

PULMONARY MICROVASCULAR ENDOTHELIAL CELLS EXPRESSES AN ENDOGENOUS BICARBONATE-SENSITIVE ADENYLYL CYCLASE THAT CONTROLS ENDOTHELIAL BARRIER INTEGRITY

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Transmembrane adenylyl cyclases generate a highly compartmentalized subplasma membrane cAMP pool, which dynamically regulates pulmonary endothelial barrier critical to maintain gas exchange. However, cAMP signaling is highly compartmentalized. Indeed, in contrast to transmembrane adenylyl cyclase activity, bacteria inject soluble adenylyl cyclases into endothelial cells generating a cytosolic cAMP pool that disrupts the barrier a major contribution toward the progression of acute lung injury and ARDS. Since bacterial adenylyl cyclases increase endothelial permeability by increasing cytosolic cAMP, we were interested to determine whether endothelial cells express the recently described mammalian soluble adenylyl cyclase. This mammalian soluble adenylyl cyclase lacks transmembrane domains and is expressed in the cytosol. Further, is insensitive to heterotrimeric Gprotein modulation, and is uniquely activated by bicarbonate and calcium to generate cAMP. We sought to determine whether this bicarbonate-sensitive adenylyl cyclase is expressed in pulmonary endothelial cells, and whether the enzyme contributes to intracellular cAMP concentrations. Using Western analysis we demonstrated the endogenous expression of this bicarbonate-sensitive soluble adenylyl cyclase in pulmonary microvascular and pulmonary artery endothelial cells. Following 10 minutes in bicarbonate free conditions, we demonstrate that adding back increasing concentrations of bicarbonate (0-100 mM) in pulmonary microvascular endothelial cells leads to increases in cAMP levels. This cAMP pool was regulated by phosphodiesterase 4 activity, such that the phosphodiesterase 4 inhibitor, rolipram, increased the bicarbonate stimulated cAMP pool. Using electrical cell impedance sensing (ECIS), we demonstrate bicarbonate dose-dependently decreases resistance across the monolayer. These data reveal bicarbonate-stimulation of AC10 activity synthesizes a rolipram-sensitive cAMP pool, which increases permeability across the pulmonary microvascular endothelial cell monolayer.

DYSFUNCTION OF REGULATORY T CELLS IN RASGRP1-DEFICIENT MOUSE MODEL OF SYSTEMIC LUPUS ERYTHEMATOSUS

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Ras guanine nucleotide releasing protein 1 (RasGRP1) regulates Ras activity and thereby mediates signals downstream of T cell receptor activation. RasGRP1-deficient mice display impaired T cell development and develop spontaneous autoimmune disease similar to systemic lupus erythematosus (SLE). The deficiency of RasGRP1 is also observed in a subset of SLE patients. To understand the role of RasGRP1-deficiency in development of SLE, we characterized a major regulator of peripheral tolerance - regulatory T cells (Treg) in RasGRP1-deficient mice. We found that in the absence of RasGRP1, though Treg development in thymus is impaired, the frequency of Treg within the peripheral CD4+ T cell compartment is increased. Despite this increased frequency, RasGRP1-deficient Treg are unable to suppress activated effector T cell (Teff) production of interferon-gamma. Treg have the propensity to differentiate into Th17 cells, which produce Interleukin-17 (IL-17) and promote inflammatory responses. In vitro culture of wild type Teff with RasGRP1-deficient Treg, in the presence of anti-CD3 stimulation, resulted in increased levels of IL-17 compared to similar cultures with wild type Treg. We speculate that RasGRP1-deficient Treg are more predisposed to become Th17 cells. Therefore, RasGRP1-deficiency impairs the development and function of Treg, potentially contributing to development of SLE.

CONDITIONAL EXPRESSION OF A PROKARYOTIC GENE IN LUNG ENDOTHELIUM

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Rationale: *Pseudomonas aeruginosa* is a leading cause of ventilator-associated pneumonia with mortality that exceeds 30 percent. Type III secretion system (T3SS) is a major virulence factor for *P. aeruginosa*. The relative risk of mortality is 6-8 fold in patients infected with *P. aeruginosa* with a functional T3SS. Four exotoxins are secreted through *P. aeruginosa* T3SS: ExoS, ExoT, ExoU, and ExoY. ExoY is an adenylate cyclase that increases cytosolic cAMP, activates protein kinase A, and phosphorylates non-neuronal tau leading to microtubule disassembly. These events result in inter-endothelial gap formation and increased microvascular lung filtration, both phenomena hallmark of exudative pulmonary edema. Currently, the study of ExoY inside eukaryotic cells relies solely on the bacterial delivery of the toxin through T3SS, limiting the thorough examination of enzyme's function and dynamics. We hypothesized that the stable and conditional expression of ExoY in endothelial cells will enable us to scrutinize its function and dynamics inside the eukaryotic cell. **Methods:** First, rat pulmonary microvascular endothelial cells (PMVEC) cultured at 37°C in the atmosphere of 5% CO₂ were infected with retrovirus containing supernatants delivering the pTet-On advance system (Clontech) followed by selection with blasticidin for 5 days. Second, blasticidin-resistant PMVEC were re-infected with a lentiviral construct containing the rat codon-optimized, ExoY gene with a hemagglutinin (HA) tag in the C-terminus. This second infection was followed by selection with puromycin. Third, double-lentiviral infected cells were treated with doxycycline in a dose and time-dependent manner. Finally, immunoblot against HA using the established protocols was used to detect ExoY expression. **Results:** Double lentiviral- infected cells conditionally expressed ExoY-HA in response to doxycycline in a dose and time-dependent manner. ExoY-HA expression was correlated with tau phosphorylation. **Conclusion:** Endothelial cells can conditionally express codon optimized prokaryotic genes. The correlation of ExoY-HA expression with tau phosphorylation suggests the conditionally expressed ExoY may be a functional protein. Supported by HL-60024, HL-66299, and HL-076125.

B7H3 INDUCES CHEMOTHERAPEUTIC RESISTANCE BY ACTIVATING JAK2/STAT3-MEDIATED PROSURVIVAL PATHWAYS IN HUMAN BREAST CANCER

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B7-H3 was previously known as an immunoregulatory protein with either stimulatory or inhibitory effects on the activation of T-cells. However, the correlation between high expression of B7-H3 and poor prognosis has been reported in many human tumor types. Our previous results also indicated a direct association between B7-H3 expression and cancer metastasis. Since metastasis and chemoresistance are closely related processes, and Taxol is one of the most effective chemotherapeutic drugs in the treatment of breast cancer, we focused on the putative role of B7-H3 with respect to Taxol sensitivity in metastatic breast cancer cell lines MDA-MB-231 and 435 and the underlying mechanisms. Our results showed that silencing of B7-H3 increased the sensitivity to Taxol in MDA231 and MDA435 cells. Further study confirmed that B7-H3 silencing sensitized breast cancer cells to Taxol-induced apoptosis. Moreover, we investigated the role of STAT3 in B7-H3 mediated effect on Taxol resistance. Our results showed that the level of STAT3 Tyr705 phosphorylation and its downstream target proteins such as Mcl-1 and Survivin were sharply decreased in B7-H3 knockdown cells. The phosphorylation level of JAK2, which was a well-known upstream component of STAT3 signaling, was also significantly decreased in B7-H3 knockdown cells. Treatment with JAK2 selective inhibitor AG490 almost completely inhibited the STAT3 Tyr705 phosphorylation in both B7-H3 expressing- and knockdown cells. Furthermore, the *in vivo* experiments showed that when treated with Taxol, the tumor volume in the nude mice injected with MDA435 B7-H3 knockdown cells reduced to a much greater extent than in mice injected with MDA435 control cells. Taken together, our data demonstrate that B7-H3 induces antiapoptosis and Taxol resistance at least partially through JAK2/STAT3 pathway in breast cancer cells. The novel findings of this study have important implication for the design of new approaches to target B7-H3 overexpressing breast cancers.

