoxins, exclusively expressed in apical membranes of enterocytes, supplies intracellular cGMP maintaining intestinal fluid and electrolyte balance. Recently, GCC emerged as a key tumor susceptibility gene whose elimination produces unrestricted epithelial cell proliferation, metabolic remodeling and genomic instability accelerating tumorigenesis in mouse models of chemical and genetic intestinal carcinogenesis. These observations suggest a novel paracrine hormone hypothesis for colorectal cancer, wherein GCC coordinates key processes underlying epithelial homeostasis and its dysregulation, through early loss of guanylin and uroguanylin expression, which represents a sentinel event in neoplastic transformation. Beyond homotypic communication between epithelial cells, heterotypic interactions with underlying mesenchymal elements maintain this homeostasis through cell-cell contact and paracrine signaling. Activated fibroblasts, a component of the mesenchymal microenvironment regulating key homeostatic processes, including deposition of extracellular matrix (ECM) and regulation of epithelial cell proliferation, epithelial cell migration, and angiogenesis, contribute to dynamic modifications of the interstitium driving cancer progression. The working hypothesis here suggests that GCC-cGMP signaling regulates epithelial-mesenchymal interactions in intestine and loss of that signaling contributes to the development of a reactive stromal niche and neoplastic transformation.

- 10) Studies on the Interaction of GRK5 and HDAC5 in Myocytes.** GW Kim, JS Martini, BR DeGeorge, and *WJ Koch. Center for Translational Medicine, Thomas Jefferson University.

Heart failure (HF) is a major health problem with the pathological endpoint characterized by a loss of cardiac pump function and less responsiveness to beta-adrenergic receptor (beta-AR) stimulation. HF is often preceded by ventricular hypertrophy, which is initially adaptive but can become maladaptive. Beta-ARs and other G protein-coupled receptors (GPCRs) play a critical role in the regulation of heart function and the hypertrophic process. GPCR kinases (GRKs) such as GRK5 regulate several receptors in the heart via phosphorylation. GRK5 has been shown to be upregulated in the failing heart. GRK5 can also reside in the nucleus of myocytes to act as a novel kinase for the transcriptional repressors histone deacetylases (HDACs), which act at the level of myocyte enhancer factor-2 (MEF2). Our goal is to map the domains on GRK5 and HDAC5 responsible for their specific binding and interaction and determine how this interaction regulates myocyte hypertrophy. We have begun to construct different domains from these proteins to use in pull-down and binding assays to map the regions responsible for the interaction. We intend to design peptide constructs from the mapped HDAC-binding domain of GRK5 to determine if they can disrupt cellular HDAC5-GRK5 binding and alter myocyte hypertrophic signaling including MEF2-mediated gene transcription. Overall, these data will address the critical role of nuclear GRK5 in cardiomyocyte hypertrophic signaling and function, via its specific interaction with HDAC5.

- 9) Regulation of the Arrestin-2/Clathrin Interaction: Tales of a Tail-less Arrestin.** R C Kern and *J L Benovic. Department of Biochemistry and Molecular Biology, Thomas Jefferson University

Following agonist stimulation the non-visual arrestins, arrestin-2 and -3, promote internalization of G protein-coupled receptors (GPCR) through direct interactions with clathrin and the adaptor protein complex 2 (AP2). Studies suggest that binding of arrestin to components of the endocytic machinery is mediated by conformational changes in the C-terminal tail. Herein, we set out to further characterize the ability of the arrestin-2 C-terminal tail to regulate the interaction between arrestin-2 and clathrin. To analyze the inhibitory role of the C-terminal tail of arrestin-2, truncation analysis was used. These results revealed two regions within the arrestin-2 C-terminus that regulate clathrin binding: 1) aa 401-408, which contains a highly acidic patch and 2) aa 409-418, which contain the site of arrestin-2 phosphorylation. Ala scanning within the acidic patch revealed that three glutamic acid residues between aa 404-406 play a role in inhibiting clathrin binding. Comparison of the arrestin-2 and -3 tails revealed that arrestin-3, which shows an increased ability to bind clathrin and promote internalization of the β 2 adrenergic receptor, has several aa variations in the acidic patch and is nine amino acids shorter. Site directed mutagenesis revealed that arrestin-2-S412D, which mimics the phosphorylated form, is almost completely defective in clathrin binding. Interestingly, arrestin-2-R169E, which mimics the activated form of arrestin-2, overcomes the binding defects seen with the C-tail and arrestin-2-S412D. This suggests that receptor binding, which leads to arrestin-2 activation, provides the primary mechanism to overcome the C-tail inhibition and promote clathrin binding. These studies reveal multiple mechanisms involved in regulating the arrestin-2/clathrin interaction, highlighting the importance of this interaction in proper regulation and trafficking of GPCRs.

- 11) Biochemical and Structural Characterization of the Pak1-LC8 Interaction.** CM Lightcap, S Sun, JD Lear, U Rodeck, T Polenova, * JC Williams, Dept of Biochem and Mol Biol & KCC, Dept of Dermatology, TJU; Dept of Chem & Biochem, University of Delaware; Dept of Biochem & Biophysics, University of Pennsylvania.

P21-activated kinase-1 (Pak1) and the dynein light chain, LC8, are coexpressed at elevated levels in breast cancer and physically interact. The Pak1 sequence, however, does not contain a 'canonical' sequence motif present in other well-characterized LC8 interaction partners. Here we describe a point mutant in LC8 (K36P) that abrogates binding to canonical LC8 recognition sequences, but retains the ability to bind Pak1. Mutational analysis reveals that the LC8 binding site in Pak1 spans residues 212 to 222. Despite the absence of a canonical sequence, diffraction and NMR data indicate that Pak1 binds to LC8 along the same groove as peptides containing canonical LC8 binding sequences. In addition, the Pak1/LC8 interaction is contingent upon the dimeric state of LC8 and Pak1 binding to the LC8 dimer renders LC8 S88 inaccessible to phosphokinases. Thus, these results confirm the Pak1/LC8 interaction, they do not support the current model whereby Pak1 binds to and phosphorylates LC8 at S88 to promote anchorage independent growth. Rather, they suggest that LC8 binding regulates Pak1 activity and/or nuclear localization.

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- 12) Guanylyl Cyclase C Integrates Metabolic and Proliferative Programming Opposing Intestinal Tumorigenesis through AKT.** J.E. Lin, A.E. Snook, P Li, S. Schulz, G.M. Pitari, *S.A. Waldman. Depart. of Pharmacology and Experimental Therapeutics, Thomas Jefferson Univ., Phila, PA 19107, USA

GCC, the intestinal receptor for the paracrine hormones guanylin and uroguanylin whose early loss is universally associated with initiation of colorectal cancer, has emerged as a tumor suppressor whose dysregulation promotes proliferation and produces genomic instability underlying intestinal neoplasia. Beyond corruption of replicative and genomic integrity, reprogramming of metabolic circuits confers a survival advantage to cancer cells globally licensing tumor initiation and progression in all tissues. Here, we show that GCC is a system's integrator, coordinating glycolytic and oxidative metabolism with proliferation underlying normal regenerative homeostasis along the crypt-surface axis, and opposing tumorigenesis, in intestine. Eliminating GCC signaling in mice expands the proliferating crypt compartment, accelerating the cell cycle by inducing critical mediators including cyclin D and phosphorylated Rb. Replicative induction is coupled with an increase in glucose transport and the glycolytic machinery and a reciprocal reduction in mitochondrial biogenesis and oxidative phosphorylation, recapitulating the neoplastic metabolic phenotype defining a tumorigenic niche. Conversely, GCC signaling reverts neoplastic proliferative and metabolic programming in human colon cancer cells, decelerating the cell cycle and switching from glycolytic to mitochondrial ATP production, reminiscent of normal enterocytes. Coordination of proliferative and metabolic circuits in vitro and in vivo is orchestrated by GCC through suppression of signaling by AKT1, a key mediator of neoplastic transformation. Pharmacologic and genetic inhibition of AKT1 signaling mimics, while constitutive activation eliminates, the ability of GCC to regulate proliferative and metabolic programming in human colon cancer cells. GCC suppresses AKT1 signaling through the phosphatase and tensin homolog (PTEN), an antagonist of phosphoinositide 3 (PI3) –kinase signaling central to activating AKT.

- 14) Factors Affecting Lipid Homeostasis in *Schizosaccharomyces pombe*.** T Okomski, L Rice, PhD, and *J T Nickels, PhD. Pharmacogenomics, Medical Diagnostic Laboratory, LLC. Department of Bioscience Technologies, Thomas Jefferson University

Sterol synthesis in *S. pombe* is similar to that of mammals, except that the end-product in *S. pombe* is ergosterol, instead of cholesterol. Sre1 is the *S. pombe* ortholog of mammalian SREBP (sterol regulatory element binding protein), which regulates lipid homeostasis through expression of genes involved in ergosterol synthesis. The goal of this study is to evaluate and further characterize the regulation of the SREBP pathway. Activity of enzymes in the sterol pathway can be inhibited via administration of Itraconazole and Lovastatin. It is expected that hmg1+ and erg11+ expression will be up-regulated in *S. pombe* in response to this condition. Additionally, we hypothesize that Arv1, a putative lipid trafficking protein, plays a role in SREBP signaling, and will thereby impact expression of the same genes that respond to azoles and statins. The method developed to examine Arv1's involvement will be achieved by deleting arv1+ in *S. pombe*, to test via real-time-PCR whether it plays a role in the regulation of hmg1+ and erg11+, as well as other gene targets in the SREBP-pathway. We show that expression of hmg1+ and erg11+ in wild-type *S. pombe* is, in fact, up-regulated when treatments are administered. We propose that *S. pombe* arv1- will respond similarly, substantiating its role in SREBP signaling. This model provides an explanation for conditions that will lead to a better understanding of the sterol biosynthetic pathway.

- 13) Uncovering G Protein-Coupled Receptor Kinase-5 as a Histone Deacetylase Kinase in Cardiomyocytes.** J Martini, P Raake, B DeGeorge, L Vinge, K Chuprun, D Harris, AD Eckhart, J Pitcher, WJ Koch*, TJU

G Protein-coupled receptor (GPCR) kinases (GRKs) are critical regulators of cellular signaling and function including in the heart. GRK2 and GRK5 are two GRKs important for cardiac regulation and both have been shown to be up-regulated in dysfunctional myocardium. Our data show that increased levels and activity of GRK5 may have unique significance due to its nuclear localization, a property not shared by GRK2. We find that transgenic mice with elevated cardiac GRK5 have exaggerated cardiac hypertrophy and early heart failure compared to control mice after pressure overload. This pathology is not present in GRK2 transgenic mice or mice with cardiac-overexpression of a mutant GRK5 that is excluded from the nucleus. Nuclear accumulation of GRK5 is enhanced in myocytes after aortic banding in vivo and also in vitro in myocytes after increased Gαq activity, the trigger for pressure overload hypertrophy. GRK5 can activate myocyte enhancer factor-2 (MEF2) in concert with Gq signals demonstrating that GRK5 regulates gene transcription via a pathway critically linked to myocardial hypertrophy. Mechanistically we show that this is due to GRK5 acting as a class II histone deacetylase (HDAC) kinase as it can associate with and phosphorylate the MEF2 repressor, HDAC5. Moreover, significant HDAC activity can be found with GRK5 immunoprecipitated from hypertrophied hearts. Our data suggest that GRK5 is a novel nuclear HDAC kinase that plays a key role in maladaptive cardiac hypertrophy.

- 15) Macrophages containing Caveolin-1 have a protective role in the development of Atherosclerosis.** S. Pavlides, J-F. Jasmin, I. Mercier, G. Llaверias, M.P. Lisanti*, P.G. Frank*, Department of Biochemistry and Molecular Pharmacology, Kimmel Cancer Center, TJU

Coronary Artery Disease is the number one cause of morbidity and mortality in industrialized nations. The initial steps of the complex process of atherosclerosis occur as a consequence of a chronic inflammation in the arteries, which eventually leads to the formation of atheromatous lesions or plaques. An important molecule for the development of atherosclerotic lesions is Caveolin-1 (Cav-1). Cav-1 has been shown to regulate cellular cholesterol metabolism and various signalling pathways. In order to specifically dissect the role of Cav-1 in macrophages, we setup an elegant system involving bone marrow transplantation. In these experiments, we used Cav-1 -/- ApoE -/- double knockout mice and ApoE -/- knockout mice. Our results suggest that a specific deficiency of Cav-1 in bone marrow-derived macrophages increases atherosclerosis. However, when we transplanted bone marrow-derived macrophages from a wild-type or Cav-1 -/- mice (donors) into Cav-1 deficient mice (recipients) atherosclerosis was not affected. These findings suggest that Cav-1 in macrophages has an anti-atherogenic role. We hypothesize that the absence of Cav-1 in macrophages leads to impaired intracellular cholesterol homeostasis causing an increased accumulation of cholesterol in macrophages thus promoting larger atherosclerotic lesions.

- 16) DACH1 regulation of estrogen receptor alpha in breast cancer.** V. M. Popov, Jie Zhou, Lawrence A Shirley Kongming Wu, Hallgeir Rui, Ratna K Vadlamudi, Chenguang Wang, and Richard G. Pestell,* KCC, Department of Surgery, TJU, Phila, PA. Department of Obstetrics and Gynecology, the University of Texas Health Science Center at San Antonio, San Antonio, TX

The Dachshund (dac) gene, initially cloned as a dominant inhibitor of the Drosophila hyperactive EGFR mutant ellipse, encodes a key component of the cell-fate determination pathway involved in Drosophila eye development. Analysis of over 2200 breast cancer samples demonstrated improved survival by some 40 months in patients whose tumors expressed DACH1. Herein, DACH1 bound and inhibited ER α function. Nuclear DACH1 and ER α expression correlated in human breast cancer (OR 3.34, 95% CI 3.02, 16.424). DACH1 expression inhibited, and DACH1 shRNA enhanced estradiol-induced DNA synthesis, cellular proliferation and apoptosis. DACH1 bound ER α in IP-Western blotting, associated with ER α in chromatin IP and inhibited ER α transcriptional activity, requiring a conserved DS domain. Proteomic analysis identified Proline, Glutamic acid and Leucine rich Protein 1 (PELP1), as a DACH1-binding protein. The DACH1 carboxyl terminus was required for binding to PELP1. Estradiol disengaged DACH1 from the PELP1/ER α complex allowing PELP1 to serve as an ER α co-activator. DACH1 expression, which is lost in poor prognosis human breast cancer, functions as endogenous inhibitor of ER α function in breast cancer cells.

- 17) Description withheld.**

- 18) Effects of Volatile Anesthetics on Tubulin Polymerization.** A. Roy, Jefferson *R Eckenhoff, J Xi. Medical College, Thomas Jefferson University and Department of Anesthesiology, University of Pennsylvania

Microtubules have long been suspected to play a role in anesthetic action. Anesthetics not only bind tubulin, the building blocks of microtubules, but they also trigger microtubule disassembly. Further, neurons increase tubulin expression in response to anesthetic exposure, suggesting tubulin dysfunction. Here, we hypothesized that halothane and isoflurane decrease tubulin polymerization into microtubules. Beta-tubulin protein was combined with varying concentrations (0-5 mM) of halothane and isoflurane and incubated for up to 1 hour at 37 oC. The progress of the polymerization was monitored using a fluorescence dye that increases quantum yield when bound to tubulin polymers. The anesthetics had no effect on the dye fluorescence in the absence of tubulin. We found that both halothane and isoflurane decreased the rate of tubulin polymerization in a concentration dependent manner. We also investigated the location of anesthetic binding within tubulin. Halothane quenches 20-30% of the tryptophan fluorescence signal of tubulin, indicating that halothane may bind near one of the four tryptophan residues within tubulin. Additional studies using H-diaziflurane, a photolabel analog of isoflurane, suggested an anesthetic binding site near the colchicine binding site within β -tubulin. Since colchicine is a known inhibitor of tubulin polymerization, this finding may explain why anesthetics decreased tubulin polymerization. Considering that tubulin polymerization is critical for processes such as mitosis, cytokinesis, vesicular transport, and synaptic function, the findings of this study may indicate a direct mechanism by which anesthetics contribute to altered cellular function.

