

ABSTRACTS

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MOST FREQUENT AMBULATORY CARE SENSITIVE CONDITIONS AMONG PATIENTS ADMITTED TO THE USA MEDICAL CENTER, 2004–2007

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Ambulatory Care Sensitive Conditions (ACSC) are conditions in which potentially fatal diseases and hospitalization can be prevented by seeking primary care. Factors include low income, minority race, poor access to primary care services, insurance coverage, lack of regular source of care, and poor education. Hospitalizations for ACSC can be used to evaluate the status of primary healthcare of the community by indicating areas with poor access to healthcare. We studied the four year rates of hospitalization for ACSC at the University of South Alabama Medical Center (USAMC), a tertiary care teaching hospital in Mobile, Alabama. Data collected included demographics, risk of mortality, ICU days, length of hospital stay, and charges. We hypothesized that there would be little change in ACSC diagnoses throughout the four year period. Discharge records from the USAMC were sorted and analyzed using the Statistical Analysis Software (SAS) program. Results show that the most frequent ACSC are Congestive Heart Failure (CHF) (29%), Bacterial Pneumonia (25%) and Uncontrolled Diabetes (18%). The majority of patients admitted for these ACSC reside in the immediate service area of USAMC, a predominantly African American, low income, medically underserved area. Though results show that there was a slight decrease in admissions for CHF and diabetes, a slight increase in admissions for pneumonia was observed over the four year study period.

EFFECT OF GLUCOSE AND INSULIN ON INSULIN RESISTANCE IN A CULTURED ADIPOCYTIC MODEL

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Insulin resistance has been strongly correlated with the onset of Type 2 diabetes, identifiable by desensitization to insulin (insulin resistance) as well as concomitant elevation of blood glucose levels. The molecular mechanisms behind the phenomenon of insulin resistance are largely unknown. This study examines the hypothesis that the diabetic state, over a longer period of time, may negatively impact insulin sensitivity. The investigation implements 3T3-L1 adipocytes to systematically gauge the impact of varying levels of glucose as well as elevated insulin on insulin sensitivity of adipocytes. 3T3-L1 cells were cultured and differentiated into adipocytes; they were then electroporated with GLUT4 reporter DNA to monitor insulin action. Cells were challenged with physiologically normal and diabetic levels of glucose with insulin both absent and present over varying periods of time. The acute action of insulin was assayed by monitoring the behavior of the GLUT4 glucose transporter, a natural target of insulin action, using epifluorescent microscopy. The assay, executed at time points ranging from day five to day ten post differentiation, yielded no concrete link between the hyperglycemic state and insulin action.

ISOLATION AND CHARACTERIZATION OF MAMMARY STEM CELLS FROM NORMAL AND H-RAS-TRANSFORMED HUMAN BREAST EPITHELIAL CELLS

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Breast cancer initiating cells have been defined as a subpopulation of cancer cells that show the capacity of self-renewal and ability to propagate in distant sites. Cancer initiating cells from tumor samples and breast cancer cell lines can be propagated as mammospheres that initiate tumors in cleared mammary fat pads when implanted in immunodeficient mice. Ras proteins and its downstream targets have been implicated in almost every aspect of breast cancer progression. Farnesylation of Ras protein to the membrane is one key step required for the propagation of oncogenic signals in breast cancer. While Ras inhibitors like farnesyl transferase inhibitors (FTIs) are effective in experimental settings, their clinical effectiveness has been questioned due to cancer recurrence and eventual expansion of metastatic disease. Breast cancer biopsies from recurrent tissues show high rates of Ras pathway activation, suggesting a potential link between H-Ras activation and biology of cancer initiating cells. We hypothesized that H-Ras activation in mammary cells can activate several features associated with mammary cancer cells. Mammospheres were isolated and characterized from normal and H-Ras-transformed human mammary epithelial cells and a comparative analysis of gene-expression profile was performed using cancer pathway focused PCR Array. Several cancer-related genes involved in cell survival, angiogenesis as well as in invasion and metastasis were upregulated in H-Ras mammospheres. One of the highlights of our findings was downregulation of pro-apoptotic Bax and upregulation of anti-apoptotic Bcl-2 in H-Ras-transformed normal breast epithelial cells.