ELUCIDATION OF THE INTERACTIONS BETWEEN THE HOST AUTOPHAGIC RESPONSE AND THE INTRACELLULAR PATHOGEN, RICKETTSIA PROWAZEKII

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Rickettsia prowazekii is the etiologic agent of epidemic typhus. The obligate intracellular lifestyle of *R. prowazekii* necessitates that it have a mechanism to evade degradation by autophagy, a mediator of innate-immunity within the host cytoplasm. Autophagy, a defense mechanism induced by innate immune effectors, nutrient deprivation, and/or intracellular damage, poses a serious threat to *R. prowazekii*, which is known to promote these triggering events. How *R. prowazekii* evades the autophagic mechanisms of the host cell is critical to our understanding of rickettsial obligate intracytoplasmic parasitism. As a first step in characterizing rickettsial interaction with the autophagic pathway, we examined lipidation of the autophagic marker protein LC3, an essential event for the progression of autophagy within the host cell. Lipidation of the LC3 protein can be distinguished by its altered electrophoretic properties via western blot or by its altered cellular distribution via immunofluorescent microscopy. A significant increase in the lipidated form of LC3 was detected following infection by both methods. By 24-48 hours post-infection, the amount of lipidated LC3 detected in rickettsiae-infected cells was comparable to that of the rapamycin-treated positive control. These data support the hypothesis that *R. prowazekii* is recognized by the autophagy-inducing machinery of the cell but is capable of evading this induced system. Current studies include analysis of the effect of inhibition of bacterial protein synthesis upon the ability of *R. prowazekii* to avoid inclusion in the autophagolysosome, the response of other autophagy associated proteins to *R. prowazekii* infection, and the effect of the functional deletion of autophagy upon the success of the rickettsial infection. Completion of these studies will provide an understanding of the efforts of this critical host-immune response to clear infection and the ability of this pathogen to overcome those efforts.

CELL-TARGETED DRUG DELIVERY FOR THE TREATMENT OF PULMONARY ARTERIAL HYPERTENSION

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Rationale:

A major limitation in the systemic treatment of pulmonary arterial hypertension with pharmacological agents is the lack of pulmonary vascular selectivity. A recent study has identified a peptide, CARSKNKDC (CAR), that specifically recognizes the vasculature of regenerating wound tissue. We hypothesized that CAR would selectively bind to injured hypertensive pulmonary artery endothelial cells, and that CAR-fasudil (a Rho kinase inhibitor) mixture would cause potent selective pulmonary vasodilation in a rat model of severe pulmonary arterial hypertension.

Methods and Results:

Rats were injected with a vascular endothelial growth factor receptor blocker, SU5416, and exposed to hypoxia for 3 weeks. They were then returned to normoxia for an additional 2 weeks. Five weeks after SU5416 injection, the rats had severe pulmonary arterial hypertension and were subjected to various examinations. Two hours after intravenous administration of fluorescein labeled CAR, immunohistochemical analysis of lung and systemic vascular tissues with showed specific distribution of the peptide to the intima of hypertensive pulmonary arteries, except for minor distribution to aorta and ventricles. CAR caused a left shift of the concentration-relaxation curve for fasudil against phenylephrine-induced contraction in isolated hypertensive pulmonary artery rings, but a right shift in the response in mesenteric artery rings. Similarly, in catheterized pulmonary arterial hypertensive rats, addition of CAR enhanced the acute pulmonary vasodilation to fasudil but reduced its systemic vasodilatory effect.

Conclusion:

The peptide CAR may be useful for pulmonary artery selective drug delivery and vasodilation in pulmonary arterial hypertension.

DEFINING TUMOR mRNA EXPRESSION SIGNATURES PREDICTING RESPONSE OF LUNG CANCERS TO RADIATION THERAPY

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Approximately 50% of all patients with lung cancer will need to receive radiation therapy (XRT) sometime during their course and some tumors will respond to XRT while others will not. Our goal is to develop tumor mRNA and protein expression signatures predicting response to XRT before a patient is treated and to integrate this with signatures predicting response to chemotherapy (CTX) to ultimately facilitate selection of the best CTX regimen, XRT, and combined CTX + XRT for each individual patient ("personalized medicine"). We determined the XRT response phenotypes for a panel (up to 50) non-small cell lung cancers (NSCLCs) and small cell lung cancers (up to 30) (SCLCs) by performing radiation survival curves using clonogenic assays to determine the surviving fraction at 2 Gy (SF2). These values are integrated with our genome wide mRNA expression profiles (Affymetrix and Illumina arrays) on these same tumor lines with biostatistical approaches to develop mRNA expression signatures associated with sensitivity and resistance to radiation. Available SF2 values in NSCLCs are distributed over a range from 0.18 to 0.67. We also determined the repair kinetics of XRT induced DNA double strand breaks (DSBs) monitored by the disappearance of γ -H2AX and 53BP1 foci (determined by immunofluorescent staining with specific antibodies and scoring fluorescent foci). A striking correlation was found with increased radiosensitivity for NSCLC containing an EGFR oncogenic mutation and these tumor lines exhibited a deficiency in DNA DSB repair (see also Das *Can Res* 67:5267, 2007). Amundson et al. (*Can Res*, 68:415, 2008) recently reported a mRNA signature predictive of response to XRT in the NCI-60 panel of tumor cell lines. However, when we applied this signature to our mRNA array data we observed that their signature predicted that SCLC would be resistant to XRT which our previous studies (Carmichael *Eur J Can Clin Oncol* 25:527, 1989) and clinical experience disagree with their findings. In conclusion: 1. NSCLCs display strikingly different radiation response phenotypes reflected in the corresponding SF2 values that will allow us to develop mRNA signatures predicting response to XRT; 2. mRNA signatures predictive of XRT response developed by other investigators in tumors other than lung cancer do not predict for lung cancer XRT response.

UPREGULATION OF LACTATE DEHYDROGENASE A BY ERBB2 THROUGH HEAT SHOCK FACTOR 1 PROMOTES BREAST CANCER CELL GLYCOLYSIS

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ErbB2 has been shown to activate signaling molecules that may regulate glucose metabolism. However, there is no evidence reported to directly link ErbB2 to glycolysis and the mechanism underlying ErbB2-enhanced glycolysis is poorly understood. In this study, we investigated the role and mechanism of ErbB2 in regulating glycolysis. We found that ErbB2-overexpressing cells possessed significantly higher level of glycolysis when compared to ErbB2-low expressing cells, and the downregulation of ErbB2 markedly decreased glycolysis. Overexpression of ErbB2 increased the expression of glycolysis-regulating molecules lactate dehydrogenase A (LDH-A) and heat shock factor 1 (HSF1). ErbB2 activated HSF1, indicated by the increased HSF1 trimer formation, and promoted HSF1 protein synthesis. HSF1 bound to LDH-A promoter. Moreover, the downregulation of HSF1 reduced the expression of LDH-A and subsequently decreased cancer cell glycolysis. Taken together, this study demonstrates that in human breast cancer cells, ErbB2 promotes glycolysis at least partially through HSF1-mediated upregulation of LDH-A. This pathway may play a major role in regulating glucose metabolism in breast cancer cells. These novel findings have important implications for the design of new approaches to target ErbB2-overexpressing breast cancers.