- 19) Cdc28 and Ime2 co-regulate the Sum1 meiotic gene repressor.** ME Shin and E Winter*. Department of Biochemistry and Molecular Biology, Thomas Jefferson University

Meiosis is a specialized program where a single round of replication is followed by genetic recombination and two chromosomal divisions to yield four haploid gametes. In the yeast, *Saccharomyces cerevisiae*, meiosis is part of sporulation which creates four gametes encapsulated in a resistant spore wall. The sole, essential cyclin-dependent kinase (CDK), Cdc28, is not only a key driving force in mitosis, but also in meiosis. Another key driver is the meiosis-specific CDK-like kinase, Ime2. Sum1 is a transcriptional repressor of middle meiosis-specific genes (MSGs) that promote the meiotic divisions. We demonstrate that Cdc28 and Ime2 are able to phosphorylate a cluster of sites in Sum1. These phosphorylation sites reside in a regulatory region adjacent to Sum1's DNA-binding domain. We hypothesize that Cdc28 and Ime2 negatively regulate Sum1 which allows for the expression of MSGs and progression through meiosis. We have created a non-phosphorylatable mutant, Sum1-4A, to examine the consequences when the two major kinases are unable to regulate Sum1. We also created a phosphomimetic mutant, Sum1-8D, to examine the consequences if Sum1 were to be constitutively phosphorylated by the two key kinases. These studies will elucidate how Cdc28 and Ime2 co-regulate Sum1 and meiosis.

- 20)** **A role for SUMO in genome stability.** H.R. Silver, X.L. Chen and *E.S. Johnson. Department of Biochemistry and Molecular Biology. Thomas Jefferson University

Conjugation of the small ubiquitin-like modifier SUMO to target proteins is essential for viability in the yeast *Saccharomyces cerevisiae* and in most other eukaryotes. About 95% of SUMO conjugation in budding yeast is catalyzed by the E3 ligases Siz1 and Siz2. Our genetic analyses have shown that the *siz1Δ siz2Δ* mutant is viable, but when combined with the homologous recombination (HR) mutant *rad52Δ* the resulting triple mutant is dead. This suggests that *siz1Δ siz2Δ* mutants accumulate DNA damage that requires the HR pathway for repair. Remarkably, the lethality of this triple mutant is suppressed by deletion of the gene encoding topoisomerase I (TOP1). We hypothesize that SIZ mutants accumulate Top1-dependent DNA damage that requires Rad52 for repair or prevention. The goal of this study is to characterize the SUMO-dependent process that is defective in this mutant. We have developed a galactose-inducible TOP1 allele to study cell cycle progression in these mutants. The GAL-TOP1 allele behaves as a *top1Δ* in the absence of galactose. Using flow cytometry and microscopy we have observed that, with the addition of galactose, *siz1Δ siz2Δ rad52Δ* GAL-TOP1 mutants arrest in early mitosis, similar to mutants that are unable to complete DNA replication. We have also detected the activation of the Rad53 intra-S phase checkpoint in these mutants. Based on these and other observations we conclude that Siz dependent SUMO conjugation plays a role in maintaining genome integrity, possibly by participating in DNA replication.

Field Topic:

Cell & Molecular Biology

- 21)** **Developing a Protocol for Rapid Identification of SNPs Associated with Dyslexia.** J. Alaimo, M Susek, J George, *A Yezerski, and J Coffin. Department of Biology and Neuroscience, King's College.

Recent studies have suggested that cerebellar function is impaired in individuals with dyslexia. Eye-blink conditioning is a method to measure cerebellar function and may discriminate between dyslexic and non-dyslexic individuals. Many susceptibility loci have been identified on 8 different chromosomes, indicating a strong genetic component for dyslexia. Specifically, KIAA0319, a locus on chromosome 6, containing two SNPs, have shown a significant relationship to a dyslexia diagnosis, but is undefined in molecular function. The ultimate goal of our research is to compare the results of cerebellar function testing to the SNPs known to be associated to dyslexia. This genotype to phenotype study will help to elucidate the molecular mechanism behind this impairment as well as clarify the efficacy of these specific methods for dyslexia diagnosis. To that end, we have developed a molecular genetic protocol for genotyping individuals tested by eye-blink conditioning. Additionally we developed a nested PCR method for genotyping DNA of low quality. Having created these consistent and sensitive genotyping methods, we have begun to compare our results to the outcomes of the eye-blink conditioning. Our preliminary data suggests a relationship between the SNPs and eye-blink conditioning. Further work could better automate the genotyping process by using fluorescent primers and fragment analysis via an automated DNA sequencer.

- 22)** **Development of a Yeast Mating Protocol for Cytotrap® Two Hybrid Interaction Analysis.** M. Bah and *E E Biswas-Fiss, Department of Bioscience Technologies, JCHP, Thomas Jefferson University, Philadelphia, PA 19107.

Purpose: We wanted to develop a simple screening procedure for Cytotrap® two-hybrid interaction analysis using a yeast mating system. **Methods:** A temperature sensitive *cdc25h* yeast strain was used, which is rescued by interaction of the hybrid proteins. A library of human retinal cDNA in pMyr vector was constructed and used to transform *cdc25h* alpha-cells. The transformed *cdc25h* cells were amplified on selective media plates, pooled and stored in aliquots at -80°C. The extra-cellular domains of the retina-specific ABC transporter, ABCR, (ECD1 aa 62-646 and ECD2 aa 1395-1680) were cloned into pSOS bait vector and used to transform *cdc25h* a-cells. The interaction was carried out by mating the a-bait strain with the alpha-library strain followed by plating onto selective media at a density of 10^5 cfu per plate. The plates were then grown at 37°C to select for positive two hybrid interaction by diploids (a/alpha) capable of growth at the restrictive temperature. **Results:** Our results indicated that there were approximately 5 colonies per plate of 10^5 diploids. The number of colonies per plate appears to be a reasonable number for this particular interaction type. Isolation of the plasmids from the diploids and sequencing will confirm these interactions. **Conclusion:** The mating protocol appears to be a viable approach for screening yeast two hybrid interactions using the Cytotrap® system, and allows for multiple parallel screenings of a given cDNA library.

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- 23) B56-containing PP2A may positively regulate apoptosis in CD4+/CD8+ thymocytes.** P. Barish, Z McCormick, N Cunningham, J McDonald, D Grant, F Albino and *J Punt. Department of Biology, Haverford College

Developing T-cells in the thymus are selected for maturation based on stimulation through their T-cell Receptor (TCR). A strong stimulation results in cell death (apoptosis) to prevent the production of autoreactive thymocytes, in a process called negative selection. Paradoxically, however, mature thymocytes which undergo the same strong stimulation through the TCR proliferate. Kinase activity has long been thought to play a significant role in the differences between these two cell populations, as many molecules key to cell fate (Nur77, Bim, Akt) undergo phosphorylation events. Recently the role of phosphatases in this signaling pathway has been investigated. Here we argue for the role of Protein Phosphatase 2A (PP2A), a complex heterotrimer, in the apoptosis of autoreactive thymocytes. Our data indicate PP2A associates with Nur77, Bim and Akt and inhibition of PP2A results in increased levels of Akt phosphorylation. We have identified a particular isoform of the B56 subunit, occurring at 50 kDa, which shows differential expression in mature and immature thymocytes. Preliminary experiments show this isoform to associate with PP2Ac in immature thymocytes, suggesting a possible role for this subunit in the dephosphorylation of Akt. Recent studies have shown certain B56 isoforms to dissociate from PP2A upon phosphorylation by ERK, suggesting a method for differential regulation of PP2A in thymocytes. Here we propose a model of B56-containing PP2A as a positive regulator of apoptosis in response to strong stimulation through the TCR in immature thymocytes.

- 25) Overexpression of A1 Adenosine Receptors Causes Decreased Cardiac Contractility and Abnormal Calcium Handling by Selectively Perturbing Caveolin 3-Associated T-tubule Structure.** PH Chung, E Cheskis, J Zhang, J Song, JF Jasmin, H Funakoshi, DE Hermann, MP Lisanti, JY Cheung, AM Feldman, *TO Chan. Medicine, TJU

Mice overexpressing the A1-adenosine receptor (A1-AR) develop a dilated cardiomyopathy and impaired myocyte calcium homeostasis. We hypothesize that overexpression of A1-AR might alter coupling between the receptor and T-tubules, containing caveolin (Cav). Mice overexpressing A1-AR demonstrated a decrease in the expression of Cav3 as measured by western blot. When compared to Cav3 in WT mice, the Cav3 protein in A1-AR mice had differential detergent solubility and higher sucrose gradient density migration. When isolated cardiac myocytes were evaluated using immunofluorescent microscopy, Cav3 in WT mice demonstrated a punctate-striated staining pattern that co-localized with the T-tubule marker, NCX. This punctate-striated pattern was lost in A1-AR myocytes. Di-8-ANEPPS T-tubule binding assay showed that T-tubule structure was not disrupted in A1-AR myocytes. Morphologic alteration of Cav3 structure was not seen in myocytes overexpressing A2A-AR. Co-expression of both A1- and A2A-ARs restored Cav3 staining pattern similar to WT mice. Our data demonstrate that diminished cardiac contractility and abnormal calcium homeostasis in mice overexpressing A1-AR is due at least in part to coupling of A1-AR and Cav3. These results suggest that the coupling of select G protein-coupled receptors and caveolins might be a novel target in the treatment of heart failure.

- 24) RAPD-PCR as a Novel Method for DNA Fingerprinting.** M E Brice, *A Yezerksi. Biology Department, King's College.

The specificity of "DNA Fingerprinting" techniques may be the root of their shortcomings. These methods use long primers to target specific variable loci which comprise less than 0.01% of the total genome. While these analyses are powerful, they may fall short when the compared DNA's are similar. Our research explored the efficacy of a potentially more comprehensive method, RAPD-PCR. While RAPD's are less specific, they do increase the proportion of the genome analyzed in the process. Our research compared the RAPD profiles using three replicates of eight tissue samples from three genetically diverse mice. Some areas of variability in the profiles indicated that this method can detect even the small mutational differences that might accrue within the same individual.

- 26) Antihypertensives as antineoplastics: potential mechanism(s) of action.** M Davis, *H Arafat, Department of Surgery, TJU.

Purpose: We recently showed that angiotensin II type 1 receptor (AT1R) is expressed in pancreatic ductal adenocarcinoma (PDA) tissues and cells, and that its blockade by an AT1R blocker, losartan, potently reduces cell survival. Here, we investigated the involvement of AT1R in PDA cell viability and explored the mechanisms involved. Methods: Human PDA cell line Panc10.01 apoptosis was analyzed by TUNEL staining. The expression of the tumor suppressor/proapoptotic p53 gene, its downstream proapoptotic targets BAX and the cyclin-dependent kinase inhibitor p21WAF1, and the antiapoptotic bcl-2 genes were examined by real time PCR. In addition, the expression of different histone deacetylases (HDACs 1-6) was analyzed by PCR and the effect of losartan on their expression was analyzed by QuantiGene assay. Results: Losartan (0.01-1mM) inhibited PDA cell growth and triggered apoptosis dose-dependently. This was associated with increased p53 gene and protein expression, increased BAX, and reduced bcl-2 gene levels. There was also a dramatic increase in the expression of p21WAF1 mRNA. Analysis of PDA cells showed they express HDACs 1-6. Losartan reduced the transcription of HDACs 1, 2, 3 by 40-60%, but did not affect the expression of HDACs 4,5,6. Conclusions: Our data demonstrate a previously undescribed involvement of AT1R in PDA cell survival, and suggest that the losartan-mediated induction of different proapoptotic signaling pathways and inhibition of HDACs transcription could represent some of the molecular basis of its action as a novel antineoplastic agent.

27) Description withheld.

28) Expression of novel *Anaplasma phagocytophilum* antigenic peptide.

D. Dimitrov, *E Biswas, PhD, Bioscience Technologies, TJU, J Hoey, PhD, Medical Diagnostics Labs

Anaplasma phagocytophilum, which is a tick-borne pathogen that infects humans and causes Human Granulocytic Anaplasmosis, has not been fully investigated for its detection mechanism. The goal of this study is to grow and discover a novel *A. phagocytophilum* antigenic peptide, which could aid in future assays. In order to achieve that, we must put its DNA into *E. coli* first and grow it on twenty large NZY media plates. This nutrient rich media helps the DNA grow into plaques, which are taken and re-plated on another twenty plates in order to separate colonies better. These new plaques are screened using IPTG-pres soaked nitrocellulose membrane, patient positive and negative sera samples, and the western blot technique. Ten positive plaques appeared as small dark circles on the membranes after screening with positive patients. Mini preps were done on the positive plaques, and then are sent for sequencing in order to make sure that this DNA is indeed from *A. phagocytophilum*. Using the tool BLAST, the sequences are read, and determined what organism the DNA comes from. The positive plaque(s) confirming their *A. phagocytophilum* origin will be the novel antigenic peptide(s), which will aid in future research and detection assays.

29) Variable expression of DNA-dependent protein kinase within neoplastic pancreatic tissues. B. Durkan, DM Pineda, SL Showalter, TN Showalter, A Witkiewicz, CJ Yeo, *J Brody. Departments of Surgery, Thomas Jefferson Pancreas, Biliary and Related Cancer Center, Thomas Jefferson University Philadelphia, PA 19107

Double-strand breaks are known to be one of the most lethal types of DNA damage which inevitably prove fatal to cells unless repaired. Non-homologous end joining is an important mechanism to repair DNA damage. The main components of this type of DNA repair include DNA protein kinase (DNA-PKcs) and Ku subunits Ku70 and Ku80. DNA-PK, utilizing a binding site for DNA free ends, allows for approximation of separated ends of the fragmented DNA thereby allowing base repair and stabilization of the DNA fragment. Previous studies have attributed a tumor suppressor function to DNA-PK. However, the ultimate role of DNA-PK in cancer biology remains controversial. Increased expression of DNA repair enzymes could aid in protecting cancer cells. We evaluated the expression of DNA-PK in pancreatic cancer cell lines utilizing immunohistochemistry, immunofluorescence, and western blot techniques. DNA-PK is up-regulated in the chromosomally unstable (CIN), Capan-1 cells, versus the PL5 cell line, a chromosomally stable pancreatic cell line. We also demonstrated, via immunohistochemistry, an up-regulation of DNA-PK in all pancreatic ductal adenocarcinomas compared to normal pancreatic tissue as well as an up-regulation of the Ku70 protein in pancreatic compared to benign tissue. These preliminary results may prove valuable in further understanding the mechanisms by which highly unstable cancerous cells, such as pancreatic cancers, avoid undergoing apoptosis and maintain chromosomal integrity. Further, as DNA-PK has been shown to be a radiosensitizer in past studies, the enzyme may prove to be a valuable target for future treatment strategies against pancreatic cancer.

30) Can Sterol Superlattices Form in Lipid Membranes in the Presence of Membrane Bound Proteins? K. A. Essuman, B Venegas and *P L-G Chong. Department of Biochemistry, Temple University School of Medicine.

Cholesterol resides in cell membranes, and is an etiological factor in many disorders. Studies show that cholesterol is not homogeneously distributed in membranes but tends to form regular distributions called superlattices. The extent of superlattice reaches a local maximum at predicted critical sterol mole fractions (Cr), and influences the activity of surface-acting enzymes. These studies, however, were done on lipid membranes without membrane bound proteins (MBPs). Since cell membranes contain many MBPs, it is of interest to investigate whether sterol superlattice persists in MBP-containing membranes. The long-term goal is to investigate how sterol superlattice formation is affected by rhodopsin, an MBP. Initially, we must determine whether the detergent octyl glucoside (OG) affects sterol superlattice. So the generalized polarization (GP) of LAURDAN fluorescence was monitored in 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine/cholesterol unilamellar vesicles with sterol mol% values near the predicted Cr value of 22.2 mol%. Vesicles were mixed with buffer containing OG, then dialyzed to remove the OG. GP-vs-sterol mol% showed a peak at 22.2 mol% cholesterol. The presence of a biphasic profile shows that the amount of detergent used does not affect sterol superlattice formation; thus, the proposed experiment is feasible. This research may eventually lead to a deeper understanding of the importance of membrane cholesterol and the etiology of cholesterol-related diseases.