VISFATIN EXPRESSION IN LEAN WOMEN VS. PREGNANT WOMEN

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Visfatin is a 52 kDa adipokine that is shown to mimic the actions of insulin. Adipokines are a group of fat cell proteins. We examined visfatin expression in a number of pregnant women to see if pregnancy influenced visfatin gene expression. We also examined visfatin in lean women as well as obese women. Fat samples were collected during delivery in pregnant women, and at hysterectomy in lean women. Our aim was to determine if visfatin is expressed more in pregnant women rather than lean women. Visfatin gene expression was measured by PCR (which measures mRNA) and we found that the amount of visfatin from pregnant women was eight-fold higher than the amount of visfatin of lean women. A western blot was used to measure the amount of visfatin protein. The data from the western blot showed that an increase in the gene expression was followed by a large increase in visfatin protein in the omental fat of pregnant women. These data suggest that visfatin may function in the regulation of maternal/fetal glucose metabolism and/or distribution.

THE MOLECULAR MECHANISM OF RESVERATROL'S ANTI-CANCER PROPERTIES

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Resveratrol is a natural compound with antioxidant properties found in grapes. Resveratrol is known to be effective as a treatment for diabetes and cardiovascular diseases. It is also known to have anti-cancer properties, therefore it has a lot of potential in cancer treatment. The cellular and molecular mechanisms by which resveratrol fights cancer are still unknown and were the subject of our research. The question our research will attempt to answer is: What is the molecular mechanism by which resveratrol inhibits the growth of cancer cells? Our hypothesis was that resveratrol can fight cancer by inhibiting properties that cancer cells use to form tumors and to spread to other tissue and organs, such as uncontrolled cellular division, migration, loss of cell adhesion and evasion of apoptosis. In this research, we used several cancer cell lines to determine the effect of resveratrol in various properties of cancer cells. For this purpose, we used the human colon adenocarcinoma HT29 cell line, the breast carcinoma MCF-7 cell line, and the bone tumor Saos-2 cell line. Normal osteoblasts (MC3T3-E1) were also used to determine if resveratrol has any effects on normal cells. These cells were cultured in the presence of various concentrations of resveratrol as well as in DMSO control vehicle and their behavior was directly observed under a microscope. Cellular behaviors that will be studied are: cell proliferation, cell viability and cell migration. Proteins were also extracted from treated and control cells and used in a technique called western immunoblot in order to determine if resveratrol is capable of affecting the levels of several proteins that are important regulators of cellular processes such as proliferation, cell adhesion, apoptosis, migration, etc. Some of the proteins we studied are the tumor suppressors p53 and retinoblastoma, and the cell adhesion molecule β -catenin.

PROBING THE PROTEIN EXPRESSIONS OF Ca^{2+} CHANNEL $\alpha 1E$ SUBUNIT IN THE INFERIOR COLLICULUS OF THE GENETICALLY EPILEPSY-PRONE RAT (GEPR)

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The inferior colliculus (IC) is thought to be critical in the initiation of auditory-evoked seizures (audiogenic reflex seizures) in the genetically epilepsy-prone rat, (GEPR), a model of genetic epilepsy in humans. However, the molecular mechanisms underlying seizure susceptibility in the GEPR is not yet fully understood. Voltage-gated Ca^{2+} channels play an important role in neuronal hyperexcitability that leads to seizures. Previous studies showed that the current density of L-,N-,R- but not P- and Q- type of the high threshold voltage-gated Ca^{2+} channels was significantly increased in IC neurons of the GEPR. Subsequent molecular studies showed that the levels of protein expression of Ca^{2+} channel $\alpha 1A$, $\alpha 1B$, $\alpha 1C$, $\alpha 1D$ subunits associated with P-,Q-, N-, and L-type Ca^{2+} channels were not altered in IC neurons of the GEPR. Here we examined whether the protein expression of Ca^{2+} channel $\alpha 1E$ subunit (associated with R-type current) was enhanced in IC neurons of the GEPR using Western blot analysis. These studies were to determine whether upregulation of protein expression of Ca^{2+} channel $\alpha 1E$ subunit was the molecular correlate for the enhancement of R-type Ca^{2+} channel current in IC neurons of the GEPR. Unfortunately, for technical reasons the Western blots did not result in anything that would allow us to quantify protein expression of Ca^{2+} channel $\alpha 1E$ subunit.