BRAIN ACIDOSIS, ACID-SENSING ION CHANNELS, AND NEURON FUNCTION.

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Brain acidosis occurs in numerous neurological diseases including epilepsy, stroke, traumatic brain injury, mitochondrial dysfunction, and various neurodegenerative diseases. However, it is unclear how acidosis regulates brain function. Currently, the lab focuses on two areas. First, since dendritic spines are important in controlling neuron function, we are investigating the role of acidosis on dendritic spines. Specifically, we are asking how acidosis regulates spines in disease paradigms like recurrent seizures or ischemia/hypoxia. Second, we are studying the trafficking mechanisms of acid-sensing ion channels (ASICs), the major class of proton receptors in the brain. Results from these studies will help us to understand the role of protons in neuron function better.

DEHYDROEPIANDOSTERONE ATTENUATES ESTABLISHED SEVERE PULMONARY ARTERIAL HYPERTENSION IN RATS.

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Rationale: There is an urgent need for better therapy of severe pulmonary arterial hypertension (PAH). We and others have shown that the naturally occurring steroid hormone dehydroepiandrosterone (DHEA) is effective in treating rodent models of PAH, including chronic hypoxia-exposed rats and mice and monocrotaline-injected pneumonectomized rats. Recent studies suggest that the VEGF receptor blocker, SU5416, plus hypoxia in rats lead to a severe form of PAH that closely resembles the human disorder both histopathologically and hemodynamically. It is also apparent that this model is resistant to various pharmacologic agents that have been reported effective in the other models. In this study, therefore, we examined whether DHEA would reverse the severe PAH in SU5416/hypoxia-exposed rats.

Methods: Male Sprague-Dawley rats were assigned to three groups as follows: (A) Normal controls, (B) Rats receiving a single subcutaneous injection of SU5416 (20 mg/kg) on day one and then exposure to 3 weeks of hypoxia (10% O₂) followed by re-exposure to normoxia for 5 additional weeks, and (C) The same as group (B) except for receiving 1% DHEA-containing food from week three to week eight (total of 5 weeks). At the end of the eighth week, all rats were catheterized and hemodynamic measurements were performed.

Results: Eight weeks after SU5416 injection, all group (B) rats developed severe PAH, as reflected by marked increases in right ventricular systolic pressure (RVSP), total pulmonary resistance index (TPRI), and, right ventricle/left ventricle + septum weight ratio (RV/LV+S), in addition to a significant reduction of cardiac index (CI). Five-week DHEA treatment significantly attenuated the markedly elevated RVSP, RV/LV+S, and TPRI. Although DHEA treatment did not normalize these parameters, it did restore the low CI to a normal level.

Conclusion: These results indicate that chronic (5-week) DHEA treatment significantly slowed the progression of severe PAH in SU5416/hypoxia/normoxia-exposed rats. The impressive effect of DHEA on CI raises a possibility that its beneficial effects may include direct improvement of cardiac function.

TYROSINE KINASE INHIBITORS ARE POTENT PULMONARY VASODILATORS IN RATS

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Background: The pathogenesis of pulmonary arterial hypertension (PAH) includes vascular remodeling and vasoconstriction. Tyrosine kinase inhibitors (TKIs) have recently been reported promising for the treatment of PAH. Their therapeutic effects are postulated to be due to inhibition of cell growth-related kinases, such as platelet-derived growth factor receptor, and subsequent attenuation of vascular remodeling. Nothing, however, has been considered regarding their potential vasodilatory activity. This study examined whether TKIs might acutely induce pulmonary vasodilation.

Methods and Results: In isolated extra-lobar pulmonary arteries from normal adult rats, imatinib, sorafenib, and nilotinib concentration-dependently reversed U46619-induced contractions. Nitro-L-arginine did not reduce their relaxant potency. Imatinib (30 μ M) reduced phosphorylation of MYPT-1 in U46619-treated arterial rings. Imatinib (50 mg/kg, ip) acutely and preferentially lowered high right ventricular systolic pressure (from 111 ± 9 to 92 ± 6 mmHg) compared to systemic arterial pressure (from 145 ± 8 to 138 ± 11 mmHg) in SUGEN 5416 (VEGF receptor inhibitor; 20 mg/kg, sc) plus chronic hypoxia-exposed severe pulmonary hypertensive rats.

Conclusion: TKIs have potent, Ca^{2+} desensitization-dependent pulmonary vasodilator activity, which could be involved in their long-term beneficial effect against PAH.

ASSESSMENT OF PULMONARY HYPERTENSION IN PATIENTS WITH SICKLE CELL DISEASE WITH TRICUSPID REGURGITANT JET VELOCITY OF ≥ 2.5 M/S ON ROUTINE ECHOCARDIOGRAM.

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Objective: To compare the diagnostic utility of transthoracic echocardiography and a tricuspid regurgitant jet velocity (TRJV) of ≥ 2.5 m/s to right heart catheterization (RHC) in defining Pulmonary Hypertension (PHT) in adult patients with sickle cell disease (SCD).

Methods: This is a retrospective chart review of adult patients diagnosed with SCD (HbSS, HbSC, and HbS β -thalassemia) at the University of South Alabama Comprehensive Sickle Cell Center (USACSCC) who have had at least two screening transthoracic echocardiograms with a TRJV of ≥ 2.5 m/sec and RHC. A TRJV of ≥ 2.5 m/sec was used to define pulmonary hypertension with transthoracic echocardiography. At right heart catheterization, pulmonary arterial hypertension (PAH) was defined as a mean pulmonary artery pressure (mPAP) of ≥ 25 mm Hg and PCWP of ≤ 15 , and pulmonary venous hypertension (PVH) was defined as a mPAP of ≥ 25 mmHg and a PCWP of > 15 mmHg. RHC and transthoracic echocardiography findings were compared. In addition, correlations between the TLC and DLCO were assessed relative to confirmed pulmonary hypertension at RHC.

Results: Twenty five (12 males and 13 females) patients with SCD met the inclusion criteria. The mean age of the study cohort was 35.16 ± 11.33 years. Twenty two patients had HbSS, 1 HbSC and 2 HbS β -thalassemia. The mean TRJV was 2.84 ± 0.46 m/s (range 2.5 – 4.6 m/s). Nine of the 25 (36%) patients had a mPAP ≥ 25 mmHg. Out of these 9 patients, 3 (33%) had PAH and 6 (66%) had PVH. When compared with patients without pulmonary hypertension, patients with pulmonary hypertension had higher TRJV (3.1 ± 0.68 vs 2.70 ± 0.16 m/s, $p=0.031$), and higher cardiac output (10.4 ± 2.7 vs 7.81 ± 1.85 L/m, $p = 0.012$). The cardiac outputs were not significantly different between patients with PVH pulmonary hypertension and those with a normal mPAP, 50% had a reduced DLCO.

Conclusion: Pulmonary hypertension was confirmed in RHC in 36 % of patients found to have TRJV of ≥ 2.5 m/s. Of those with confirmed pulmonary hypertension, 1/3 had PAH, and 2/3 had PVH. Restrictive lung physiology is common in the presence and absence of pulmonary hypertension in SCD adult patients. Decreased DLCO does not appear to be a predictor whether an adult SCD patient will have pulmonary hypertension.

Clinical implication: The prevalence of pulmonary hypertension in SCD patients is significant. Transthoracic echocardiography is a good screening test for pulmonary hypertension in SCD patients, but RHC remains the standard for diagnosis of pulmonary hypertension. Pulmonary hypertension should be confirmed by RHC before implementation of medical management in adult patients with SCD.