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- 31) Monocarboxylate Transporter 4 is required for cell migration in vitro following wounding of the retinal pigment epithelium.** SM Gallagher and *NJ Philp; Dept of Anatomy, TJU, Philadelphia, PA 19107

Epithelial cell migration during wound healing depends on the coordinated interactions of cytoskeletal proteins and integrins. Extracellular pH (pHe) affects cell attachment and migration by modulating the strength of integrin-mediated adhesion to the extracellular matrix. In addition, increased lactate in the extracellular space promotes expression of the matrix components collagen and hyaluronan, which are important for cell migration. Wound healing is also accompanied by increased glycolytic metabolism and lactate production, suggesting that proton-coupled monocarboxylate transporters (MCTs) could be critical regulators of pHe and migration. In the present study, we examined whether MCT4 plays a role in retinal pigment epithelial (RPE) cell migration during wound healing. We found that MCT4 and β 1-integrin colocalized at the basal membrane of RPE cells in vitro and formed a stable complex in coimmunoprecipitation assays. In wounded RPE cells, MCT4 and β 1-integrin colocalized in lamellapodia at the leading edge of migrating cells. Silencing MCT4 slowed migration in RPE cells following wounding and decreased attachment to laminin. The results of these studies clearly demonstrate that MCT4 associates with β 1-integrin at the leading edge of migrating cells and may stabilize integrin-mediated attachment to extracellular matrix. Thus, the role of MCT4 during cell migration may be to increase extracellular lactate as well as to modulate pHe.

- 33) A Role for Guanylyl Cyclase C in Colon Cancer Metastasis.** Deana Guzzi, Adam Berger, David Zuzga and *Scott Waldman. TJU

Metastasis is a complex process consisting of a cascade of molecular events that permit a tumor cell to escape from a primary tumor site and seed distant organs. Guanylyl Cyclase C (GCC), previously identified as a marker for metastatic colon cancer cells, is a regulatory protein expressed by colorectal cancer cells that regulates diverse cellular processes such as proliferation, metabolism, and cytoskeletal architecture. It was shown that activation of GCC in human colon cancer cells with its exogenous ligand, heat-stable enterotoxin ST, decreased pulmonary metastatic seeding and peritoneal carcinomatosis of human T84 colon cancer cells in mice. Here, the role of GCC signaling in colorectal cancer cell metastasis was further explored. CT26 murine colon cancer cells expressing either GCC or GCCTm, a truncated protein lacking a catalytic domain, were treated in vitro with ST and 105 cells were injected into the peritoneum or the tail vein of male nude mice. Mice that received an intraperitoneal injection were examined for carcinomatosis in the abdomen while those that received a tail vein injection were examined for metastatic nodules in the lung. There was no significant difference in carcinomatosis in mice injected with CT26-GCC or CT26-GCCTm. Furthermore, there was no significant difference in carcinomatosis between PBS- or ST-treated CT26-GCC cells. In striking contrast, in vitro ST treatment of CT26-GCC cells reduced the frequency of tumor metastasis in the lung 34% compared to PBS-treated cells ($p=0.02$), recapitulating results obtained with human colon cancer cells. Thus, GCC and cGMP signaling appear to reduce the ability of human and murine colorectal cancer cells from forming pulmonary, but not peritoneal metastases. In the context of the universal over-expression of GCC by metastatic colorectal cancer cells, these observations suggest that GCC ligands may be efficacious therapeutic agents to selectively prevent pulmonary metastases in patients with colorectal cancer.

- 32) Investigation of the role of PAM-1 and PTL-1 in meiotic exit and anteroposterior polarity in the *Caenorhabditis elegans* embryo.** B Godoy, C Reeves, K Susman, and *R Lyczak. Department of Biology, Ursinus College.

In *Caenorhabditis elegans* the puromycin sensitive aminopeptidase (PSA) PAM-1 has been shown to play a key role in embryonic meiotic exit and anterior-posterior (AP) axis determination. Mutations in *pam-1* lead to delays in meiotic exit and AP axis establishment, which results in the production of many dead embryos. Despite the implication of PAM-1 in early embryonic axis specification, the mechanism by which it acts is currently unknown. We believe PAM-1 is involved in protein degradation, and that a buildup of its target protein(s) contributes to the abnormal embryonic phenotypes and increased lethality. PTL-1 is a microtubule associated protein homologous to the human Tau, which has been shown to reach excessive levels in Alzheimer's disease. Work in other systems suggests that Tau may be a target of PSA's. Thus, we hypothesize that PTL-1 may be a PAM-1 target in *C. elegans*. We propose that the buildup of PTL-1 in *C. elegans* embryos leads to some of the abnormalities in *pam-1* mutants. To test this, we have created a *pam-1*; *ptl-1* double mutant strain and will observe the phenotype. We expect a rescue of some of the *pam-1* phenotypes, namely those that depend on proper microtubule function. We hope to visualize the AP axis and measure the timing of meiotic exit through GFP tagging of the spindle apparatus. We hope to determine whether PTL-1 is a target of PAM-1 and how it contributes to delayed meiotic exit and inhibition of axis formation in *pam-1 C. elegans*.

- 34) Microtubule instability may prevent polarization of MTOC in immature thymocytes.** Emily Hinchcliff, Mary Chang, Nicole Cunningham, & *Jennifer Punt. Biology Department Haverford College, 370 Lancaster Ave, Haverford, PA, 19041

In response to T cell receptor (TCR) and CD28 costimulation, mature single positive (SP) thymocytes proliferate, while immature CD4+CD8+ thymocytes undergo apoptosis. A key difference between these two cell types involves cytoskeletal rearrangement. Specifically, within minutes after stimulation, mature T cells reorganize and polarize their microtubule organizing center (MTOC) to the site of stimulation. Interestingly, immature CD4+CD8+ thymocytes do not reorient their MTOC in response to TCR signals. We are working to understand both the causes and consequence of this developmental difference and have evidence that microtubule stability (regulated largely by microtubule associated proteins, such as GSK-3 β) may play an important role. Preliminary data, in fact, suggest that the tyrosination of tubulin, an indicator of microtubule instability, may demonstrate differences that underlie the differential MTOC polarization. Tyrosinated tubulin levels are higher at rest in immature T cells, which also display a markedly more diffuse microtubule staining pattern. When we increase stability, we observe that DP ability to polarize is restored. We propose a model whereby decreased Akt activity in immature CD4+CD8+ thymocytes enhances microtubule instability, thereby interfering with the ability of molecular motors to move the MTOC to the site of stimulation.

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- 35) Determining the role of subcellular localization of heterotrimeric G protein betagamma subunits in Gbetagamma-mediated Golgi vesiculation.** R Irannejad and *PB Wedegaertner, Department of Biochemistry and Molecular Biology, TJU

Heterotrimeric G proteins are responsible for transducing signals initiated at the plasma membrane (PM) by G protein-coupled receptors. However, a number of studies indicate that G proteins can internalize and recycle to the PM once activated, thus begging the question of whether G protein subunits signal at endomembrane locations. As an example of potential signaling at endomembranes, a series of studies have implicated Gbetagamma in activating protein kinase D through PKCeta at the Golgi membrane to initiate a pathway causing fission of Golgi vesicles. However, it is not clear whether the betagamma signal is generated at the Golgi membrane rather than another subcellular location. The goal of our study is to identify the site of Gbetagamma activation in this pathway. Because overexpressed Gbetagamma, which causes Golgi vesiculation, localizes to both PM and endomembranes, the site of Gbetagamma action is unclear; thus, we generated Gbetagamma that were selectively targeted to distinct subcellular locations. Constitutively targeted PM-betagamma, ER-betagamma, Golgi-betagamma and cytosolic-betagamma were expressed in HEK293 cells, and Golgi vesiculation, as a read-out of betagamma activation, was determined by fluorescent microscopy. Our data indicate that Golgi-betagamma, but not PM-betagamma, ER-betagamma and cytosolic-betagamma, induces Golgi vesiculation. Moreover, we are implementing a system for rapamycin-inducible recruitment of Gbetagamma to distinct subcellular locations, and our initial data shows that PM recruitment of Gbetagamma activates appropriate signaling.

- 37) Novel Intermediate Filament-Linked N-Cadherin/ γ -Catenin Cell Junction Involved in the Establishment of Differentiated Lens Cytoarchitecture.** S Leonard, *AS Menko. Thomas Jefferson University

Tissue morphogenesis and maintenance of complex tissue architecture requires a variety of cell-cell junctions. Typically, cells adhere to one another through cadherin junctions, both adherens and desmosomal junctions, which are strengthened by association with cytoskeletal networks during development. While both β - and γ -catenins link classical cadherins to the actin cytoskeleton, only γ -catenin links desmosomal cadherins to intermediate filaments (IFs). Here we provide the first biochemical evidence that γ -catenin also mediates interactions between classical cadherins and the IF cytoskeleton in vivo. We discovered that in the developing lens, which has no desmosomes, vimentin was associated with N-cadherin complexes. To determine whether vimentin was linked to N-cadherin/ γ -catenin or N-cadherin/ β -catenin complexes we developed a novel double immunoprecipitation technique that makes possible identification of multiple members of a single protein complex. This approach revealed that vimentin linked specifically to N-cadherin/ γ -catenin complexes. These unique junctions were not observed until after lens cell differentiation was initiated. γ -catenin had a distinctive localization to the cell vertices at the basal aspects of the hexagonally shaped differentiating fiber cells, a region devoid of actin. We believe this unique vimentin-linked N-cadherin/ γ -catenin junction provides tensile strength necessary to establish and maintain structural integrity to tissues that lack desmosomes.

- 36) Protein-Protein Interactions of Arid2 and Prox1.** L H Isaacs, X Chen, T P Patel, *M K Duncan. Department of Biological Sciences, University of Delaware, Newark, DE 19716

Prox1 is a homeodomain transcription factor that is important for the regulation of lens, liver, pancreatic, and lymphatic system development¹. Prox1 also regulates the tumor suppressor genes p27KIP1, p57KIP2, and E-cadherin². Previously, Arid2 (also called BAF200 and zipzap) was isolated from a yeast-two-hybrid assay as a potential Prox1 interacting protein. Arid2 belongs to the ARID family of proteins that are important for cell development, gene expression, and cell growth regulation³. It is also a vital component of SWI/SNF complexes that function in chromatin remodeling. This work seeks to test the hypothesis that Prox1 and Arid2 actually interact and to determine the biological relevance of this interaction. A GAL4 yeast two-hybrid assay was used to detect an interaction between proteins by the activation of reporter genes that are transcribed if the two proteins are able to join. The plasmids Prox1-pGBKT7 and Arid2-pACT2, the yeast two-hybrid shuttle vectors, were transformed into E. coli and minipreps were performed to purify the DNA from the cells. The plasmids were then transformed into two different yeast strains of two different mating types. These were mated and plated on selectable media lacking nutrients that the reporter genes are responsible for making to screen for interactions. Yeast colonies did grow on selectable media, suggesting that Prox1 and Arid2 do interact. Furthermore, fluorescent immunohistochemical analysis was performed on four-week C57B6 mouse lens, which showed that Arid2 and Prox1 are colocalized in the young mouse lens. This study will continue to determine whether protein-protein interactions between Prox1 and Arid2 occur in vivo and to determine how Arid2 affects Prox1 function. This research is funded by the Howard Hughes Medical Institute.

- 38) Investigating the role of the novel DNA Helicase Pfh1 in Maintaining Genomic Stability.** Karen LeSuer and Stephen Dunaway*. Drew University, Madison, NJ 07960

Genomic instability is a hallmark of human cancer. Helicases play important roles in maintaining genome stability because they are involved in DNA replication, recombination and repair. The Pif1 family of 5'-3' DNA helicases is conserved throughout eukaryotic evolution and is involved in maintaining the stability of both nuclear and mitochondrial genomes. Members have been implicated in maintenance of nuclear and mitochondrial DNA stability, including involvement in telomere and Okazaki fragment processing, and in inhibition of gross chromosomal rearrangements. pfh+ is the only Pif1 family member in fission yeast. Pfh1 acts as a monomeric 5'-3' helicase and is believed to be involved in nuclear DNA replication. It has been found to be essential for cell viability and pif1- cells terminally arrest at the G2/M checkpoint suggesting that Pfh1 may be important for genomic stability in fission yeast. In this study we attempted to elucidate further functions and pathways for Pfh1 in S. pombe. To ascertain Pfh1 functions, we performed a high copy suppressor screen using a cDNA library in a cold sensitive strain of Pfh1, pfh1-R20, to look for rescue events of the strain at restrictive temperatures. In the event of a rescue, the responsible gene could be isolated for indications of Pfh1 functions. We have identified 5 possible candidate cDNA fragments from the screen which we are presently characterizing.

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- 39) Quantitative In Situ Protein Analysis of Hormone Pathways in Breast Cancer Tissue Microarrays.** J Lin, T H Tran, F E Utama, A Witkiewicz, and *H Rui. Dept of Cancer Biology, KCC, Dept of Bioscience Technologies, Dept of Pathology, TJUH, TJU

Hormone Pathways have been shown to influence breast carcinogenesis. Prolactin (PRL), a key peptide hormone regulating breast epithelial growth and differentiation, activates signaling via the transmembrane PRL receptor (PRLR). PRLR is expressed in a majority of breast cancers. Estrogen and progesterone receptors, two nuclear regulators of normal and malignant breast epithelia, are expressed in approximately 70% of breast cancer tissues. Stat5, a substrate of the PRL-activated Jak2 tyrosine kinase, is phosphorylated (P-Stat5) and activated following PRL stimulation in target tissues. P-Stat5 is reduced in high-grade breast carcinomas. The Map-Kinase pathway mediates growth-promoting effects of many growth factors. Thus, it is expected to be active in large numbers of breast cancer tissues. This study takes a quantitative approach to analyze expression levels of hormone-related proteins in an array containing approximately 200 normal or malignant human breast tissues, utilizing 'Cutting Edge Matrix Assembly' (CEMA) tissue arrays and Automated Quantitative Analysis, AQUA. Expression levels of different proteins were determined using immunohistochemical (IHC) analysis, followed by fluorescent detection methods to quantify protein target levels. Quantitative data on biomarker levels across normal, DCIS, IDC1, IDC2, IDC3 and lymph node metastases, are presented. While ER and PR levels were increased in breast cancer, levels of phospho-Stat5 were significantly reduced. P-MEK and HER2 showed levels of expression with different grades of breast cancer. Ongoing studies are correlating levels of these markers with molecular subtypes of breast cancer and breast cancer outcomes.

- 41) Molecular and Mechanical Solutions to Problems Creating a Tissue-Engineered Stem Cell Vascular Graft.** SE McIlhenny, ES Hager, N Moudgill, IM Shapiro, *PJ DiMuzio, Dept. of Surgery, TJU

Background: Adipose-derived stem cells (ASC) acquire endothelial characteristics in response to growth factor and shear force. However, the lack of endothelial nitric oxide synthase (eNOS) expression and low ASC retention on vascular grafts following fluid flow are of concern. To address these problems, we determine the effect of shear force on cell retention and integrin function, as well as preliminarily evaluate eNOS transfection. Methods: Isolated human ASC were cultured in ECGS media to induce endothelial differentiation. Within a bioreactor, ASC were seeded onto vascular grafts and gradually exposed to shear force over 3d (0-9dynes). Retention was determined by confocal microscopy image analysis. Integrin expression was determined by RT-PCR and ELISA. ASC transfection with eNOS was accomplished using an adenovirus; subsequent expression was determined by RT-PCR and immunoblot. Results: Linearly increasing shear force resulted in a 30-fold increase in ASC retention compared to step-wise increases. Shear increased $\alpha 5\beta 1$ and $\alpha v\beta 3$ integrin expression 2.25- and 1.4-fold, respectively, over statically cultured cells, and $\alpha 5\beta 1$ blocking significantly decreased ASC attachment. Following transfection, ASC expressed eNOS message and protein. Conclusions: These studies suggest: 1) linear application of shear force yields increased ASC retention on vascular grafts, 2) retention is the result of integrin upregulation, 3) eNOS transfection is successful and should encourage anti-thrombogenic properties.

- 40) Interplay between Notch and TCR signaling in T-cell development.**

Akriti Mathur, Amrita Batheja, Shivani Gandhi, Nicole Cunningham and *Jennifer A. Punt
Biology Department, Haverford College, Haverford, PA 19041

Cell fate decisions in T cell development are mediated by a myriad of different receptors. Of these, the T-Cell Receptor (TCR) and the Notch receptor seem to play central roles. TCR signal strength regulates negative and positive selection, as well as CD4/CD8 lineage commitment. Notch, a trans-membrane receptor and transcription factor, also regulates T-cell decisions at some of the same developmental junctures. The data we have gathered show that Notch and the TCR have the ability to influence one another's activity in vitro. We have previously been able to show that Notch activation via a Notch-1 antibody inhibits TCR signaling in immature and mature thymocytes. Here, we confirm the occurrence of that same phenomenon in a co-engagement dependent manner through Notch activation by its Jagged1 ligand, a more physiologically accurate model of interaction. This effect can be partly reversed with the addition of γ -secretase inhibitor, an inhibitor of the generation of active or Intracellular Notch (ICN), in mature but not immature T-cells. Our data are consistent with results from multiple labs that demonstrate cross-talk between TCR and Notch, although they add to the controversy about the direction and consequences of the interplay.