SELECTIVE NOTCH 1 INHIBITION IN LEUKEMIA STEM CELLS

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Self-renewal is the ability to regenerate. Regenerating tissues rely on self-renewing stem cells for survival. An important stem cell self-renewal gene is Notch 1. Notably, 50% of patients with T-cell acute lymphoblastic leukemia (T-ALL) harbor a Notch 1 activating mutation that may be active in leukemia stem cells (LSC) responsible for therapeutic resistance. These patients have higher relapse rates. We hypothesized that unlike normal hematopoietic stem cells (HSC), LSC were reliant on Notch 1 to self-renew whereas normal HSC were not. Our studies tested this hypothesis in three ways using a novel gamma secretase inhibitor (GSI; PF-4014) to block Notch 1 activation. First, we performed Q-PCR analysis of Notch 1 expression in normal HSC and LSC before and after treatment with a GSI after a 3-day, 5-day and 1-week culture period. Using this method, we saw that normal HSC were resistant to the Notch 1 inhibiting drug (PF-4014; 100 nM) whereas LSC were not. Also, immunocompromised mice have been transplanted with LSC, and will be treated with this GSI to determine if it inhibits LSC self-renewal more than normal HSC self-renewal. Using this GSI, we have established that a therapeutic index exists between LSC and normal HSC.

DIABETES, HIGH CHOLESTEROL, AND HYPERTENSION: THE CAUSE OF MICROALBUMINURIA

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Mentor: Chen Yen-Wang, PhD

Microalbuminuria is the earliest sign of diabetic nephropathy although it also increases the risk of cardiovascular diseases in both diabetic and non-diabetics. Microalbuminuria may be induced by uncontrolled hypertension or diabetes. The purpose of this study was to associate microalbuminuria with factors such as blood pressure, fasting blood glucose, age, sex, and cardiac index such as total cholesterol level, triglyceride, etc. We focused on Filipinos since the population is known to have a higher prevalence of diabetes and hypertension.

To determine whether diabetes, hypertension, or cardiac index had any connection with the level of microalbumin, we tested adult Filipinos who had one or more of the factors. We measured cholesterol level, blood pressure, height, weight, and microalbumin level. The participants' medications were recorded to examine if their current medications had any effects on the levels of microalbuminuria.

ANALYZING THE PROTEIN EXPRESSION OF Ca^{2+} ACTIVATED POTASSIUM CHANNEL (SK1) IN INFERIOR COLLICULUS NEURON OF THE GENETICALLY EPILEPSY PRONE RAT (GEPR)

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Defective genes encoding for ion channels including Ca^{2+} channels have been involved in the physiopathology of human epilepsy. Interestingly, increases in the current density of high threshold voltage-activated Ca^{2+} channels have been found in inferior colliculus (IC) neurons of the genetically epilepsy prone rat (GEPR), a relevant model of inherited generalized epilepsy. Ca^{2+} current is known to have activated potassium (K^+) current that initiates repolarization of action potential and generates hyperpolarization. Such mechanism may represent an intrinsic inhibitory mechanism that would maintain normal physiological excitability. Preliminary studies have shown that the current density of Ca^{2+} -activated K^+ channels was significantly decreased in IC neurons of the GEPR. Ca^{2+} -activated K^+ channels comprise large (BK) conductance and small (SK) conductance Ca^{2+} -activated K^+ channels. There are three subtypes of Ca^{2+} -activated K^+ channels including SK1, SK2, and SK3. Here, we determined whether the downregulation of SK1 subtype of the small conductance Ca^{2+} -activated K^+ channels, account, in part, for the decrease of the Ca^{2+} -activated K^+ current density in IC neurons of the GEPR. Western Blot analysis showed that the levels of protein expression of SK1, subtype of Ca^{2+} -activated K^+ channel was significantly reduced in IC neurons of the GEPR, suggesting that these channels may play an important role in inherited seizure susceptibility in GEPR.