SHORT NON-CODING RNA AND THE UNFOLDED PROTEIN TRANSCRIPTION FACTOR XBP1

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Short non-coding RNAs (sncRNAs) are functional RNA molecules that have limited or no protein-coding potential. In higher eukaryotes, these sncRNAs serve various biological roles, which include guiding chemical modifications of other RNAs, repressing translation of target mRNAs, and acting in the RNAi pathway to interfere with expression of specific genes. Thus, there is much interest in delineating how sncRNAs regulate cellular processes and signaling responses. The unfolded protein response (UPR) is mediated by intracellular signaling pathways activated by the accumulation of unfolded proteins in the endoplasmic reticulum (ER). The UPR plays important roles in the proper development and function of specialized secretory cells such as antibody-secreting B-cells. One branch of the UPR is initiated by an ER bound endoribonuclease called IRE1. Upon activation, IRE1 unconventionally splices a 26 nucleotide (nt) intron from the mRNA encoding X-box Binding Protein 1 (XBP1), resulting in the translation of XBP1(S), a transcription factor that up-regulates expression of many secretory pathway genes and mediates expansion of the ER in specialized secretory cells. Hence, B-cells lacking XBP1 are defective in antibody secretion. In addition, XBP1 is required for embryonic development and has been implicated in promoting survival of cells exposed to physiologic stresses like hypoxia that disrupt normal ER function. Whether the expression or activity of this critical transcription factor is regulated by sncRNAs is unknown. Using bioinformatics, we identified a miRNA, miR-30c-2*, as a candidate for targeting the 3'UTR of *Xbp1* mRNA. Additionally, we found that the 26nt intron excised from *Xbp1* mRNA by IRE1 exhibits characteristics of sncRNAs. Therefore, we are investigating the potential roles of these two sncRNAs in the UPR and in the differentiation of antibody-secreting B-cells.

ROLE OF G6PD IN HYPOXIA-INDUCED PULMONARY HYPERTENSION

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One of the major causes for pulmonary hypertension (PH) is alveolar hypoxia. A reduction in oxygen tension causes pulmonary vasoconstriction by a variety of actions on endothelium and smooth muscle. Chronic mountain sickness, COPD and sleep apnea are common etiologies of PH associated with hypoxemia. It is well known that acute hypoxia evokes contraction of pulmonary artery (PA) to match the ventilation-to-perfusion ratio. We have shown that Glucose-6-Phosphate Dehydrogenase (G6PD) promotes acute Hypoxic Pulmonary Vasoconstriction (HPV). We, therefore, hypothesized that G6PD could play a role in development of chronic hypoxia-induced PH. To investigate our hypothesis we used 3 weeks hypoxia rat model. Rats exposed to hypoxia (to Denver atmosphere) had elevated PA pressure, right ventricular hypertrophy and showed a phenomenal increase in NADPH levels, as compared to those exposed to normoxia. G6PD is the major source of NADPH in cells, and as expected G6PD activity and protein levels were higher in hypoxia treated animals than normal animals. Also DHEA, an inhibitor of G6PD, suppressed the development of PH in rats exposed to hypoxia. Further we investigated the location of increase in G6PD in lungs of PH rats by immunohistochemistry, and found that PA smooth muscle cells (PASMC) expressed higher G6PD. So, we exposed cultured PASMC to hypoxia (3% O₂, 5% CO₂) and CoCl₂ (100 μM), which mimics hypoxic conditions, for different time periods (1-to-72 hrs) and measured the protein expression, activity of G6PD and NADPH levels. Activity and protein levels of G6PD showed a significant increase between 1 and 8 hrs of hypoxia and COCl₂ treatment as compared to untreated PASMCs. In conclusion, we propose that increased G6PD expression and activation in PASMC by hypoxia plays an important role in the promoting hypoxia-induced vasoconstriction and in the development of PAH.

GLYCOMORPHOLOGY OF THE PULMONARY VASCULATURE: ENDOTHELIAL CELL GLYCOCALYX AND ENDOTHELIAL BARRIER FUNCTION.

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Rationale: The endothelium forms a semi-permeable barrier between constituents of the blood and underlying tissue. The endothelial glycocalyx consists of glycoproteins, glycolipids and proteoglycans which coat the cell surface. The carbohydrate network that contributes to the glycocalyx is very complex, and to date, the structure of these complex carbohydrates and their role in endothelial barrier function are poorly understood. Methods: We have begun to probe the molecular identities of endothelial cell surface carbohydrates using fluorescently-tagged lectins in a systematic way, revealing the presence of α -2,3-Gal and α -2,6-Gal/GalNAc terminal sialic acid residues that differed dependent upon the cell type. Cultured rat pulmonary microvascular endothelial cells (PMVECs) and pulmonary artery endothelial cells (PAECs) were treated with neuraminidase from *Clostridium perfringens* (2 U/mL) for one to three hours, and inter-endothelial cell gaps were evaluated by light microscopy at 20x magnification. Neuraminidase induced changes in endothelial barrier integrity were also evaluated using ECIS™. In these experiments, PMVECs and PAECs were individually treated with neuraminidase from *Clostridium perfringens* (2 U/mL) or *Vibrio cholera* at lower neuraminidase doses (0.6-0.9 U/mL) for four hours and the changes in resistance were monitored as a function of time. Results: A resulting dramatic loss of cell-cell and cell-matrix adhesion was noted by the formation of large inter-endothelial cell gaps subsequent to a dose-dependent treatment with neuraminidase from *Clostridium perfringens*. In the ECIS™ experiments, a decrease in resistance was observed in PAECs with neuraminidase from both species, whereas in PMVECs, only the neuraminidase from *Vibrio cholera* promoted a sustained decrease in resistance. Neuraminidase from *Vibrio cholera* preferentially cleaves α -2,3-terminal sialic acid / glycocalyx residues, in contrast to the similar rates of α -2,3- and α -2,6- sialic acid / glycocalyx hydrolysis observed when cells were treated with *Clostridium perfringens*. Conclusion: Overall these observations support the hypothesis that the glycocalyx of PMVECs and PAECs contains terminal sialic acid residues that play an important role in endothelial barrier function.

MODULATION OF AIRWAY SMOOTH MUSCLE PHENOTYPE BY MICRORNAS AND THE P38MAPK PATHWAY

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Inflammation is a major component of many lung diseases including asthma. Airway smooth muscle is thought to contribute to the innate immune response in the lung by synthesizing and secreting cytokines, chemokines, and growth factors. The secretion of these signaling proteins is a characteristic of the synthetic phenotype of smooth muscle. The p38MAPK pathway is known to enhance cytokine expression by regulating transcription and by regulating mRNA stability. Recent evidence from others suggests that miR-16 is an important component of the machinery that destabilizes transcripts downstream of the p38MAPK pathway. MiR-16 binds to transcripts with AU-rich elements (ARE) in the 3' untranslated region and targets these transcripts for degradation at processing bodies with the assistance of the ARE-binding protein, tristetraprolin. Tristetraprolin is phosphorylated by the p38MAPK substrate, MAPK activated-protein kinase 2, and when dual phosphorylated, it is sequestered by 14-3-3 and unable to bind AU-rich transcripts. Transcripts of numerous pro-inflammatory molecules including IL-6, IL-8, and GM-CSF contain AREs in the 3'-untranslated regions. Increased expression of miR-16 would theoretically antagonize the development of the secretory component of the synthetic phenotype in smooth muscle. Smooth muscle can be induced to develop into the contractile phenotype by the overexpression of myocardin. Overexpression of myocardin by adenovirus transduction of cultured primary human airway smooth muscle cells resulted in increased expression of miR-16, as measured by qRT-PCR. Thus, miR-16 expression increases when smooth muscle cells are driven towards the contractile phenotype. Activation of the p38MAPK pathway with a pro-inflammatory mixture containing TNF α , IL-1 β , and IFN γ (Cytomix) also induced the expression of miR-16 in cultured primary human airway smooth muscle cells, as measured by qRT-PCR. The p38MAPK inhibitor, SB239063, reduced cytomix-stimulated miR-16 expression. We propose a signaling pathway in which p38MAPK activation induces the expression of miR-16 to function in negative feedback. p38MAPK pathway negative feedback would limit ARE-containing pro-inflammatory gene expression in smooth muscle cells. To further test this negative feedback model, we will perform miR-16 gain of function studies in human airway smooth muscle cells to assay ARE-containing pro-inflammatory gene expression in the presence and absence of SB239063.