- 42) 17-allylamino-17-demethoxygeldanamycin is more toxic to human tumor cells at low pH.** D Mikhalkova, C Stork, and *R Coss. Thomas Jefferson University

The ability of the Hsp90 inhibitor, 17-allylamino-17-demethoxygeldanamycin, 17AAG, to kill human tumor cells cultured at low pH more than cells at pH 7.3 was investigated. This laboratory has demonstrated enhanced toxicity of 17AAG to human melanoma DB1 cells at pH 6.7. 17AAG was tested on three additional cell lines: A549 and H1299 lung tumor cell lines, and the LoVo colon tumor cell line. Cells were cultured and treated at pH 7.3 and pH 6.7 in vitro. Protein expression was assessed by immunoblot analysis and survival by colony formation. 17AAG (200 nM) was more toxic to the two lung tumor lines cultured at pH 6.7. Survival of treated A549 cells and H1299 cells at pH 6.7 was 42% (vs 72% at pH 7.3) and 26% (vs 61%), respectively. There was no effect of 17AAG on LoVo colon cancer cells. 17AAG induced Hsp70 and Hsp27 in both lung lines but not in the LoVo cell line. 17AAG dramatically sensitized DB1 cells cultured at pH 6.7 to ionizing radiation and concomitantly reduced levels of RAD51. The ability to inhibit RAD51 dependent homologous recombination repair (HRR) was tested in the lung and colon tumor cells by measuring RAD51 expression. RAD51 expression was decreased in treated H1299 and A549 cell lines cultured at pH 6.7 but not in LoVo cells. RAD 51 levels were minimally reduced in cells at pH 7.3. These results suggest that the H1299 and A549 cell lines at pH 6.7 will be sensitized to ionizing radiation, and that tumors with large acidic components may be more sensitive to 17AAG than tumors with small acidic compartments.

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- 43) A Role for GSK3 β in Platelet Function.** S E Neves and *D Woulfe. Center for Translational Medicine, Thomas Jefferson University

GSK3 β is a substrate of the ser-thr kinase Akt, which has previously been shown to play a positive role in platelet activation. Our laboratory has recently shown that GSK3 β negatively regulates platelet activation, although the specific mechanism by which it affects platelets is not known. A potential effector of GSK3 β is Bcl-3, which has been shown to be negatively regulated by GSK3 β in other cell types. It has recently been demonstrated that the procoagulant activity of platelets can be regulated by the mitochondrial transition pore (MPTP), which in turn affects phosphatidyl serine (PS) exposure on the platelet surface, a known marker of platelet procoagulant activity. The goal of our study was to determine if GSK3 β affected platelet function through MPTP formation and PS exposure and to determine the effects of GSK3 β on Bcl-3 in platelets. Using JC-1 staining and annexin V binding assays, we found increased MPTP formation and PS exposure in platelets treated with GSK3 β inhibitors. Using Western blot assays in human and mouse platelets, we also show that Bcl-3 phosphorylation decreases with GSK3 β inhibitors and in the presence of platelet agonists. Although Bcl-3 has recently been shown to inhibit the activation of Bim, whether Bcl-3 can impact mitochondrial permeability and PS exposure in platelets remains to be explored. We propose that GSK3 β affects platelet activation through inhibition of MPTP formation and PS exposure and that GSK3 β negatively regulates Bcl-3 in platelets.

- 45) The Effects of miR-181a on Bim Expression Levels in Developing T Cells.** E C Rabinowitz and *J Punt. Department of Biology, Haverford College.

MicroRNAs are short, 19-23 nucleotide RNAs that help regulate the translation of specifically targeted mRNA transcripts. Determining the roles these small RNAs play in the complex process of T cell development has only begun, although findings suggest that specific miRNAs regulate discrete developmental stages. In particular, miR-181a appears to play an important role in the fate of immature double positive (DP) thymocytes. At the DP developmental time point, thymocytes undergo T cell receptor mediated positive and/or negative selection events that ultimately generate a population of functional T cells that protect against invading pathogens while remaining non-reactive to self antigens in the body. Computational target prediction programs suggest miR-181a may regulate the expression of various genes crucial to these cell fate decisions, such as Bim – a thoroughly researched pro-apoptotic activator. Another miRNA, the miR-15/16 family, also could potentially provide important regulatory roles by targeting CD28 – a costimulatory protein required for T cell activation. We are specifically interested in the roles these miRNAs serve in negative selection and are using an in vitro model to examine their influence. By transfecting mouse thymocytes with specific miR-181a mimics (for miRNA overexpression) or inhibitors (for miRNA knockdown) and then assessing their effects on TCR-induced pro-apoptotic events, we can address our hypothesis that miR-181a regulates Bim involvement in T cell positive and negative selection.

- 44) TGF β regulation of ANKH (progressive ankylosis) expression in ATDC5 cells.** P A Oca, R Zaka, A S Dion, and C J Williams*, Dept Med, Ctr for Trans Med, TJU

To evaluate the expression of ANKH in response to TGF β during chondrogenesis in ATDC5 cells, an in vitro model of growth plate differentiation, cells were subjected to differentiation with and without TGF β . The progression of differentiation was monitored using markers of chondrogenesis. Inhibitors of TGF β signaling were used to identify the signaling pathway employed in TGF β regulation of ANKH expression. Finally, inhibition of the L type calcium channel α 1c (Lt1c) was studied to determine its role in the regulation of ANKH expression in response to TGF β . TGF β produced an increase in ANKH expression at day 14 (proliferation) and day 32 (mineralizing hypertrophy) of culture. To determine the TGF β -activated signaling pathway, cells with and without TGF β were incubated in the presence of specific inhibitors. The TGF β treatment activated the Ca²⁺-dependent protein kinase C (PKC) signaling pathway. Since previous reports of TGF β stimulated chondrogenesis implicated Ltcs in Ca²⁺-dependent PKC signaling, we explored the activity of the Lt1c in the TGF β -stimulated ANKH response in ATDC5 cells. Our results indicated that inhibition of Lt1c with nifedipine inhibited the TGF β stimulated increase in ANKH expression only during hypertrophy. Expression of ANKH during chondrogenesis demonstrates a bimodal response to TGF β during the proliferative and hypertrophic phases of differentiation. The TGF β response is mediated by the Ca²⁺-dependent PKC signaling pathway and requires influx of calcium via Lt1c only during hypertrophy.

- 46) Five RNA Editing Sites in Shab Alter Pore Block and the Kinetics and Voltage Dependence of Activation.** Y Ryan¹, R Maloney², R A Reenan² and *R Horn¹
¹Department of Molecular Physiology & Biophysics, Thomas Jefferson University, ²Department of Molecular Biology & Biochemistry, Brown University

RNA editing creates single base changes in mRNA. Editing can result in modified amino acid sequence and protein function. Sequence analysis of the Shab potassium channel of *Drosophila melanogaster* revealed five RNA editing sites; four are $\geq 50\%$ edited (I583V, T643A, Y660C and I681V), and one is edited more in larval than in adult flies (T671A). We examined the biophysical consequences of editing these sites using five mutant constructs, each containing the genomic (unedited) base at one site in the background of a channel in which all other sites are edited and using two-microelectrode voltage clamp in *Xenopus* oocytes. Each 'unedited' mutation slowed activation kinetics and three exhibited a significant hyperpolarized shift in their midpoints of activation. Four of the mutations are relatively conservative. The V $\rightarrow$ I substitution at position 681 is a minimal side chain modification; however it exhibited the most striking change in deactivation kinetics. Deactivation was slowest in the 'genomic' construct (all sites unedited). The editing site at position 660 aligns with the Shaker 449 residue, which is important in tetraethylammonium (TEA) block. The aromatic (genomic) residue tyrosine at this position in Shab enhances TEA block 14-fold compared to cysteine (edited). These results show that both the editing site position and the substituted amino acid are important for channel function.

- 47) Invadopodia in Collective Migration**
Robyn T. Sussman¹ and *Sue Menko. Department of Pathology, Anatomy and Cell Biology, Thomas Jefferson University, Philadelphia, PA 19107

Invadopodia are cytoskeletal projections into the matrix that have thus far been described in metastatic cancer cell lines. Cortactin, a protein involved in actin polymerization has been used as a marker for invadopodia. We have shown that invadopodia exist in non-malignant migratory cells in an ex-vivo lens system used to study posterior capsule opacification (PCO). PCO is a common complication after cataract removal where the cells migrate across the cleared posterior capsule through a process known as collective migration, a mechanism this system has in common with epithelial cancers. We have found that in the original attachment zone of the PCO culture, cortactin is cortical at cell-cell borders and co-localizes with actin. However, at the leading edge of migratory cells, cortactin is no longer co-localizing with actin and extends beyond the actin cytoskeleton. Cortactin here is localized to invadopodial anchors into the basement membrane, allowing the cells to pull themselves as they migrate into the center of the culture. These findings suggest that invadopodia are also found in non-cancerous migrating cell and may be studied in ex-vivo systems as well as cell lines.

- 49) Identification of a Novel Splice Variant, Cdx2(+RS), Defines CDX2 as the First Tissue-Specific Gene that Regulates Transcription and pre-mRNA Processing.**
Matt E. Witek and *Scott A. Waldman. Department of Pharmacology and Experimental Therapeutics, Thomas Jefferson University, Philadelphia, PA, 19107

Gene expression is a dynamic and coordinated process that couples transcription with pre-mRNA processing. In this paradigm, tissue-specific transcription factors regulate the expression of a limited set of cell-type specific transcripts, which are subsequently amplified by alternative splicing, a process controlled by the S/R-rich (SR) family of splicing factors. This model of gene expression increases proteome complexity and functional diversity. The intestine-specific transcription factor Cdx2 regulates development and maintenance of the intestinal epithelium by inducing expression of genes characteristic of the mature enterocyte phenotype. Here, sequence analysis of Cdx2 mRNA revealed an alternatively spliced transcript, Cdx2(+RS), that encodes a protein in which the 85 carboxyl terminal aa in the wild-type protein are replaced with a 45 residue domain enriched in S and R (RS domain). Both proteins were concomitantly expressed in normal colonic mucosa, colorectal adenocarcinomas and all colon cell lines examined. Cdx2 and Cdx2(+RS) exhibited, overlapping but not identical, sub-cellular distribution patterns with limited nuclear co-localization. Cdx2(+RS) was unable to activate the Cdx2-dependent promoter of GCC nor did it regulate the expression or transcriptional activity of Cdx2. The RS domain of Cdx2(+RS) was required for nuclear localization and co-localization with the putative splicing factors SC35. This co-localization was dependent of the transcriptional state of RNA polymerase IIa. Cdx2(+RS) altered splicing patterns of CD44v4v5 and Tra2- β 1 mini-genes in LovoCdx2-/- cells in the presence and absence of Cdx2. Cdx2(+RS) modulated the splicing pattern of the endogenous Cdx2-dependent intestine-specific gene GCC. This suggests that Cdx2(+RS) functions as a novel component of the pre-mRNA processing machinery and defines CDX2 as the first gene that encodes protein products that regulate tissue-specific gene expression at the levels of transcription and pre-mRNA processing.

- 48) Purinergic activation of PKC α translocation in Osteoblast.**

P. Timothee A. Matamoros, and *V Fomin. Department of Biological Sciences, University of Delaware.

Osteoblasts respond to mechanical load with a rapid increase in intracellular calcium concentration ([Ca²⁺]_i) which is essential for load induced bone formation. Soon after the increase in [Ca²⁺]_i, bone cells release ATP, via exocytosis, which stimulates the bone cells in an autocrine and paracrine manner through activation of purinergic receptors (P2 X and P2Y). Protein Kinase C was shown to be important in the regulation of osteoblast's response to mechanical load. PKC activation results in its translocation to the membrane, which further potentiates [Ca²⁺]_i. The objective of this study is to investigate whether activation of osteoblast purinergic receptors cause PKC α translocation to plasma membrane region. The experiments were designed to monitor PKC activation, by infecting the osteoblasts with a cDNA construct of PKC α -GFP. We found that BzATP, added at 0.5mM caused the translocation of PKC to the plasma membrane. BzATP effect was blocked by inhibition of ROCK(Rho-associated kinase) using Y27632. When stimulating the cells with 0.5mM ATP, there was no translocation of PKC to the membrane region. Due to the specificity of BzATP to P2X purinergic receptors, we conclude that PKC is effectively stimulated via P2X receptors, and not P2Y receptors due to possibly higher density of the P2X receptors on the cell surface. Since ROCK is implicated in regulation of actin cytoskeleton our finding may also suggest that the translocation of PKC occurs with the involvement of the osteoblast cytoskeleton.

- 50) The 11S Proteasomal Activator REGgamma Increases the Aggregation of Expanded Polyglutamine Androgen Receptor.** J Yersak¹, Y Liu¹, M Rechsteiner², *D Merry¹. ¹Department of Biochemistry and Molecular Biology, Thomas Jefferson University; ²Department of Biochemistry, University of Utah.

Recent studies in SBMA have illuminated the importance of nuclear degradation in the altered metabolism of the expanded AR, where both nuclear localization and proteasomal function is required for AR cleavage and aggregation. The nucleus is the site of SBMA pathogenesis and contains only the proteasome as its degradation machinery. Our focus is on the role of nuclear proteasomal activators in SBMA pathogenesis. To determine whether the 11S REG γ could alter nuclear AR metabolism, we overexpressed REG γ in our inducible PC12(ARQ112) cell model. Overexpression resulted in a significant increase in expanded AR aggregation. These data suggest that overexpression of REG γ causes a change in the metabolism of ARQ112. Next, we tested whether REG γ is required for expanded ARQ112 aggregation. siRNA knockdown studies showed a significant decrease of nuclear AR aggregation in REG γ -depleted cells, suggesting that REG γ plays a role in expanded AR nuclear aggregation. Finally, we sought to understand whether REG γ affects primary motor neuron viability in our SBMA primary motor neuron model. We hypothesized that overexpression of REG γ would exacerbate DHT- and polyglutamine-dependent motor neuron cell death. These studies are still ongoing. Through these combined studies, we expect to better understand the mechanism of REG γ mediated ARQ112 aggregation and its subsequent effect on the biochemical state of ARQ112.

- 51) A central function for perlecan in skeletal muscle and cardiovascular development.** JJ Zoeller¹, A McQuillan¹, J Whitelock², S-Y Ho³, and RV Iozzo^{1*}
¹Dept of Pathol, Anat and Cell Biol, and the Cancer Cell Biology and Signaling Program, KCC, TJU, Phila, PA19107
²Graduate School of Biomedical Engineering, University of New South Wales, Sydney, Australia 2052

Perlecan's developmental functions are difficult to dissect in placental animals because perlecan disruption is embryonic lethal. In contrast to mammals, cardiovascular function is not essential for early zebrafish development because the embryos obtain adequate oxygen by diffusion. Here, we use targeted protein-depletion coupled with protein-based rescue experiments to investigate the involvement of perlecan and its C-terminal domain V/ndorepellin in zebrafish development. The perlecan morphants show a severe myopathy characterized by abnormal actin filament orientation and disorganized sarcomeres, suggesting an involvement of perlecan in myopathies. In the perlecan morphants, primary intersegmental vessel sprouts, which develop through angiogenesis, fail to extend and show reduced protrusive activity. Live videomicroscopy confirm the abnormal swimming pattern due to the myopathy and the anomalous head and trunk vessel circulation. The phenotype is partially rescued by micro-injection of human perlecan or ndorepellin. These findings indicate that perlecan is essential for the integrity of somitic muscle and developmental angiogenesis and that ndorepellin mediates most of these biological activities.

Field Topic:

Clinical

- 52) Linear Eversion Carotid Endarterectomy.** R Beard, E Hager, and *J Lombardi, Division of Vascular Surgery, TJUH.