LIPOSOME MEDIATED TRANSFER OF MITOCHONDRIA HARBORING FOREIGN MITOCHONDRIAL DNA INTO CULTURED FIBROBLASTS

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Mitochondria are organelles that play a key role in cellular energy metabolism through oxidative phosphorylation (OXPHOS) and the production of ATP. Mutations in mitochondrial DNA (mtDNA) that negatively affect the function of oxidative phosphorylation enzyme subunits or mitochondrial protein translational mechanisms can result in severe impairment in tissues with high energy requirements (central nervous system, heart, skeletal muscle, etc.). Our ultimate goal was to produce mouse models of human mitochondrial DNA disease. For last year's 2007 NIH STEP-UP research project, a hypothesis was tested that isolated, viable mitochondria can be transferred into cultured mouse fibroblasts using synthetic liposomes. Further analysis, however, showed that the foreign mitochondria were eliminated after several passages. The current research focus tested the hypothesis that cultured fibroblasts can stably incorporate transferred mitochondria after depletion of endogenous *Mus musculus domesticus* mtDNA. Using rhodamine-6-G (R-6-G) and the liposome-mediated transfer technique previously described, mitochondria from livers of xenomitochondrial D7 lineage mice harboring *Mus terricolor* mtDNA were transferred into cultured fibroblasts. After passaging the transfected cells, PCR was employed using primers specific to *M. m. domesticus* and *M. terricolor* mtDNA to test for the presence of stable cell lines harboring *M. terricolor* mtDNA. Proof that viable mitochondria can be stably transferred into cultured cells using synthetic liposomes will pave the way for adapting this technique to transfer genetically engineered mitochondria into embryonic stem cells for development of live heteroplasmic mice that will serve as models of human mtDNA diseases.

EFFECT OF PIOGLITAZONE ON THE CONTROL OF OXIDATIVE STRESS AND RENAL FIBROSIS IN A RAT MODEL OF TYPE 2 DIABETES

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Thiazolidinediones, such as pioglitazone, are insulin-sensitizing agents widely used to treat patients with type 2 diabetes mellitus. Thiazolidinediones are peroxisome proliferator-activated receptor- γ (PPAR γ) agonists. Previous results have shown that PPAR γ agonists may have a protective effect on renal function, however, its effect on renal oxidative stress and fibrosis have not been fully examined in type 2 diabetic nephropathy. In this study, we aimed to evaluate whether a PPAR γ agonist, pioglitazone (PGT), protects from renal fibrosis and oxidative stress. The young, male, and obese Zucker fa/fa rats, rat models of insulin resistance, received either regular chow (OZR) or chow with PGT at a low dose of 0.6 mg/kg for 5 months (OZR-PGT). The young, male, and lean Zucker fa/fa rats (LZR) animals were used as a lean control group. Animals were sacrificed, glucose levels were measured. The kidneys were removed, fixed in formalin, and processed for paraffin embedded sections. Masson trichrome staining was done to determine the development of fibrosis and nitrotyrosine was determined by immunohistochemistry as a marker of oxidative stress. No fibrosis was present in the LZR group while the OZR showed interstitial fibrosis accompanied by glomerulosclerosis and macrophage infiltration. These effects were significantly reduced in the OZR-PGT group. Nitrotyrosine expression was highly increased in the OZR and was significantly reduced in the OZR-PGT, similar to the levels found in the LZR group. Pioglitazone treatment may improve the renal function by decreasing inflammation and fibrosis due to a reduction of oxidative stress and inhibition of nitric oxide production.