OPTIMIZING PROTEIN ENRICHMENT OF PROSTATE-SPECIFIC ANTIGEN IN HUMAN URINE SAMPLES USING HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY AND IMMUNOPRECIPITATION

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Prostate-Specific Antigen (PSA) is a secreted kallikrein-like serine protease used as a biomarker for early diagnosis of prostate cancer. Though prostate specific, PSA is not prostate cancer specific, and its concentration in blood can fluctuate with factors unrelated to cancer. Currently, for a patient to be recommended for biopsy, the level of PSA in blood sera must be 2-4ng/mL. This threshold is controversial, because of the percentage of false positives leading to unnecessary biopsies performed in healthy patients. The status of glycosylation in PSA can be useful in discriminating between PSA secreted in benign and cancerous conditions. To study variations in glycosylation of PSA, an effective method for protein enrichment is critical to avoid errors in glycoanalysis. This research examines immunoprecipitation and high-performance liquid chromatography (HPLC) in tandem with mass spectrometry to isolate PSA in human urine specimens. First morning void urine samples were processed through C2 liquid chromatography and fractionated by HPLC using C3, C8, and C18 columns with varying organic gradient. To optimize isolation of PSA through immunoprecipitation, three antibodies (2 mouse monoclonal, and one rabbit polyclonal) were compared. Quadrapole time-of-flight Mass spectrometry was used to identify and quantify PSA in each experiment.

RESPONSES OF IMMATURE B CELLS LACKING RASGRP1 AND CONTRIBUTIONS TO MURINE LUPUS

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Systemic Lupus Erythematosus (SLE) is an autoimmune disease characterized by the production of antibodies directed against self-tissues. In humans and in mice, deficiency in a molecule called RasGRP1 results in SLE. Recognition of pathogen-associated molecules by toll-like receptors (TLRs), normally providing immunoprotection from pathogens, was recently shown to activate autoreactive B cells to produce antibody. This study examines the role of RasGRP1 in activation of autoreactive B cells, and its involvement in TLR signaling pathways. Splenic B cells from wild-type (WT) and RasGRP1-deficient (KO) mice were isolated and loaded with carboxyfluorescein succinimidyl ester (CFSE), a fluorescent dye used to measure proliferation. While B cell receptor (BCR) stimulation alone induced negligible proliferation of WT and KO B cells, TLR7 stimulation induced measurable proliferation of both WT and KO B cells. Surprisingly, TLR7 stimulation induced greater proliferation within immature B cells of both WT and KO mice. Whereas the TLR7-induced proliferation of immature WT B cells was not increased by addition of BCR stimulation, approximately 70% of immature KO B cells proliferated under the same conditions. Further, as assessed by ELISpot, we found that KO B cells produced a higher frequency of autoreactive antibody secreting cells in response to TLR7 alone and in combination with BCR stimulation compared to WT B cells. The results suggest that TLR7 is critical for activation of autoreactive B cells lacking Rasgrp1, specifically at the transitional stage of development.

N-MYC INTERACTOR (NMI) AND THE EPITHELIAL-MESENCHYMAL TRANSITION

Devine, D

The epithelial-mesenchymal transition (EMT) is a process in which differentiated epithelial cells change to a more mesenchymal phenotype. Mesenchymal cells formed in this way are less differentiated and more proliferative than their epithelial progenitors and are capable of forming tumors. The Wnt signaling pathway can induce epithelial cells to undergo this transition, thus increasing the possibility of those cells becoming tumorigenic. The Wnt pathway is highly regulated by a diverse set of mechanisms. Dkkopf-1 (DKK1) is a secreted protein that represses Wnt signaling by blocking interaction of Wnt receptor (Frizzled) with its co-receptor (LRP5/6). Recently our lab discovered that Dkkopf-1 expression is substantially up-regulated by N-myc interactor (NMI). NMI protein sequence does not show characteristic features of a transcription factor—it does not contain any predicted intrinsic nuclear localization sequence, DNA binding domain or a transactivation domain. However, NMI seems to form complexes with several known transcription factors. Formation of these protein complexes can increase or decrease transcription of target genes. Our lab has also revealed that NMI can impede tumor growth in mice. Furthermore, recent data generated by our lab suggests that NMI can also inhibit EMT in cancer cells. With this in mind, we hypothesize that N-myc interactor reduces tumor growth rates by diminishing Wnt-dependent activation of the epithelial-mesenchymal transition.

INHIBITION OF LACTATE DEHYDROGENASE-A RE-SENSITIZES TAXOL-RESISTANT CANCER CELLS TO TAXOL

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Background: Taxol is one of the most effective chemotherapeutic agents for the treatment of patients with breast cancer. Despite impressive clinical responses initially, the majority of patients eventually develop resistance to Taxol. Lactate dehydrogenase-A (LDH-A) is one of the predominant isoforms of LDH expressed in breast tissue, which controls the conversion of pyruvate to lactate and plays important role in glucose metabolism. In this study we investigated the role of LDH-A in mediating Taxol resistance in human breast cancer cells. Results: Taxol-resistant subclones, derived from the cancer cell line MDA-MB-435, sustained continuous growth in high concentrations of Taxol while the Taxol-sensitive cells could not. The increased expression and activity of LDH-A were detected in Taxol-resistant cells when compared with their parental cells. The downregulation of LDH-A by siRNA significantly increased the sensitivity of Taxol-resistant cells to Taxol. A higher sensitivity to the specific LDH inhibitor, oxamate, was found in the Taxol-resistant cells. Furthermore, treating cells with the combination of Taxol and oxamate showed a synergistical inhibitory effect on Taxol-resistant breast cancer cells by promoting apoptosis in these cells. Conclusion: LDH-A plays an important role in Taxol resistance and inhibition of LDH-A re-sensitizes Taxol-resistant cells to Taxol. To our knowledge, this is the first report showing that the increased expression of LDH-A plays an important role in Taxol resistance of human breast cancer cells. This study provides valuable information for the future development and use of targeted therapies, such as oxamate, for the treatment of patients with Taxol-resistant breast cancer.

P38 MAPK-DEPENDENT REGULATION OF MMPS DURING CORONARY COLLATERAL GROWTH.