Atherosclerosis occurring at the bifurcation of the carotid artery leads to plaque formation and carotid stenosis. Plaque removal is the objective of carotid endarterectomy, the most frequently performed surgery for stroke prevention. The objective of this study is to describe a new variation of eversion carotid endarterectomy whereby the plaque is everted from a linear incision over the common carotid artery. We did a retrospective review of 66 patients from 2005 to 2007 who underwent linear eversion carotid endarterectomy (LEE) by two surgeons. Preoperative comorbidities and postoperative complications (stroke, bleeding, restenosis) were recorded. Sixty-six patients between the ages of 50 and 91 underwent LEE for carotid stenosis with 22 (33%) being symptomatic at the time of surgery. Preoperative comorbidities included hypertension (81%), hyperlipidemia (67%), coronary artery disease (64%), diabetes (32%) and peripheral vascular disease (26%). Eight patients (14%) were converted at the time of surgery from LEE to patch closure, while the 58 remaining patients successfully underwent LEE. Postoperative complications included 3 neck hematomas (5%) with 2 requiring evacuation, 1 postoperative superficial wound infection (1.7%), 1 myocardial infarction (1.7%) and 1 ipsilateral stroke in an asymptomatic patient (1.7%). There were no postoperative deaths and no restenosis reported in any of these patients within the 2 year follow-up period. We concluded LEE is a safe and durable alternative to traditional carotid eversion or patch closure for carotid endarterectomy.

- 53) Postoperative Auras and Risk of Recurrent Seizures.** T Chandrasekar^{1,2}, AD Sharan^{1,3,4}, MR Sperling^{1,4,*}
¹Comprehensive Epilepsy Center, ²Jefferson Medical College, ³Department of Neurosurgery, and the ⁴Department of Neurology, Thomas Jefferson University, Philadelphia, PA.

Purpose: Little is known about the prognostic values of persistent auras following temporal lobe surgery in determining the recurrence of complex partial seizures (CPS) or generalized tonic-clonic seizures (GTCS). Methods: In a retrospective analysis of temporal lobectomy patients who had preoperative auras associated with CPS or GTCS and who were seizure-free following surgery, the predictive value of auras was assessed at three months and two years following surgery. Medication use, in relation to the presence of auras, was also assessed. Results: Of the 347 patients seizure-free for 3 months following surgery, 247 were aura-free and 101 had persistent auras. KM analysis yielded no difference in outcome ($p = 0.65$) and current outcome class was also similar ($p = 0.99$). Patients with persistent auras were somewhat more likely to be on medication at the time of recurrence ($p = 0.06$) and at last follow-up ($p = 0.09$). Of the 224 patients seizure-free for 2 years following surgery, 163 were aura-free and 61 had persistent auras. KM analysis yielded no difference in outcome ($p = 0.86$) and current outcome class was also similar ($p = 0.35$). Patients with persistent auras were more likely to be on medication at the time of recurrence ($p < 0.001$) and at last contact ($p < 0.01$). Conclusions: Persistent auras following temporal lobectomy do not appear to have a predictive value in the recurrence of CPS or GTCS. However, these findings may be influenced by AED use, with was greater in patients with persistent auras following surgery. It may be safest to state therefore that with AED treatment, persistent auras are not associated with a worse prognosis.

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- 54) Education and Blood Pressure Level in Urban African Americans.**
SC Czarkowski, H Kushner, and *B Falkner, Department of Medicine, Thomas Jefferson University, BioMedical Computer Research Institute, Inc., Thomas Jefferson University.

Minority groups and individuals of lower socioeconomic status (SES) are disproportionately affected by cardiovascular diseases (CVD). Education level is one factor used to study the association between SES and health status. African Americans show disproportionately higher rates of hypertension when compared to their white counterparts. The purpose of this study was to determine if a relationship of education and blood pressure (BP) is detectable in African Americans. Data were drawn from 509 young, adult African Americans spanning three sequential investigations on BP and cardiovascular risk factors. Data included years of education, anthropometric, and BP measurements. Each participant was classified into one of four education levels (<high school (hs), hs, 1-2 years post-hs, >2 years post-hs). Despite demographic variation in age, gender, body mass index (BMI), and attained education between studies, there was no significant effect of education on systolic BP (SBP) or diastolic BP (DBP). However, in multiple linear regression models a statistically significant but small education effect was detected. For each additional year of education above the mean, a decrease of 1.1 ± 0.49 mm Hg for SBP was detected ($P = 0.03$). In this study on urban, young, adult African Americans, education (as an indicator of SES) had a small but statistically significant effect on BP. Of the other variables examined, BMI was shown to have the greatest statistically significant effect on BP. The factors that contribute to the disproportionately high rates of disease, mortality, and morbidity affecting minority populations and individuals are still unclear.

- 56) Prophylaxis, screening and the incidence of periprocedural deep vein thrombosis among neurosurgical patients at an academic medical center.**
PC Henwood, *G Tzanis, L Thomson, J Fink, WK Kraft, RH Rosenwasser, and *GJ Merli. JMC; Dept of Internal Medicine, Center for Vascular Disease, TJUH; JHN.

We sought to determine the incidence of deep vein thrombosis (DVT) in neurosurgical patients who received periprocedural surveillance compression venous ultrasonography (CVUS) of the lower extremities at least twice weekly, in addition to prophylaxis with both unfractionated heparin 5000 units subcutaneously every twelve hours and external pneumatic compression sleeves. We conducted a retrospective review of 7,298 CVUS studies carried out on 2,593 patients from January 2003 – January 2007 at the Jefferson Hospital for Neuroscience. We recorded the total number of CVUS studies, patients, and abnormal studies on a cumulative and annual basis. Of 2,593 patients who had lower extremity CVUS performed, there was a 7.4% incidence of proximal DVT. The total incidence for both proximal and distal lower extremity DVT was 9.7%. However, patients with distal DVT were nearly all identified during 2005 and 2006; this is most likely due to more comprehensive CVUS screening of the calf veins during this time period. The previous literature has focused on patients who had craniotomy for brain tumors in trials with much smaller patient numbers. Further evaluation of this cohort, and variables associated with it, will aid in determining the efficacy of our DVT prophylaxis and screening protocol, and help identify subpopulations with increased risk of venous thromboembolism.

- 55) Therapeutic or Adverse Effect of Reversing Anticoagulation After Closed Head Injury (CHI).**
B Frank, M Maltenfort, *J Harrop. Department of Neurosurgery, TJU

Closed head injury (CHI) is a common presentation after trauma. When patients present to the emergency department (ED) with CHI, standard practice is to stabilize the patient, initiate intracranial pressure (ICP) monitoring, and stop the patients bleeding, if necessary. Standard of care to coagulate the patient is to transfuse large volumes of fresh frozen plasma (FFP). Unfortunately, these large volumes can have adverse effects of fluid overload, pulmonary congestion, pneumonia, intubation, etc. Therefore, there is a question of whether this treatment algorithm is beneficial in the population at least 65 years of age. The goal of this study is to evaluate outcomes of CHI patients receiving FFP to assist in control of bleeding. A retrospective cohort review of 120 patients presenting to the ED at TJUH from 2003-2007 is underway. Patients will be divided into two groups, FFP treated and non-FFP treated. We anticipate patients treated with FFP have an increased incidence of adverse effects, most importantly pulmonary complications and re-hemorrhage, many the result of fluid overload, and an increased length of stay. Additionally, we believe patients treated with FFP would experience a higher mortality rate secondary to the increased incidence of adverse effects and because of secondary injury. These hypotheses will be tested with appropriate statistical methods once ongoing data collection is complete, and the results presented at the conference.

- 57) Dose-response Relationship between Maternal Methadone Dose and Decreased Neonatal Head Circumference.** K. Horowitz, E. Hayes, N. Seligman, M. O'Neill, B. Leiby, K. *J. Baxter, Thomas Jefferson University.

OBJECTIVE To determine if maternal methadone dose is correlated with changes in neonatal head circumference.

STUDY DESIGN Retrospective cohort study of neonates born from 2000-2006 exposed to methadone in utero. Maternal methadone dose was defined by the maintenance dose at time of delivery. Head circumference was measured at birth. A mixed effects linear regression was used to model the association between head circumference and gestational age at delivery, adjusting for those variables found to be significant, and stratified separately by dose at delivery. The adjusted mean head circumference was calculated for each range of methadone levels at gestational ages 28, 32, 36, and 40 weeks, and compared with the mean population head circumference.

RESULTS 259 neonates were examined (Maternal dose 1-50mg (n=17); 51-100mg (n=76); 101-150mg (n=98); 151-200mg (n=46); >200mg (n=22)) After adjusting for those variables which were found to be significant (race, cocaine, and age), a significant decrease in neonatal head circumference was demonstrated with increased methadone dose in a dose-response relationship ($p=0.01$).

CONCLUSION Higher maternal methadone dose is associated with a significant decrease in neonatal head circumference.

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- 58) Treatment of Uncomplicated Vaso-occlusive Crises in Children with Sickle Cell Disease in a Day Hospital.**
Aditi Kamdar, Jean L. Raphael*, M. Brooke Beavers, Donald H. Mahoney, Brigitta U. Mueller. Baylor College of Medicine, Pediatrics, Texas Children's Cancer Center, Houston, TX, and JMC-TJU, Phila.

Day hospital management for patients with sickle cell disease experiencing uncomplicated vaso-occlusive pain crises has been described in adult populations as an alternative care delivery system. The objective of this study was to characterize and descriptively assess the benefits of a day hospital exclusively designed for children.

We retrospectively studied all admissions to the Day Hospital at the Texas Children's Sickle Cell Center since its inception in 2000. A Day Hospital admission was defined as a minimum of two consecutive days of aggressive pain management as an outpatient, including intravenous hydration and analgesics, supported by home treatment over night with oral analgesic and anti-inflammatory agents. We gathered data on demographics, incoming pain score, provider type, opioid administration, length of stay, and needs for higher level care.

A total of 35 patients, ages 2-19, accounted for 80 episodes during the study period. The median incoming pain score was 8 on a scale from 1 to 10. The median length of stay was 2 days. The return rate for acute care within 48 hours for persistent symptoms was 7%. Seventy-one percent of patients admitted to the Day Hospital were treated without requiring transfer to inpatient care for escalating pain or medical needs.

We conclude that a dedicated Day Hospital facility has the potential to provide patient-centered, effective, and timely management of vaso-occlusive crises in children as well as adults.

- 60) Home allodynia testing: day to day correlation between headache intensity and allodynia.**
T F Madden, N L Rosen, and *W B Young. Neurology, TJU, Philadelphia, PA.

This study evaluates the temporal relationship between cutaneous allodynia and migraine headache pain. Allodynia is the abnormal experience of pain in response to a normally non-painful stimulus. Symptoms and signs of allodynia are common in episodic and chronic migraine and the presence of allodynia influences the selection and timing of medicine administration. Brush allodynia can potentially be tested by the migraineur at home, giving the patient more control over his or her therapy. Chronic migraine patients with ophthalmic trigeminal nerve (V1) distribution cutaneous allodynia on the date of an office visit were asked to complete an eight week log. The log captured both the migraine severity and allodynia on a daily basis. Eleven patients with chronic migraine and cutaneous allodynia completed the study. From the eleven patients, a total of 587 allodynia observations were recorded and patients recorded a headache intensity of 1 or more on 77% of the days and an allodynia score of 1 or more 68% of the days. As a group, the correlation of headache to allodynia on any given day was 0.658. Day to day correlation (Spearman) of headache intensity and allodynia was reasonably consistent between individuals ranging from .582 to .896. This suggests physiologic consistency of the relationship between headache and allodynia. We also conclude that brush allodynia testing can be routinely self-administered at home as the correlations are similar for both home and office testing.

- 59) Day Hospital versus Inpatient Management of Uncomplicated Vaso-occlusive Crises in Children with Sickle Cell Disease.** Aditi Kamdar, Jean L. Raphael*, Tao Wang, Hao Liu, Donald H. Mahoney, Brigitta U. Mueller. Baylor College of Medicine, Pediatrics, Texas Children's Cancer Center, Houston, TX, Jefferson Medical College, Philadelphia, PA, USA

Day hospital management for patients with sickle cell disease experiencing uncomplicated vaso-occlusive pain crises has been utilized as an alternative care delivery system to inpatient hospitalization. The objective of this study was to determine whether day hospital management results in shorter length of stay compared to inpatient care.

We conducted a retrospective cohort study with 35 day hospital admissions and 35 inpatient admissions for children with sickle cell disease presenting with uncomplicated vaso-occlusive crises. A day hospital admission was defined as a minimum of two consecutive days of aggressive pain management as an outpatient, including intravenous hydration and analgesics, supported by home treatment over night with oral analgesic and anti-inflammatory agents. We gathered data on demographics, pain scores, length of stay, admission charges, and needs for persistent care.

Day hospital care resulted in a 39% reduction of the average length of stay compared to inpatient admissions. Multivariate linear regression demonstrated that the location of patient care for vaso-occlusive crises was a significant predictor of length of stay ($P < 0.0006$) after controlling for patient characteristics, severity of illness, and disease history.

We conclude that a dedicated day hospital facility has the potential to provide efficient and timely management of uncomplicated vaso-occlusive crises through reduction of length of stay. This delivery care system may be particularly relevant for children who may be significantly impacted by inpatient hospitalization.

- 61) Outcome of repeat two-stage reimplantation for infected total knee arthroplasty.**
K McHale, K Azzam, J Purtil, M Austin, *Javad Parvizi. Rothman Institute, TJU

This study aims at measuring the outcome of repeat two-stage reimplantation for the management of reinfection after total knee arthroplasty and identifying risk factors for failure. Between 2000 and 2005, patients who had reinfection after two-stage reimplantation of total knee arthroplasty were retrospectively reviewed using our institutional database. A subgroup of 17 patients (10 females and 7 males) treated with a second resection-reimplantation protocol was followed up prospectively. Detailed data including demographics, comorbidities, intraoperative data, organism profile, and complications were extracted to evaluate potential outcome predictors. At an average of 40 months follow-up (range 24 to 83 months), 15 patients were infection free accounting for a success rate of 88.2%. Of these 15 patients, 10 patients were receiving suppressive antibiotic therapy. There were 2 reinfections (11.8%). These two patients were treated with a third two-stage protocol successfully and were infection-free after the 3rd two-stage exchange arthroplasty. The latest average knee society knee score was 53 and the average functional score was 56. Recurrence of infection after two-stage exchange arthroplasty for an infected total knee arthroplasty requires aggressive measures to control infection. This study demonstrates that although the functional outcome of a second reimplantation is generally modest, it is still a viable knee-salvage option provided such surgery is only undertaken after strict criteria are met.

- 62) Paraspinous Muscle Flap Reconstruction of Complex Midline Back Wounds: Risk Factors and Incidence of Post-flap Complications at a Busy Regional Spine Center.** AF Mericli, NA Tarola, JH Moore, SE Copit, J Fox, GA Tuma*. Division of Plastic and Reconstructive Surgery, Jefferson Medical College, Philadelphia, PA, USA

The purpose of this study is to examine the effect of various preoperative risk factors on postoperative complications after paraspinous muscle flap reconstruction of non-healing midline back wounds. An 11-year, retrospective, office and hospital chart review was conducted. There were 92 patients in the study, representing the largest reported series to-date for the paraspinous muscle flap procedure. Mean follow up was 120 days. Several wound-healing risk factors were present in this patient population: 72% were malnourished, 40% had hypertension, 36% were obese, 33% had a history of smoking, 28% had diabetes, 16% were on chronic steroids, 15% had a history of more than two previous spine surgeries, and 9% had a history of radiation to the wound area. Risk factors associated with a statistically significant ($p < .05$) increased rate of post-flap wound complication included emergent initial spine surgery, a history of more than two spine surgeries, hypertension, radiation to the wound area, smoking, and quadriplegia. This patient population possesses multiple comorbidities making complex wound healing difficult. Several specific risk factors are associated with an increased rate of post-reconstruction wound complication. It may be beneficial for patients possessing these risk factors to undergo prophylactic flap placement at the time of initial spine surgery.

- 64) High Prevalence of Low Bone Mineral Density (LBMD) in a Cohort of Children with Nephrological and Rheumatological Conditions.** N Nair, S Balladares, F Ramirez, A Cepero, R Rivas-Chacon, A Paredes*. Nephrology and Rheumatology, Miami Children's Hospital, Miami, Florida., and JMC-TJU

Symptomatic childhood osteoporosis is emerging as a clinical problem of increasing prevalence. Genetics, diet, lifestyle, chronic illnesses and medications play a pivotal role in the development of bone health early in life. The incidence of LBMD in children at risk has yet to be elucidated. In this pilot project, we studied the prevalence of LBMD in a cohort of patients from our Nephrology and Rheumatology clinics. Subjects are 6-17 years, separated into at risk (AR) and no risk (NR) for LBMD. AR subjects have various chronic Nephrological and Rheumatological disorders. NR subjects are those lacking family history of osteoporosis, recurrent fractures, exposure to glucocorticoids, or chronic diseases. BMD is determined by peripheral Quantitative Computerized Tomography using pediatric software (true volumetric measurements of total and trabecular densities in mg/cm³). Subjects with total and trabecular BMD $< -1SD$ from the mean for matched age and sex are identified as LBMD. 274 patients were screened (117 NR and 157 AR) Mean age of NR group is 9.92 $\pm$ 3.16 y, M= 51, F= 66, mean age for AR group is 12.06 $\pm$ 3.11 y, M=61, F=96. In the AR group, LBMD is found in 35 (22.3%) patients (Tubulopathies 9, Glomerulopathies 7, Autoimmune 19) of which 24 (15.3%) have BMD $< -1SD$, 11 (7%) have BMD $< -2SD$ and 24 (68.6%) had exposure to prednisone. Of the 94 patients in the AR group with a history of past or present use of prednisone, 24 (25.5%) had LBMD (16 $< -1SD$ and 8 $< -2SD$). In the NR group, 6 (5.1%) had LBMD (5 $< -1SD$ and 1 $< -2SD$). In conclusion, this pilot study indicates a 22.3% vs. 5.1% prevalence of LBMD in children and adolescents AR vs. NR for osteopenia / osteoporosis. Routine evaluation of bone density should be considered in children at risk for LBMD.

- 63) Self-retained vs traditional retractors for c-section in obese women: a randomized controlled trial.** L Moroz, G Bowers, EJ Hayes, J O'Brien, Tina Carroll, *JK Baxter. Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, TJU

Objective: To determine whether use of a self-retained disposable retractor during cesarean deliveries of obese women decreases operative time and blood loss and improves surgeon satisfaction during the procedure. Methods: We conducted a randomized, controlled trial of self-retained disposable retractors versus traditional retractors for non-emergent cesarean deliveries of women with body mass index (BMI) greater than 35 kg/m² (Class II obesity). Outcomes analyzed were operative time, blood loss, incision length, and the surgeon's assessments of the procedure. Results: Over ten months, 30 patients were randomized to the self-retained retractor and 30 patients to the traditional retractor. Demographic characteristics, past surgical history, and modifications of the delivery did not vary significantly between groups. Surgeons who used the self-retained retractors reported improved visualization ($p = 0.007$), overall satisfaction ($p = 0.006$), and a preference to use the self-retained retractor again (p less than 0.001). There was a decrease in mean blood loss with use of the self-retained retractor group (928 ml) compared to the traditional retractor group (1104 ml), although this difference did not reach statistical significance. Operative time was an average of 68 minutes using the self-retained retractor and 72 minutes using the traditional retractor ($p = NS$). There was no difference in incision length between the groups. Conclusions: Self-retained disposable retractors improve visualization and surgeon satisfaction, without increasing incision length, and may decrease blood loss during cesarean delivery of obese women.

- 65) Panitumumab and Hypertrichosis: Effects of an EGFR-Inhibitor on Hair Growth.** M. Nugent, E.P. Mitchell*; Kimmel Cancer Center Thomas Jefferson University, Philadelphia, PA; Thomas Jefferson Medical College, Philadelphia, PA

Panitumumab is a human mAb that targets EGFR and that has been approved by the US FDA for treating patients with metastatic colorectal cancer. Panitumumab produces skin toxicities that include hypertrichosis and trichomegaly. The mechanism behind the EGFR-induced hair growth is poorly understood. We attempted to find patterns that distinguished the group of patients that developed hypertrichosis from the group of patients which did not. The dermatological manifestations of 24 patients on four clinical studies were noted. All skin-related toxicities were graded using the CTC for Adverse Events version 3.0. When comparing skin toxicities of the patients, we used the worst severity grade that they had developed while on study. Hypertrichosis was defined as excessive hair growth that grew in places that did not normally have hair and that was not induced by androgens. Trichomegaly was defined as excessive growth of the eyelashes. Patients were separated into a group that had developed hypertrichosis and a group that did not. They were then separated according to gender, race and therapy regimen. 41.6% of patients developed hypertrichosis or trichomegaly. It took 3.46 months after the first dose of Panitumumab for patients to develop hypertrichosis. No significant differences were noted in gender, race, or median age of patients with and without hypertrichosis. There is no correlation between hypertrichosis and severity of the other skin toxicities, monotherapy or combination therapy, or presence of anti-tumor response. Panitumumab may alter the growth cycle of the hair follicle in both men and women, causing excessive hair growth in atypical sites. The characteristic trichomegaly and hypertrichosis develop after a median duration of 3.46 months of therapy. Hypertrichosis and trichomegaly show no relationship to the other skin toxicities. A higher response rate was observed in patients with hypertrichosis, but this is related to the duration of anti-tumor therapy.

- 66) A Comparison of the Comorbidities of Subjects with New Daily Persistent Headache and Transformed Migraine.** J. Scena, W Cohen, C Stingo, A Jagolino, and W Young*, Jefferson Headache Center, TJUH.

NDPH has been distinguished from TM by nature of onset and the presence of migrainous features, but no cross-sectional study has compared them based on comorbidities. 50 NDPH patients and 50 age and gender matched TM patients were enrolled and diagnosed using Silberstein-Lipton criteria. Irritable bowel syndrome (IBS), restless leg syndrome (RLS), and fibromyalgia were evaluated using standard diagnostic criteria. Depression was evaluated with the BDI-II. 36 of 50 pairs were female. Mean age was 37.5 (NDPH) and 36.3 (TM). NDPH patients had less migrainous features than TM patients (3.4 vs. 4.0, $p=0.007$). There were no significant differences in prevalence of depression; IBS; RLS; or fibromyalgia. Logistic regression for diagnosis showed p values of 0.009 (migrainous features), 0.388 (IBS), 0.788 (RLS), 0.797 (fibromyalgia) and 0.223 (depression). Cluster analysis identified 2 clusters within each diagnostic group. For NDPH, the prevalence was: 0.0%-89.5% (RLS), 20.0%-42.1% (IBS), 0.0%-31.6% (fibromyalgia) and 20.0%-52.6% (depression); 3.31-3.79 (migrainous features). For TM, the prevalence was: 26.3%-58.3% (RLS), 0.0%-100% (IBS), 5.3%-41.7% (fibromyalgia) and 13.2%-41.7% (depression); 3.87-4.58 (migrainous features). The prevalence of comorbidities did not differ between NDPH and TM patients. Cluster analysis identified two patient clusters: one with more comorbidities and migrainous features and one with less. These findings suggest a common underlying pathophysiology.

- 68) Palliative Care in a Case of Extensive Post-operative Gastric Bypass Surgery Complications.** Stephen Stache, Janine V. Kyrillos, David Axelrod*. Thomas Jefferson University Hospital, Jefferson Medical College, Philadelphia, PA

This report discusses the case of a 57 year old white female who underwent gastric bypass surgery in February of 2005. The patient's postoperative course was quite extensive and initially significant for inability to tolerate any oral intake, severe malnutrition, dumping syndrome, and chronic hypokalemia. The patient had numerous hospital admissions to treat her underlying malnutrition and by November of 2005, she was so chronically malnourished that a PICC line was placed to provide total peripheral nutrition to help correct her malnutrition. Nine months later the PICC line became infected and the patient had a lengthy hospital admission for the treatment of severe sepsis. After recovering from the infection the patient's health continued to decline and she developed chronic myalgias, gait disturbance and peripheral neuropathy likely secondary to vitamin deficiencies, further complicated by Mallory-Weiss tears, a gastric ulcer and the need for a jejunostomy tube for nutrition. The patient's extensive two year treatment course culminated on her last hospital admission in September of 2007 when the patient and her family decided to forego further invasive and aggressive treatment. Palliative care became the primary goal of treatment and the patient was eventually transferred to hospice care where she died peacefully several weeks later. This case serves as a sample of a rare, yet potentially catastrophic complication of gastric bypass surgery. This case also illustrates the importance of close post-operative follow-up by a medical team with knowledge of post-operative morbidities of gastric bypass surgery. Recent studies demonstrate that post-operative outpatient complications of gastric bypass surgery are common and have the potential to become disabling and irreversible. During medical management of these potentially life-threatening complications, palliative care should be a consideration of the medical team. Furthermore, it is imperative that the patient's quality of life be a priority when making treatment decisions.

- 67) Clinical Predictors In Conservative Management of Vestibular Schwannoma.** Sharma P, Malhotra PS, Fishman MA, Pequignot E, Dasgupta A, Grumbine FL, Tholey R, Dam V, Willcox TO*. Department of Otolaryngology, Thomas Jefferson University.

Vestibular schwannomas are intracranial extra-axial tumors that arise from the schwann cell sheath investing either the vestibular or cochlear nerve that eventually occupy a large portion of the cerebellopontine angle. The goals of this study were to examine clinical variables at presentation that may predict which patients fail conservative management in an attempt to better delineate those patients most suitable for such treatment. A retrospective chart review was performed of 204 patients who elected observation primarily. Presenting symptoms, symptom progression, tumor size, audiologic measures, and global clinical outcomes were recorded for each patient with follow-up ranging from 1 month to 16 years. Disequilibrium as a presenting symptom appeared more often in patients who failed observation (58% vs. 32% overall, $p=0.039$), as did new onset disequilibrium. Subjective hearing also worsened more frequently in the group requiring intervention. Presenting tumor size differed for patients who failed conservative management and those continuing surveillance, with mean 14.0 mm vs. 9.03 mm ($p=0.0007$). Neurological complications, clinical outcomes, and overall morbidity compared favorably to those treated primarily with surgery or radiotherapy.

- 69) Sex-based analysis of abdominal disease prevalence and procedure utilization in Regione Emilia-Romagna.** R. Szumski and *DZ Louis, Center for Research in Medical Education and Health Care, TJU.

Sex-based differences in health care can be attributed to various constructs such as biological factors, physician attitudes, physician formed differential diagnoses when faced with a chief complaint, and patient utilization of health care services. The aim of this study was to evaluate sex-related differences in patterns of care for common abdominal diseases. Hospitalization rates for five abdominal diseases were classified by sex, severity stage, and surgical treatment when applicable. The abdominal conditions examined were appendicitis, cholecystitis, diverticulitis, pancreatitis, and abdominal aortic aneurysm. For both appendicitis and cholecystitis, women had a higher rate of hospitalization at lower stages of disease, 6.47 and 16.30 per 10,000 residents respectively, while men were hospitalized and diagnosed with the conditions at more severe stages of disease. There were also differences in proportions by sex when treatment procedures were examined. Laparoscopic appendectomies and cholecystectomies were performed at higher proportions on women compared to men. Sex differences in rates of diagnosis and hospitalization at various stages of illness may imply problems with the recognition of disease at low stages in men. Treatment regimens that differ between sexes may be due to a variety of rationales. Further studies should explore sex differences by severity and treatment procedures to identify efficacy, physician attitude and sex bias towards diagnostics and treatments.

- 70) Consequences for the health of communities: increased emergent global path length from local arbitrary homophily.** GE Weissman, and *K Armstrong. Jefferson Medical College and Department of Medicine and Leonard Davis Institute of Health Economics, University of Pennsylvania.

The purpose of this study is to demonstrate the effect of local arbitrary homophily on emergent global network structure, and provide a quantitative basis for investigating the consequences of social structure in a public health context. We ran a "Strangers' Banquet" simulation: beginning with 200 nodes divided into two arbitrarily colored subgroups, and a completely empty graph, we then populated the graph in steps, adding edges according to a homophilic association game, and measured connectivity after each new connection. We ran the simulation over the full range of homophilic preference [0, 1.0] and connectivity. This study demonstrates that for the case of high discrimination and low edge density – common features of most real social networks – the emergent network will have a distinctively larger characteristic path length. We conclude that individual (nodal) preferences and behaviors directly affect global characteristics, i.e. quality, of networks. This points to individual social choices and structures as important targets in community health interventions. This work also suggests that common markers in public health like race and geography are in part proxies for social position. Finally, this study fills the gap of a strong quantitative framework for investigating other social determinants of health such as patient-physician trust, access, and discriminatory referral patterns.

Field Topic:

Genetics

- 71) GRK2 Ablation Augments the Contractile Effect of Phenylephrine.** HI Cohn, DH Harris, S Pesant, M Pfeiffer, R Zhou, & AD Eckhart*. Dept of Med, TJU

G protein-coupled receptor kinase 2 (GRK2) is a ubiquitous serine/threonine kinase that phosphorylates and desensitizes agonist-bound receptors including β -adrenergic receptors (ARs). GRK2 is elevated in hypertension and we were interested in whether inhibition of GRK2 would rescue high blood pressure (BP). A surgical model of high BP, the two kidney-one clip (2K1C) model, in mice resulted in a 30% increase in BP, a 50% increase in vascular smooth muscle (VSM) GRK2 RNA levels, and a 3-fold increase in circulating catecholamine levels. However, VSM-specific GRK2 ablation, by either GRK2 knockout (GRK2KO) or peptide inhibition (GRK2ct), failed to rescue the hypertension. Importantly, β AR BP responses were enhanced in both the GRK2KO and GRK2ct mice suggesting that β AR signaling was restored. We also observed augmented signaling of α 1ARs. Acute agonist studies revealed a further increase in BP with phenylephrine (PE) infusion in GRK2KO and GRK2ct mice vs controls. In addition enhanced α 1AR constriction was observed in GRK2KO and GRK2ct thoracic aorta vessels. Increased α 1AR signaling due to lack of GRK2 or GRK2 inhibition was ablated with either an α 1AAR or α 1DAR specific inhibitor. α 1BAR antagonists provided no effect on the enhanced vessel constriction. Our data illustrate that although lack of GRK2 or GRK2 inhibition in VSM improves β AR mediated dilation, it also enhances α 1AAR and α 1DAR mediated vasoconstriction suggesting that these α 1AR are a target of GRK2 and that GRK2 is not an appropriate antihypertensive therapeutic target.

- 72) Intestinal polyp suppression by a novel modifier locus in the ApcMin mouse model of colorectal cancer.** SC Nnadi, R Koratkar, RA Watson, J Innocent, J Brunner, AM Buchberg and *LD Siracusa. Kimmel Cancer Center, Dept of Microbiology and Immunology, Thomas Jefferson University

Colorectal cancer in humans is a leading cause of cancer death in the U.S. Familial adenomatous polyposis (FAP) is an autosomal dominant genetic disorder characterized by hundreds to thousands of benign polyps in the colon, which if left untreated may become malignant. The mutant adenomatous polyposis coli (ApcMin/+) mouse is a powerful model to study the initiation, growth, and progression of intestinal and colorectal cancer. Genetic background greatly influences polyp number, size, and position in ApcMin/+ mice, and modifier genes can alter these phenotypes. Our laboratory demonstrated that the Pla2g2a and Atp5a1 genes are responsible for the protective Modifier of Min 1 (Mom1) and Mom2 phenotypes, respectively. Hybrid progeny from C3H/HeJ (C3H) females crossed to C57B6/J (B6) ApcMin/+ males showed an ~80% decrease in polyp number compared to B6 ApcMin/+ mice. In addition, a similar finding involving the Mus castaneus (CAST) genome showed a similar decrease in polyp number. These findings indicate the presence of additional resistant modifier loci (distinct from Mom1 or Mom2) in the C3H and CAST genomes. We have conducted several crosses to limit the boundaries of a new modifier (known as Mom4). We describe the genetic strategies being used to identify the gene(s) responsible for these protective effects. The study of modifier genes will impact the prevention, diagnosis, and treatment of human colorectal cancer. Research supported by funding from the NCI.