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An alternative way to bypass blocked coronary arteries is through the development of coronary collaterals, which is induced by transient, repetitive ischemia (RI) in healthy but not in metabolic syndrome animals. The process of coronary collateral growth requires extracellular matrix (ECM) remodeling, which allows for migration and proliferation of endothelial and vascular smooth muscle cells, and thus, outward vessel remodeling. Matrix metalloproteinases (MMPs) contribute to ECM remodeling by degrading ECM proteins. One of the signaling pathways that may regulate MMPs is the p38 MAPK pathway. We have previously determined that the activation of p38 MAPK was required for RI-induced coronary collateral growth. The goal of the present study was to elucidate p38 MAPK's role in the regulation of MMPs and, consequently ECM remodeling as well as how this is altered in the metabolic syndrome. A pneumatic occluder was implanted over the left anterior descending coronary artery (LAD) in both the metabolic syndrome (JCR) and healthy Sprague-Dawley (SD) rats. The RI protocol consisted of 8 40 sec occlusions; every 20 minutes, repeated every 8 hrs for 0, 3, 6, or 9 days. Coronary collateral growth was measured in the LAD-dependent (ischemic) and the normal (non-ischemic) zones using microspheres. p38 MAPK activation and MMP 1, 2, and 9 expression were determined by Western blot, MMP activity by zymography, and ECM remodeling by immunohistochemistry. A specific pharmacological inhibitor of p38 MAPK, SB203580, was given IV to assess p38 MAPK's role in MMP activation. Inhibition of p38 MAPK activation was confirmed by Western blots using anti-phospho MAPKAPK-2 antibodies. The expression and activation of MMP 2 and 9 was increased on days 3 and 6 of RI in the LAD-dependent zone of SD rats and correlated with p38 MAPK activation (day 3) and the start of coronary collateral growth (day 6). Inhibition of p38 MAPK decreased the activation of MMP 2 and 9. In contrast, RI did not alter expression or activation of MMP 2 or 9 in the JCR rats, correlating with lack of p38 MAPK activation. MMP 2 and 9 activation correlated with decreased elastin, laminin and type III collagen content. In conclusion, coronary collateral growth is dependent on p38 MAPK-dependent regulation of MMP expression and activation. Furthermore, compromised coronary collateral growth in the metabolic syndrome may be partially due to the lack of p38 MAPK-dependent activation of MMPs, which results in decreased ECM degradation, which could prevent EC and VSMC migration and the development of coronary collaterals.

DEVELOPMENT OF A MATHEMATICAL MODEL TO STUDY THE MECHANISMS UNDERLYING CYCLIC AMP LOCALIZATION

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Cyclic AMP (cAMP) is an ubiquitous second messenger that regulates a wide range of cellular responses. Increasing evidence suggests that signaling specificity within cAMP signaling pathways can be achieved by cAMP compartmentalization, implying that cAMP signals are localized in discrete regions of the cell. However, the cellular mechanisms underlying cAMP compartmentalization are not well understood. Studies indicate that the spatial spread of cAMP signals is affected by localized adenylyl cyclase (AC) and phosphodiesterase (PDE) activities, buffering from cAMP binding proteins, cell geometry, and restrictions on cAMP diffusion. To better understand the potential contributions of these mechanisms to cAMP compartmentalization, we developed a mathematical model describing the spatial and temporal distribution of cAMP signals in pulmonary microvascular endothelial cells (PMVECs). Models were developed in *Virtual Cell* environment to simulate cellular processes including cAMP synthesis, hydrolysis, buffering and diffusion. Model parameters were based on experimental data. A homogenous rate of cAMP diffusion was assumed. Geometries used in the model were obtained from cells with distinct shapes, confluent and migrating PMVECs. Confluent PMVECs display the typical fried egg appearance whereas migrating PMVECs have protruding processes. Simulations demonstrated that only small cAMP gradients were generated when the effective diffusion coefficient (D) was $300 \mu\text{m}^2/\text{s}$ – D for cAMP in aqueous solution. This indicates that AC and PDE activities and buffering by PKA cannot solely account for the segregation of cAMP signals with $D = 300 \mu\text{m}^2/\text{s}$. However, lowering D 100- to 10000-fold allowed the generation of substantial cAMP gradients. These simulations are consistent with a conceptual model in which cellular geometry and structural elements allow localized AC and PDE activities to generate physiologically significant cAMP gradients. We are currently using this model to guide the design of future experiments to study the contribution of physical barriers, such as f-actin cytoskeletal network, to the kinetics and spatial spread of cAMP signals.

AUTOMATED ANALYSIS OF FLUORESCENT CALCIUM INDICATOR ACTIVITY

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Calcium signaling is involved in many vital physiological processes, including the regulation of vascular tone. Currently, there is no freely available tool for analyzing multiple regions of intracellular calcium dynamics within tissue. Here we present a software application, Eventpro, which allows the automated detection and temporal analysis of regions of fluorescent intensity in time lapse image sequences. We integrated a novel java application with image processing algorithms based on ImageJ open source software into a single user interface. We demonstrated the efficacy of Eventpro by applying it to image sequences of porcine coronary arteries loaded with the fluorescent calcium indicator Fluo-4. The amplitude and time course of calcium dynamics detected by Eventpro were consistent with results obtained by manual analysis of user selected regions of interest using Perkin Elmer Ultraview software. Eventpro provides the ability to automatically and objectively detect multiple regions of fluorescent activity based on predefined criteria within time lapse image sequences. This software represents a novel development in image analysis that reduces both work time and subjectivity compared to conventional methods.

DEVELOPMENT OF CELL CO-CULTURE MODEL OF NEOINTIMA FORMATION

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Normal adult pulmonary arterial smooth muscle cells (PASMCs) have a contractile/differentiated phenotype. During the development of pulmonary arterial hypertension, PASMCs dedifferentiate from a contractile to a synthetic phenotype. This phenotypic switching promotes PASMC growth, migration, and extracellular matrix deposition. Collectively, these events can lead to arterial wall thickening and formation of neointimal lesions. It is generally believed that neointima formation, a cardinal sign of human severe pulmonary arterial hypertension, results, at least partly, from pulmonary arterial endothelial cell (PAEC) dysfunction and “abnormal” signaling to the neighboring PASMCs. However, the exact mechanisms by which dysfunctional PAECs alter the phenotype and function of PASMCs and thereby cause neointima formation are poorly understood. Thus, we proposed to develop an *in vitro* neointimal model to study the pathogenesis of these lesions in detail. To this end, we co-cultured fluorescent-labeled PAECs and PASMCs on opposite sides of an 8µm pore size transwell insert. After 72 hrs of co-culture, the level, pattern, and distribution of migrating PASMCs towards PAECs were determined by confocal imaging. PAEC scrape wounding and platelet-derived growth factor (PDGF) were used as stimuli for PASMC migration. The results showed a high level of PASMC migration in response to these stimuli when PASMCs were cultured alone, compared to when they were in co-culture with PAECs. The level of migration was markedly reduced in presence of a confluent monolayer of PAECs. Although PASMCs appeared more rounded in presence of PDGF, the level of migration was not stimulated. Conversely, when PAECs were less confluent, we observed a high rate of migration of PASMCs towards PAECs. PASMCs appeared to form a vessel-like structure when they were close to large colonies of PAECs. PASMCs were also more spindle-shaped or elongated when they were close to PAECs. In contrast, the migrating PASMCs appeared more rhomboidal or swollen when they were distant from PAECs. In summary, this preliminary study supports the feasibility of developing an “*in vitro*” model of neointima formation, which resembles the behavior of PAECs and PASMCs *in vivo* and thus could be useful for studying the cellular and molecular mechanisms of occlusive neointimal lesions in severe pulmonary arterial hypertension.