- 73) Intramolecular Interaction and Coordinated Phosphorylation of the Androgen Receptor Alters Its Metabolism in a Cell Model of Spinal and Bulbar Muscular Atrophy.** CR Orr, Y Liu, SC Jenkins and DE Merry*.
Department of Biochemistry and Molecular Biology, Thomas Jefferson University

Spinal and bulbar muscular atrophy (SBMA) is a hormone-dependent, neurodegenerative disease caused by expansion of a polyglutamine (polyQ) tract in the androgen receptor (AR). Affected motor neurons exhibit neuronal intranuclear inclusions consisting of aberrantly cleaved and accumulated AR. Trafficking and activity of the AR is modulated by phosphorylation. To better understand how phosphorylation may alter the metabolism of the "expanded" AR, we used a cell model of SBMA to assess phosphorylation of AR with site-specific phospho-antibodies. Western analysis of phosphorylated Ser81 revealed enhanced phosphorylation of a polyglutamine expanded AR. Treatment with roscovitine, an inhibitor of Ser81 phosphorylation, revealed that inhibition of phosphorylation at Ser81 and Ser308 is polyQ length-dependent, occurring only on an AR with a normal polyQ tract. This suggests that phosphorylation of Ser81/308 is aberrantly regulated by polyQ expansion. Furthermore, using antagonists or mutations of AR that disrupt its amino/carboxyl terminal interaction reduced Ser81 and Ser308 phosphorylation as well as abrogated inclusion formation. These data are the first to correlate inclusion formation with phosphorylation of these sites. Preliminary data show phospho-mimetic mutants of Ser81/Ser308 increase inclusions, implicating a direct role of phosphorylation in the altered metabolism of the expanded AR in SBMA.

- 75) Analysis of a Genetic Signature Characteristic of Meis1 Expression.** J J Roth and *A M Buchberg, Department of Microbiology and Immunology, Kimmel Cancer Center, Thomas Jefferson University

Meis1 was identified as a common site of viral integration in ~15% of myeloid leukemias found in BXH-2 mice. Of these leukemias, nearly 95% have a viral co-integration at either the HoxA7 or HoxA9 locus, suggesting that these genes may cooperate to induce leukemia. Meis1 is a homeodomain containing transcription factor that binds to DNA as either a homodimer or as a cofactor with members of the Pbx, Pdx and Hox families of proteins depending on the context of expression. Overexpression of Meis1 in conjunction with HoxA9 has been seen in a variety of myeloid leukemia cell lines and primary human samples of acute myeloid leukemia. In addition, Meis1 and HoxA9 are major downstream targets in leukemias associated with ALL/MLL translocations. Expression of HoxA9 in hematopoietic stem cells leads to leukemic transformation after a long latency and co-expression of Meis1 accelerates this leukemogenesis. Few downstream targets of Meis1 and HoxA9 have been identified. Meis1 and HoxA9 have been shown to regulate c-Myb, FLT3, and CD34, all of which are expressed in many leukemias. In an effort to categorize additional downstream targets, we generated murine bone marrow cells overexpressing Meis1 in the presence or absence of HoxA9. Microarray analysis was performed on RNA isolated from these cells. A combination of bioinformatics and statistical analyses were used to look for genes of interest with differential regulation in the presence or absence of Meis1/HoxA9. From here these data will be used to generate a genetic signature characteristic of Meis1 expression and shed light onto other pathways that Meis1 and HoxA9 might play a role in.

- 74) Sirt1 determines androgen signaling and prostate cellular growth in vivo.** Powell, MJ, Casimiro, MC, Yu, Z, Katiyar, S, Marampon, F, Parlow, AF, McCue, PA, Cardiff, RE, He, X, Popov, VM, McBurney, MW, *Pestell, RG. KCC, Depts of Cancer Biol and Oncol, TJU, Harbor-UCLA Med Ctr, CA, Dept of Path and Lab Med, Ctr for Compar Med, University of California, and University of Ottawa, Canada

The Sirtuin family consists of a group of NAD⁺-dependent histone deacetylases that are conserved from archaeobacteria to eukaryotes. SIRT1 is the human homolog of the yeast Silent Information Regulator 2 (Sir2) gene. SIRT1 deacetylates multiple substrates including histones, transcription factors, as well as nuclear receptors and their co-activators such as the androgen receptor (AR) and p300. This study's purpose is to determine the functional role of Sirt1 in androgen signaling and prostate cellular growth and development in vivo. Methods used within this investigation include knockout mice, RT-PCR, western blotting, microarray and miRNA chip analysis, immunoprecipitation, immunohistochemistry, serum hormone ELISA, H&E histological staining of paraffin embedded tissue sections, gross mouse and organ weight measurements, as well as KEGG, Biocarta, GSEA, and ASSESS analysis of array data from both microarray and miRNA chip data sets. Herein, homozygous deletion of the Sirt1 gene in mice resulted in altered size of androgen-responsive tissues including the anterior and ventral prostates. Genome-wide expression demonstrated that Sirt1 deletion reduced growth factor signaling and enhanced androgen-responsive gene expression in the prostate. Sirt1 directly regulated miRNA that were also regulated by DHT. These studies provide a mechanism by which Sirt1 regulates miRNAs that directly govern DHT-dependent cellular proliferation and growth as well as the expression of genes essential for normal prostate development such as Nkx3.1.

- 76) Activation of AR by DDE: A possible mechanism for hormone-refractory prostate cancer development.** Supriya Shah, Janet Hess-Wilson, Hannah Daly, Siobhan Webb, Sonia Godoy-Tundidor, Karen Knudsen*. Thomas Jefferson University, Department of Genetics, Philadelphia.

Prostate cancer is the second leading cause of mortality in US. Previous research indicates that prostate cancer cells need Androgen receptor (AR) activation for survival. Therefore the first line of treatment for Metastatic prostate cancer is Androgen deprivation induced either by depleting the androgens or by inhibiting AR. This approach is successful initially since the cells undergo cell cycle arrest or die which even reduces the size of tumors upon the therapy. However, eventually these tumors become androgen independent due to inappropriate activation of AR by non-canonical ligands. Since there is no effective therapy for androgen independent tumors this leads to increased morbidity. Identification of agents responsible for inappropriate activation of AR is necessary for better management of disease. AR from relapsed tumors shows mutations in the ligand binding domain. Various chemical compounds and environmental pollutants mimic estrogenic properties and are therefore called Endocrine Disrupting Compounds (EDCs). We've previously shown that EDC compound such as BPA can induce cellular proliferation of prostate cancer cells, decrease the relapse time and increase tumors. Here we study another EDC called DDT which was widely used as most potent pesticide. Even though DDT is banned in US from years, the non-biodegradable DDT is eroded from soil to water sources in more toxic forms DDE/DDD and therefore still imposes a threat. Using reporter assays we show that DDE can activate mutated AR commonly found in the prostate cancer cells. Further analysis by ChIP and Q-PCR indicated AR translocation to promoter region of target genes. The growth assays and BrdU incorporation assay show that DDE is able to increase proliferation in the prostate cancer cells in absence of endogenous hormones. These results indicate that DDE can activate AR and thus may contribute to the development of hormone refractory prostate cancer.

- 77) Role of Caveolin-1 in Proliferation, Migration, and Tumorigenesis in Pam212 keratinocytes.** C M Trimmer, F Capozza, S Katiyar, R G Pestell, and *M P Lisanti, Kimmel Cancer Center, TJU.

Caveolin-1 is the main component of cell membrane invaginations called Caveolae and has been recently described to have a critical role in cell transformation and tumor formation. Mice lacking Cav-1 expression have been shown to be more prone to the development of skin tumors when exposed to the carcinogenic compound DMBA. To further determine the role of Caveolin-1 in the development of skin cancer, we examined the effect of altered Cav-1 expression on a murine keratinocyte cell line Pam212. For this purpose, we transduced Pam212 cells by means of retroviral strategy to stably over-express or down-regulate Cav-1 protein. The over-expression of Cav-1 resulted in decreased proliferation and migration in Pam212 cells. Moreover, when Pam212 cells over-expressing Cav-1 were xenografted intradermally into Nude mice, reduced tumor growth and incidence were observed. In contrast, siRNA mediated Cav-1 knock-down caused an increase in cell proliferation with up-regulation of Cyclin D1 protein and hyper-activation of the Erk pathway. Consistently, Pam212 cells with down-regulated Cav-1 formed larger tumors when cells were xenografted intradermally into Nude mice. These results provide further support for the idea that Caveolin-1 functions as a tumor-suppressor in the development of skin cancer, as over-expression of this protein decreases characteristics of transformation and tumorigenicity, while down-regulation of Cav-1 increases these characteristics in a murine keratinocyte cell line.

Field Topic:

Infectious Diseases

- 78) The Use of Atorvastatin (Lipitor) to Prevent Hyperglycemia and HIV-1 Associated Cytotoxicity in Human Astrocytes.** L Donnelly, E Acheampong and *Z. Parveen. Dept. of Medicine, Division of Infectious Disease, Thomas Jefferson University.

Patients receiving Highly Active Antiretroviral Therapy (HAART) frequently develop insulin resistance, hyperglycemia, and diabetes as a side effect of the treatment. Hyperglycemic conditions and HIV-1 Nef are linked to an increase in reactive oxygen species (ROS) causing cytotoxicity and cell death. Atorvastatin (Lipitor) is being evaluated for the ability to prevent endothelial dysfunction in diabetic patients. Here we are testing its ability as anti-ROS in primary human astrocytes exposed to various in vitro glyceic conditions, including 5 (normal), 10, 15, and 20 mM glucose with addition of 10uM of atorvastatin for twelve hours. After treating the astrocytes with various glucose concentrations and statin, astrocytes were washed and incubated with normal medium for 12 hours before transduction with HIV-1 Nef expressing virus. 48 hours later the transduced and non-transduced cells treated with hyperglycemic conditions were subjected to ELISA, western blot, RNA analyses, and immunocytochemistry. The cytotoxicity of astrocytes treated with hyperglycemia, and transduced with HIV-1 Nef was measured by ELISA using total nitrates, and 8-iso-prostaglandin F2 alpha as indicators of ROS. Our results suggest that Atorvastatin is capable of regulating the highly upregulated oxidative state of astrocytes due to hyperglycemia and HIV-1 Nef protein. The western blot indicates that Atorvastatin protects the astrocytes from caspase 9 under similar conditions.

- 79) Description withheld.**

Sigma Xi Student Research Day 2008 Poster Abstracts

- 80) A Novel Assay to Detect Post-integration Repair During Retroviral Infection.** J R Haynes and *R Daniel. Department of Medicine, Division of Infectious Diseases, Thomas Jefferson University, Philadelphia, PA 19107

The replication cycle of a retrovirus entails entry of the virus into the host cell and integration of the newly transcribed DNA into the host genome. Integration of retroviral DNA involves a staggered cut of host cell DNA followed by the joining of 3' -ends of viral DNA to host DNA by the retroviral enzyme integrase, creating short single, stranded DNA gaps that flank retroviral DNA. The repair of these gaps is facilitated by the host enzymes during the process of post-integration repair and involves the filling in of missing nucleotides, trimming of short viral DNA flaps and the ligation of 5' -ends of viral DNA to the newly synthesized 3' -end of host DNA. In this study we seek to further characterize the process of post-integration repair by establishing a time frame for completion. This would be achieved by extracting DNA at established post infection time points to be used in a novel assay which utilizes S1 endonuclease and Alu-PCR to monitor the presence or absence of the single stranded gaps. Our preliminary data shows the completion of post-integration repair between 24 and 48 hours post infection. These findings will enhance our understanding of the integration process of retroviral infection.

- 81) Description withheld.**

- 82) Multiple Modes of Resistance to HIV-1 Entry Inhibitor C37.** K M Kahle and *M J Root, Department of Biochemistry and Molecular Biology, Thomas Jefferson University

The HIV-1 entry inhibitor C37 targets the gp41 N-terminal heptad repeat region (N-HR) of Envelope (Env), thereby blocking trimer-of-hairpins formation crucial to viral membrane fusion. Due to the transient exposure of the C37 target, antiviral potency depends on both C37 binding affinity and association rate with gp41. Consequently, C37 variants of higher affinity do not possess enhanced potency against nonresistant Env. Here, we describe the selection of HIV-1 resistant to wild type C37 and a higher affinity variant C37KYI. Through propagation of HIV-1 in increasing inhibitor concentrations, two patterns of resistance involving both Env subunits, gp41 and gp120, emerged. For viruses propagated up to moderate levels of C37, mutations were observed primarily in the gp41 N-HR. These mutations disrupt the binding affinities of both C37 and C37KYI, but only conferred resistance to the wild type peptide. Resistance to similar levels of C37KYI appeared to be linked to additional mutations in the gp41 C-HR that temporally limit C37 target exposure. Increasing C37 and C37KYI concentrations elicited substitutions in the Env surface subunit gp120. At extremely high inhibitor levels, the resistance profiles for the two inhibitors begin to converge, incorporating mutations from both affinity- and kinetically-driven mechanisms. The results demonstrate how equipotent fusion inhibitors having the same mechanism of action can elicit resistance through distinct pathways.

Field Topic:

Molecular Pathology/Pathology/Anatomy

- 83) Antibiotic Tethering to Bone Grafts Confers Protection from Infections.** C Ketonis, A Aiyer, J Parvizi and *N Hickok. Department of Orthopedic Surgery, Thomas Jefferson University

Bone grafting is considered the gold standard for repair of bone defects, with approximately 2.2 million/year of these orthopaedic procedures worldwide. However allograft impaction can lead to complications such as infection. With up to a 30% incidence, infection remains the most devastating complication, requiring reoperation, debridement, metallic implantation and in some cases amputation. The purpose of this study was to minimize the incidence of infection by developing an antibiotic modified bone graft that could confer long-term bactericidal activity with no adverse effects. We developed a novel chemical scheme using naturally occurring amines on the bone surface to covalently anchor two linkers and vancomycin. A uniform antibiotic distribution was observed using anti-vancomycin antibodies, and this distribution remained stable over time. Furthermore, the modified allograft resisted bacteria colonization by *S. aureus* for 2-12 hrs using fluorescent detection of bacteria and plating; up to 85% fewer bacteria survived as compared to control. Importantly, the modified bone remains biocompatible as shown by cell numbers, toxicity assays, fluorescent imaging and SEM. We show that we can successfully and stably tether vancomycin on allografts and that they prevent colony formation and growth of bacteria without an impact on biocompatibility. We propose that this technology can provide clear benefits over the currently limited techniques in preventing bone graft infections.

Field Topic:

Neurosciences

- 84) Gap Junction Uncouplers Do Not Disrupt the Electric Organ Discharge (EOD).** E Alex and * J Sidie. Department of Biology, Ursinus College

In weakly electric fish (*Eigenmannia virescens* – Transparent Knife Fish), the electric organ discharge (EOD) is driven by a pacemaker nucleus of ~110 neurons located in the medulla. The neuronal cell bodies in this neural network are all connected by low resistance gap junctions. The effect of this coupling is to insure synchrony in the neuronal discharge pattern. This network exhibits unusual temporal stability (coefficient of variation of EOD frequency ~ 0.0003). The purpose of this investigation is to use known gap junction uncouplers to disrupt the network and thereby gain insight into the nature of this temporal stability. We studied the effect of 1-octanol, carbenoxolone, flufenamic acid, 18 α -glycyrrhetinic acid, elevated Ca^{++} , and pH (all concentrations = 10⁻⁵ – 10⁻³M). None of these actions disrupted the EOD. Octanol acts as a general anesthetic and over time significantly depresses the EOD but does not destabilize the synchrony of the composite waveform. It is possible that the gap junctions of the medullary pacemaker are resistant to these known uncouplers and may possess unique membrane properties.

- 85) Chronic cocaine induced anxiety-like behavior and trafficking of the delta-opioid receptor in the nucleus accumbens of female Sprague-Dawley Rats.** LM Ambrose-Lanci, EM Unterwald, RC Sterling and EJ Van Bockstaele*. Dept. of Neurosurgery, TJU, Temple University, Dept of Psychiatry & Human Behavior, TJU

The present study investigated the effects of chronic cocaine administration on anxiety-like behavior and delta opioid receptor (DOR) receptor trafficking in the nucleus accumbens (NAcb) of female rats. Female rats received binge-pattern (3 injections/day) cocaine (15 mg/kg, i.p.) or saline (1ml/kg, i.p.) for 14 days and subsequently underwent a 48 hour withdrawal period. To measure anxiety-like behavior, animals were tested using the elevated plus maze and. Blood estradiol levels were assessed to correlate estrogen level with anxiety scores. Following behavioral testing, animals were transcardially perfused and tissue sections containing the NAcb core (NAcbC) and shell (NAcbS) regions were processed for immunogold-silver localization of DOR using electron microscopy. Semi-quantitative analysis revealed that cocaine withdrawal caused an increase in the percentage of DOR localized to intracellular compartments in the NAcbS. In contrast, in the NAcbC of female rats, there was an increase in DOR associated with the plasma membrane following withdrawal from cocaine. Estradiol level did not correlate with anxiety-like behavior on the elevated plus maze. Since, estradiol is a naturally occurring variable, additional cohort are needed to increase group numbers to better ascertain the influence of high and low estrogen levels on anxiety-like behavior during cocaine withdrawal in female rats. The present findings did demonstrate a re-distribution of DOR in the NAcb following withdrawal from chronic cocaine administration.

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- 86) Recovery of Visual Function Following Optic Nerve Transection.** H Amin, A Hockenberry and *Dr. E Gruberg. Dept. of Biology, Temple University

The research was concerned with studying how frogs perceive stationary objects and where in the brain these objects are processed. Previous studies in the frog species *Rana pipiens* have shown that behavioral functions related to visual processing (i.e. stationary object perception, response to prey) are localized in different areas of the brain. For instance, removing the optic tectum results in a frog's inability to respond to prey and looming stimuli, but the frog can still respond to stationary objects. Transected optic nerve axons of *Rana pipiens* regenerate with great accuracy so that individual fibers reconnect to their original destinations. The destinations of these axons are at different distances from the transection and so, in principle, different visual functions could recover at different times. We investigated the time course of recovery of those visual functions in order to determine the likely location of the brain region concerned with processing visual information about stationary objects. It was found that after optic nerve transection, subsequent recovery of the visual recognition of prey objects, looming objects, and opaque barriers occurred almost simultaneously: 38, 41, and 36 days post-surgery respectively. It was concluded that our results did not confirm the assumption that regenerating optic nerve fibers take longer times to go greater distances, suggesting that visual functions are mediated by different brain structures and that there are different classes of optic nerve fibers that mediate these functions.

- 88) Redefining implicit and explicit memory: the functional neuroanatomy of post-surgical left temporal lobectomy epilepsy patients.** S Grodofsky and *J Tracy. Department of Neurology, Thomas Jefferson University

This study employed functional magnetic resonance imaging (fMRI) to characterize poorly understood implicit memory neural pathways involved in solving word stem procedures in normal controls (NC) and post-surgical left temporal lobectomy epilepsy patients (LTLE). 12 LTLE patients and 16 NCs were asked to memorize a list of 40 words and entered a 1.5 Tesla MRI to complete word stem language memory tasks while whole-brain BOLD contrast functional images were collected. The fMRI images were analyzed using software program SPM 05. The behavioral paradigm used Jacoby's Process Dissociation Process (PDP) that reliably separates intentional memory (explicit) from automatic processes (implicit). As expected, LTLE patients displayed impairment in explicit memory, but intact implicit memory function compared to NCs. When isolating explicit memory, fMRI bold signal response displays neural activity in the left medial temporal lobe and left insula in NCs and absent in LTLE patients. In contrast, patients relied upon parietal structures for task completions, unlike NCs. When isolating implicit memory, the evidence is inconclusive, but data so far suggests activity in left inferior frontal gyrus for LTLE patients but absent in NCs. This suggests that the absence of a functional left medial temporal lobe forces epilepsy patients to accomplish implicit memory by utilizing brain structures that in NCs are typically dedicated to explicit memory and word retrieval activities.

- 87) Circumventricular organs are potential site for adult neural stem cells in the brain.** LB BENNETT, M YANG, CE MARSHALL, R MEHTA, *L IACOVITTI
Farber Institute for Neurosciences, Department of Neurology, Thomas Jefferson University, Philadelphia, PA

Neurogenesis in the adult mammalian nervous system has been described in the subventricular zone (SVZ) of the lateral ventricle wall and subgranular zone (SGZ) of the hippocampus. The neural stem cells (NSCs) most widely believed to give rise to neurons in these regions are nestin+, GFAP+ germinal astrocytes. In the current study, using confocal microscopy and triple-label immunocytochemistry, we unexpectedly found that in addition to the SVZ and SGZ, subsets of cells distributed along the midline of the third and fourth ventricles of the adult rat brain also co-labeled for the NSC markers nestin, GFAP, and vimentin. Interestingly, these cells resided exclusively in a group of brain structures collectively known as the circumventricular organs (CVOs). To begin to explore the possibility that CVOs contain NSCs, we further studied the ability of CVOs to behave as NSCs in culture. We found that CVO cells grown in suspension culture were capable of forming neurospheres. Moreover, these cells were mitotically active, staining simultaneously for nestin and the proliferation marker ki67. When these cells were grown in adherent cultures and exposed to a neuronal differentiation medium, they also differentiated into neurons. These results raise the intriguing possibility that CVOs, with their proximity to capillaries and ventricles and their NSC-like staining, may represent novel sites for the generation of new neurons in the adult brain. If so, CVO cells could represent a novel cell resource in neurodegenerative disease therapy.

- 89) Temperature and Temporal Stability in a Brainstem Neural Network.** Luk, Elizabeth and James Sidie*, Ursinus College, Collegeville, PA

General anesthesia can be defined as a condition which includes immobility (loss of movement), amnesia (loss of short-term memory), analgesia (absence of pain sensation), and hypnosis (inducement of sleep/drowsiness). These functions are controlled by widely dispersed areas of the brain. Although general anesthetics are considered to be among the most dangerous drugs given to patients, their mode of action is currently unknown. The purpose of this investigation was to study the mechanism of general anesthetic action of the alkyl alcohol 1-decanol utilizing weakly electric fish as a model assay system. The Electric organ discharge frequency (EODf) was monitored and analyzed at different temperatures and in the presence/absence of 10-4M decanol. An analog/digital conversion circuit permitted the online computation of frequency, standard deviation, and time interval histogram of the EODf. EODf is directly driven by the medullary pacemaker nucleus, a neural network of ~110 brainstem neurons. The fish were reared and acclimated to 25C, then EODf was analyzed over time for ~20,000 - 40,000 events (nerve impulses). Coefficient of variation (s.d./mean) was calculated: EODf c.v. at 15C. ~0.0003; EODf c.v. at 25C. ~0.0003; at 35C. EODf c.v. ~ 0.0004. These values are 10-1000x more stable than other neuronal firing patterns. The EODf c.v. does not vary when the fish are exposed to 10-4M decanol, despite the observation that the EODf declines ~35% / 20 min. These observations indicate that anesthetics and cold temperature effectively depress electrical activity (nerve impulse frequency) in the fish's brainstem but that the temporal stability of the underlying neural network remains intact. Apparently the connections (gap junctions) between neurons are not affected by temperature or anesthetics. Supported in part by Merck/AAAS/Ursinus.

Sigma Xi Student Research Day 2008 Poster Abstracts

- 90) Distribution of Melatonin Receptor Subtypes in Area X of *Sturnus vulgaris*.** J Margulis, and *GE Bentley, Department of Biology, University of Pennsylvania and Department of Integrative Biology and Helen Wills Neuroscience Institute, UC Berkeley

Adult vertebrate neuroplasticity was first discovered in the telencephalic song control system of songbirds, such as the European starling (*Sturnus vulgaris*). It is now an accepted phenomenon in mammals, including primates. In songbirds, the concentrations of gonadal steroids such as testosterone and estradiol fluctuate annually as a result of the birds undergoing seasonal "puberty" to prepare for mating. Consequently, there are annual changes in the size and structure of components of the steroid-sensitive song control system. This seasonal neuroplasticity is important because a male's reproductive fitness is strongly correlated with the variety and complexity of his songs. Melatonin has an inhibitory role in these seasonal changes, acting via receptors (MelR) in several song control nuclei. Here we focus on Area X, a basal ganglia structure that is important for song learning and recognition. The present study aimed to determine the possibility of varying roles of the three MelR subtypes (Mel 1A, 1B and 1C) in inhibition of song learning as well as differential sensitivities to gonadal steroids. We attempted to map the potential differential distributions of MelR subtypes using in situ hybridization and polymerase chain reaction (PCR). With this approach, we showed that MelR subtypes 1B and 1C were more strongly expressed in Area X. We thus propose that these subtypes are more important in inhibition of seasonal neuroplasticity.

- 92) Neuroprotective role of IGFBP-3, PACAP-38 and other proteins upregulated in the VTA in Parkinson's disease.** S Phani, *L Iacovitti, Farber Institute for Neurosciences, TJU

Parkinson's disease and its characteristic symptoms are thought to arise from the progressive degeneration of specific midbrain dopamine (DA) neurons. In humans, DA neurons of the Substantia Nigra (SN) show selective vulnerability, while neighboring DA neurons of the Ventral Tegmental Area (VTA) are relatively spared. In this study, we aimed to determine the genes/proteins in the VTA that confer neuroprotection. Using our own microarray analysis as well as those that have been previously published, we found several candidate genes that were elevated in the VTA. These include IGFBP-3, PACAP-38, CRH, among others. To test these factors, we have developed an in vitro bioassay in which the VTA and SN are cultured separately into relatively pure populations. Cultures are established from transgenic mice taking advantage of midbrain GFP expression driven by a human Tyrosine Hydroxylase (hTH) promoter. The midbrains of these mice are microdissected at embryonic day 13 into VTA and SN populations and plated using cortical glial conditioned media. Preliminary findings have shown that SN cells at the embryonic stage require the addition of VTA conditioned media for survival. To elucidate the factors present in VTA CM that promote cell survival, SN cells were plated in glial CM containing additives such as growth factors and other specific factors upregulated in the VTA compared to the SN. Of the factors tested, none achieve the levels of cell survival obtained with VTA CM. Possibly, a combination of factors from the VTA is needed for SN cell survival. An alternative is the interesting possibility that a novel factor present in the VTA is required for normal SN survival.

- 91) A Prospective study of Synaptic Plasticity following a Right Frontal lobectomy.** KZ Osipowicz and *JI Tracy, Department of Neurology, TJU

A prospective study of reorganization of the neural correlates of motor function was carried out prior to and following a right frontal lobectomy. Functional brain mapping during a left hand motor movement task (single finger opposition) and left foot motor movement task (pedal press) was done prior to surgery, six months following surgery, and one year following surgery using functional magnetic resonance imaging (fMRI). At the same scanning intervals, seven slice functional correlation magnetic resonance imaging (fcMRI) was collected during a rest condition with the region of interest (ROI) selected to cover the right sided motor homunculus. Presurgical motor activation was mapped out to the expected regions of the right motor homunculus and rich basal state connectivity. Postsurgical activation showed a reduction in activation and connectivity without associated neurological deficit. The second postsurgical scan revealed an increase in activation and connectivity. This data indicate a reduction in activity and connectivity is essential to maintain neurological function following brain injury, and a temporally dependent synaptogenic return to basal connectivity.

- 93) Distribution and trafficking of the cannabinoid receptor CB1 in the rat noradrenergic locus coeruleus.** JL SCAVONE, K MACKIE and EJ VAN BOCKSTAELE*. Dept of Neurosurgery, Jefferson Hosp. for Neurosci., TJU and Psychological and Brain Sci., Indiana Univ., Bloomington, IN

Cannabinoid receptor CB1 is widely distributed and modulates signaling throughout the brain. In the present study, the distribution of the cannabinoid receptor CB1 was examined in the locus coeruleus (LC), where it may function to mediate noradrenergic neuron responsivity. Using an antibody directed to the entire C-terminus of the receptor, light and electron microscopic approaches were utilized to characterize the distribution of CB1 in the LC. Dual immunofluorescence microscopy revealed the presence of CB1 in tyrosine hydroxylase (TH) labeled neurons. In addition, CB1-positive cells lacking detectable TH immunoreactivity could also be identified amongst the noradrenergic neurons. Immunoelectron microscopy studies confirmed CB1 receptor localization to somatodendritic processes and revealed a smaller subset of presynaptically distributed receptors within this region. While a greater percentage of CB1 receptors were localized to the plasma membrane of axon terminals, CB1 receptors were primarily localized to the cytoplasm of somatodendritic processes. Experiments are ongoing to characterize trafficking patterns of CB1 following administration of a CB1 agonist. In summary, the present study provides neuroanatomical evidence for the presence of cannabinoid receptors in the LC, and suggests a cellular substrate for CB1 modulation of post-synaptic signaling within this region.

- 94) Ret/PTC 3 Induction of NF κ B in Inflammation and Cancer.** Neely, Robert J., Rothstein, Jay L., *May, Michael J., Department of Immunology and Microbial Pathogenesis, Thomas Jefferson University, and Department of Animal Biology, University of Pennsylvania

The expression of the oncogenic fusion protein Ret/PTC 3 (RP3) has been found in both Hashimoto's thyroiditis and in the differentiated thyroid malignancy, papillary thyroid carcinoma (PTC). RP3 is an oncogene formed from a translocation involving the N-terminal portion of RFG and the C-terminal kinase domain of the receptor tyrosine kinase c-RET. The full length c-RET receptor, once activated by ligand binding and autophosphorylation of its kinase domain, signals via the ras/raf/MAPK and PI3-K/AKT pathways. This signaling has been reported to be important in the activation of NF κ B. Intriguingly, it was recently shown that the c-RET receptor plays a role in Peyer's patch development which has been reported to be dependant on the non-canonical NF κ B pathway. However, the RP3 fusion protein does not contain the transmembrane portion of the full-length receptor and only 20% of expressed RP3 has been observed to be membrane bound. These findings therefore leave in question the signaling mechanisms induced by RP3 that underlie its pro-inflammatory function. To address this question, we have generated epithelial cell lines stably expressing RP3 and found that the classical NF- κ B pathway is robustly activated in these cells. Furthermore, RP3 expression enhances p100 processing to p52 in the RP3 expressing cells. We are currently delineating the mechanisms of RP3's activation of the NF κ B pathways as understanding these may lead to the development of potential novel therapeutic targets.

Field Topic:

Physiology and Immunology

- 95) Intercellular transfer of influenza hemeagglutinin leads to presentation of one epitope on MHC Class II while another is not presented.** JS Testa and *LC Eisenlohr, Kimmel Cancer Center, TJU

Influenza is a major concern due to its potential to cause a pandemic. Annual vaccines are relatively effective, but are a major problem. Thus, there is much room for needed improvement such as a reformulation to replace annual vaccines. In order to generate better vaccines, the immune response to this virus must be better understood. Antigen processing and presentation of viral antigens, a major focus of our laboratory, is a critical aspect of the host response as it leads to a strong T cell response which facilitates neutralizing antibody development and cell mediated killing of virally infected cells. Studies reported here examine the ability of cells to transfer an influenza epitope intercellularly for presentation. Specifically, we show with an in vitro system that one MHC Class II (MHCII) epitope from hemeagglutinin (S1) can be "cross-presented" while another cannot (S3). This phenomenon was also shown to be dependent on classical MHCII loading machinery which is consistent with how S1 is normally presented from cells expressing hemeagglutinin. Further, conditioned media from cells expressing the antigen could mediate cross-presentation but not following .22 μ m filtration. These results suggest that the transfer of soluble antigen from cells expressing antigen to cells lacking antigen can lead to presentation. These data describe an interesting presentation mechanism for the processing and presentation of influenza MHCII epitopes which may be useful in vaccine redesign, and would help to eliminate annual vaccines.

- 96) FIELD TOPIC: HISTORY OF SCIENCE**

**-The Forgotten Female Physicians -
-Philadelphia: Medicine's Pioneering City.**
CJ Myers, and S Mangione*, JMC-TJU

-The Forgotten Female Physicians -

Though historically some of the most well known and lauded physicians have been male, women have been making significant contributions to the practice of medicine since the time of the ancient Greeks. Living in the shadow of their male counterparts, their knowledge and skill has often been overlooked and undervalued. While some, such as Maria Montessori, Virginia Apgar and Elizabeth Blackwell did eventually realize some recognition for their accomplishments, the memory of others, such as Susan LaFlesche Picotte, Trotula, Agnolice, and Elizabeth Garrett Anderson have nearly been lost to the ravages of time. Their stories, and those of other oft-overlooked women in medicine, will be explored here.

-Philadelphia: Medicine's Pioneering City-

The city of Philadelphia is home to two of the nation's oldest medical schools and several of its most accomplished hospitals. Since its founding, the city has fostered an atmosphere of scientific progress. Medically, it is a city of firsts; the nation's first hospital, first medical school, first medical professional organization. In addition, it has been the site many epidemics that furthered the medical community's knowledge of diseases such as yellow fever and Legionnaire's disease. Philadelphia has been home to many famous physicians, including Benjamin Rush, Silas Weir Mitchell, and Nobel Prize winner Michael Brown. Philadelphia's long, distinguished history, diverse population and openminded mindset have laid the groundwork for one of the most advanced cities in the nation, the medical history and contributions of which will be explored here.