THE ROLE OF HEDGEHOG SIGNALING IN CANCER VASCULARITY

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Introduction: Vascularization is a critical component of cancer progression. Once a tumor reaches a diameter of 2mm, it employs one or more mechanisms in order to establish vasculature required for it to survive. Furthermore, tumors that are vascularized provide increased opportunities for cancer cells to detach from the mass and metastasize hematologically. Several pathways that promote vascularization are active in many cancers. The Hedgehog (Hh) pathway is activated when one of the three Hh ligands Sonic, Indian, or Desert Hedgehog (SHH, IHH, DHH respectively) bind to extracellular domain of its receptor PTCH. This binding eventually results in the translocation of the transcription factor GLI1 from the cytoplasm to the nucleus where it upregulates several genes including the Hh ligands themselves, cyclin D1 and osteopontin. **Rationale:** Functionally, Hh promotes vascularization during fetal development and injury yet is also constitutively active in several cancers. Therefore, it was of interest to understand the mechanism the Hh pathway plays to promote vascularization in cancer. **Methods:** We generated stable transfectants that were silenced for GLI1 expression, thus truncating Hh signaling (melanoma cell line, MDA-MB-435 termed 435-GLI1-KO). We also engineered the breast cancer cell line, MDA-MB-231 to stably express SHH (termed 231-SHH), thus constitutively activating Hh signaling. We generated stable transfectants that express the empty vector as controls. We used the Modified Boyden Chamber to assess how Hh signaling influences migration and invasion of endothelial cells. Briefly, 40, 000 rat lung derived endothelial were seeded into filter inserts (8um pore), coated with either gelatin (migration) or Matrigel (invasion). The inserts were lowered into wells of a 24 well plate containing conditioned media from each experimental group. After the allotted time intervals for migration (5 hours) and invasion (16 hours), migrating and invading cells on the underside of the insert were stained and counted. **Results & Conclusions:** We observed that conditioned media from 435-GLI1-KO cells was inefficient in promoting migration and invasion of endothelial cells compared to the vector control. Contrarily, conditioned media from cancer cells, 231-SHH that have constitutive Hh signaling were able to promote migration and invasion of endothelial cells significantly more so than the vector control group. The results suggest Hh signaling and its downstream targets may promote tumor vascularization by influencing endothelial cell behaviors necessary for blood vessel formation. Further experiments are underway to analyze the secretome of these cells in order to identify differentially expressed/secreted candidate proteins that can influence endothelial cell behavior.

PROTECTION BY 5-METHYLTETRAHYDRO-FOLATE (5MTHF) AGAINST UV-INDUCED DNA DAMAGE.

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Folates are vitamins which act as cofactors for several metabolic pathways, including *de novo* synthesis of three of the DNA bases, adenine, guanine and thymine. UV irradiation (UVI) causes DNA damage mostly through excitation of cellular photosensitizers, such as flavins, that generate singlet oxygen ($^1\text{O}_2$) species which react with DNA. Our laboratory has shown, in vitro, that 5MTHF, the most abundant form of folates in the blood, is a diffusion-limited quencher of photosensitization reactions. Moreover, at physiological concentrations, 5MTHF removes $^1\text{O}_2$ species before they react with DNA (1). Skin is the largest tissue in the human body and the most exposed part to UVI and its harmful effects. Exposure to UVI is one of the leading causes of skin cancer which is diagnosed in more than one million people annually in the United States. The hypotheses which we are testing are: (i) Increased 5MTHF levels in the skin help to protect against UV-induced DNA damage, and (ii) Dark skin color protects against endogenous 5MTHF degradation during exposure to UVI. We are in the process of determining 5MTHF content of dark and light human skin and the ability of skin to take up exogenously added 5MTHF. We shall then study the effect of UVI on the degradation of 5MTHF in human skin. Ultimately, the effect of UVI on DNA damage in isolated dark and light human skin untreated and treated with different doses of 5MTHF will be ascertained. Our research may lead to a better understanding of the mechanism of carcinogenesis in skin and natural protection, and may reveal a means of enhancing this protection. Acknowledgements: We would like to thank Drs. Outlaw, Luterman and Dyess for providing samples of skin removed during surgery, and Dr. Chouteau for his invaluable help in coordinating these activities. LZH is supported by a Graduate Research Scholar Program Fellowship funded by Alabama EPSCoR.

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EFFECT OF GSK1016790A, A POTENT TRPV4 AGONIST, ON LUNG ENDOTHELIAL PERMEABILITY

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Activation of the vanilloid transient receptor potential channel TRPV4 with 4 α -phorbol-12,13-didecanoate (4 α PDD) causes Ca²⁺ entry-dependent lung injury in rat and mouse lung. However, 4 α PDD has no impact on pulmonary arterial pressure despite TRPV4 expression in pulmonary vascular smooth muscle. In this study, we compared the effect of GSK1016790A, a potent and selective TRPV4 agonist, with that elicited by 4 α PDD. Lungs isolated from wild type or TRPV4^{-/-} mice were perfused with Earle's buffer/4% albumin at constant flow. The filtration coefficient (K_f, an index of endothelial permeability) and hemodynamics were measured before and after GSK1016790A or 4 α PDD treatment. At 3nM (n=4), GSK1016790A had little impact on permeability. However, K_f increased 5.5- and 10.6-fold with 10nM (n=6) and 30nM (n=5) doses, respectively. The permeability response to GSK1016790A was accompanied a transient increase in pulmonary arterial pressure. In contrast, 4 α PDD (10 μ M, n=4) increased K_f 3.2-fold without a concomitant pressor response. Neither 30nM GSK1016790A (n=4) nor 10 μ M 4 α PDD (n=4) had impact on permeability or perfusion pressure in lungs from TRPV4^{-/-} mice. In summary, GSK1016790A is a much more potent stimulus than 4 α PDD for TRPV4-mediated acute lung injury and pressor responses in isolated mouse lung. Supported by GlaxoSmithKline and HL066299.

ROLE OF IMMUNOPHILIN FKBP51 AND FKBP52 IN STORE OPERATED CALCIUM ENTRY

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In the endothelium, activation of store operated calcium entry (SOCE) promotes interendothelial cell gap formation leading to endothelial barrier disruption. Mammalian transient receptor potential proteins 1 and 4 (TRPC1, 4) are subunits of SOCE channels and contribute to an endothelial cell calcium-selective SOC entry channel (*I_{soc}*). Rat pulmonary artery endothelial cells (PAECs) and pulmonary microvascular endothelial cells (PMVECs) both express *I_{soc}* channel subunits, and *I_{soc}* activation is differentially regulated in PAECs and PMVECs. In PAECs *I_{soc}* is activated by the plant alkaloid thapsigargin, while in PMVECs thapsigargin alone does not activate *I_{soc}*. FK506 binding proteins (FKBPs) are immunophilins which may play a role in regulation of SOCE. FKBP52 has previously been shown to bind to TRPC1 and TRPC4 in SF9 cells. FKBP51 can be upregulated by glucocorticoid treatment. FKBP51 also often exhibits opposite effects to those of FKBP52. We hypothesized that FKBP52 binds to TRPC1 and TRPC4 subunits of the *I_{soc}* channel, and facilitates channel activation, whereas upregulation of FKBP51 by glucocorticoids inhibits channel activation. We first probed for expression of FKBP52 and FKBP51 in PAECs and PMVECs. Both PAECs and PMVECs constitutively express FKBP52. Interestingly, FKBP51 was found to be predominantly expressed in PMVECs, and not PAECs. Glucocorticoid treatment upregulates FKBP51 expression in both PAECs and PMVECs. Secondly, we observed that SOCE was reduced in PAECs following glucocorticoid treatment. These data suggest that FKBP51 and FKBP52 are important in regulation of SOCE.

IFN- γ INHIBITS CENTRAL NERVOUS SYSTEM MYELINATION THROUGH BOTH STAT1-DEPENDENT AND STAT1-INDEPENDENT PATHWAYS

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Immune cytokine interferon- γ (IFN- γ) plays a crucial role in immune-mediated demyelination diseases such as multiple sclerosis and experimental autoimmune encephalomyelitis (EAE). Our previous studies have shown that enforced expression of IFN- γ in the central nervous system (CNS) inhibits developmental myelination or remyelination in EAE demyelinated lesions. While many of the cellular actions of IFN- γ result from its activation of the signal transducer and activator of transcription (STAT)1 pathway, recent studies have shown that STAT1-independent pathways regulate some facets of IFN- γ biology. In this study, we dissected the role of STAT1-dependent and STAT1-independent pathways in IFN- γ -induced hypomyelination using a genetic approach. We found that the induction of the STAT1-dependent, IFN- γ -responsive genes in response to this cytokine was abolished in the CNS of STAT1 null mice. Moreover, STAT1 deletion diminished oligodendrocyte loss, the reduction of myelinated axons and inflammatory response in the CNS of transgenic mice that ectopically express IFN- γ in the CNS. Nevertheless, IFN- γ -induced reduction of myelin sheath thickness in the CNS of these mice was not altered by STAT1 deletion. Collectively, these data demonstrated that both STAT1-dependent and STAT1-independent pathways are involved in the detrimental effects of IFN- γ on the myelination process.

MiR-125b CONFERS THE RESISTANCE OF CANCER CELLS TO TAXOL THROUGH SUPPRESSION OF BAK1

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Paclitaxel (Taxol) is one of the most effective chemotherapeutic agents for treatment of cancer patients. Despite impressive initial clinical responses, the majority of patients will eventually develop some degree of resistance to Taxol-based therapy. The mechanisms underlying cancer cells resistance to Taxol are not fully understood. microRNA (miRNA) has emerged to play important roles in tumorigenesis and drug-resistance. However, the interaction between the development of Taxol resistance and miRNA has not been previously explored. In this study, we utilized a miRNA array to screen differentially expressed miRNAs, comparing Taxol-resistant and their Taxol-sensitive parental cells, and investigated the role and mechanisms of miR-125b in Taxol resistance of both melanoma and breast cancer cells. We found that miR-125b was up-regulated in Taxol-resistant cells, with its overexpression causing a marked inhibition of Taxol-induced cytotoxicity and apoptosis, and a subsequent increase in the resistance of cancer cells to Taxol. Moreover, we demonstrated that Bak1 is a direct target of miR-125b in both melanoma and breast cancer cells, and down-regulation of Bak1 suppressed Taxol-induced apoptosis and led to an increased resistance to Taxol. Furthermore, restoring the expression of Bak1 by either miR-125b inhibitor or specific siRNA recovered Taxol sensitivity, overcoming miR-125-mediated Taxol resistance. Taken together, our data strongly supports a central role for miR-125b in conferring Taxol resistance through the suppression of Bak1 expression. This finding has important implications in the development of targeted therapeutics for overcoming Taxol resistance in a number of different tumor histologies.

ROLE OF DNAJB6 IN RESTRICTING BREAST TUMOR PROGRESSION

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Breast cancer is one of the leading causes of death in women. We found that expression of mammalian relative of DnaJ (MRJ/DNAJB6), a member of the heat shock protein 40 family, is lost as the grade of infiltrating ductal carcinoma increases. Analysis of several breast cancer cells lines revealed that the expression levels of the large isoform of MRJ, MRJ(L), was downregulated. Overexpression of MRJ(L) suppressed invasion and migration and reduced tumor growth rate in cancer cell xenografts. Ectopic expression of MRJ(L) resulted in modulation of the secreted proteome encompassing several key proteins important for regulation of tumor progression and metastasis. This analysis revealed that the level of Dickkopf homolog 1 (DKK1), a secreted antagonist of the Wnt/ β -catenin pathway, was elevated in MRJ(L) overexpressors. Hence we hypothesized that MRJ(L) upregulates DKK1, which in turn downregulates the activation of the Wnt/ β -catenin pathway. Here we show that the DKK1 sequence in metastatic breast cancer cells does not show any mutations. This implies that upregulated DKK1 protein is capable of functionally suppressing the Wnt/ β -catenin pathway. We also demonstrate that the DKK1 luciferase reporter is activated five fold by MRJ(L). Furthermore we found that MRJ(L) downregulates β -catenin dependent transcription as assessed by luciferase reporter assays and β -catenin levels are significantly lower in MRJ(L) expressing cells. In conclusion, our observations show that MRJ(L) negatively regulates Wnt/ β -catenin signaling.

ANP INDUCED TESTOSTERONE PRODUCTION IN MA-10 MOUSE LEYDIG TUMOR CELLS

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Atrial natriuretic peptide (ANP) is a hormone that activates the membrane guanylyl cyclase, natriuretic peptide receptor A (NPR-A), in mammalian cells. One effect of this well understood. The cGMP produced by NPR-A may increase testosterone through the action of cGMP dependent protein kinase (PKG), or by activation of cAMP dependent protein kinase (PKA). In contrast, luteinizing hormone-induced testosterone production, has been extensively studied. Luteinizing hormone triggers cAMP production and subsequent activation of PKA. PKA phosphorylates the steroidogenic acute regulatory protein (STAR). STAR performs the rate limiting step in steroidogenesis: importing cholesterol to the mitochondria. Phosphorylation by PKA at serine 195 increases STAR activity. PKG will phosphorylate STAR at serine 100, but not serine 195, in vitro. It is not known whether phosphorylation at serine 100 affects STAR activity, or whether it occurs in response to ANP stimulation in an intact Leydig cell. In this study, we will measure steroid production using enzyme immunoassays to evaluate the potential contributions of PKA and PKG to ANP-induced testosterone production. We will also use phosphomimetic and non-phosphorylatable mutants of STAR to evaluate the potential effects of phosphorylation by PKG on STAR activity.

ROLE OF MERLIN IN BREAST CANCER

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Introduction: Breast cancer is the second most common type of cancer among women, affecting about 1 in 8 women during their lifetime. Breast cancer is also the second-leading cause of cancer-related death in women. Drugs currently employed to fight this disease still cause adverse side effects in the years following treatment. For these reasons, it is vital to identify novel treatments for this disease. Inactivating mutations of the neurofibromatosis 2 (NF2) gene, NF2, result in predominantly benign neurological tumors such as schwannomas and meningiomas. Several investigators have shown that the NF2 gene product, Merlin, is recruited to the cell surface and can interact with the ERM (Ezrin, Radixin, Moesin) proteins and inhibit cell proliferation. However, the tumor suppressive function of merlin in breast cancer remains uncertain. Purpose of the research: To elucidate the role of Merlin in breast cancer. Methods: We assessed the regulation of the WNT pathway by Merlin in breast cancer by monitoring sub-cellular localization of the WNT pathway transcription factor, β -catenin, and by assessing its effect TCF/LEF transcriptional activity. Results & Conclusions: We find that Merlin alters the sub-cellular localization of β -catenin concomitant with suppression of the TCF/LEF transcriptional activity in breast cancer cells. This suggests that Merlin likely modulates the Wnt pathway in breast cancer.

AUTOANTIBODIES IN PULMONARY HYPERTENSION AND METABOLIC SYNDROME

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Endothelial cells (ECs) line the interior of blood vessels and control the transport of gases and solutes into the blood stream. Endothelial dysfunction, or the loss of normal endothelial function, has been linked to multiple diseases, such as atherosclerosis, diabetes, and hypertension. Endothelial cell dysfunction is suggested to contribute to the muscularization of pulmonary vasculature, leading to pulmonary hypertension (PH). Endothelial dysfunction is also found in metabolic syndrome (MS) patients, a condition characterized by excessive abdominal fat and high blood sugar levels that increases the risk of developing cardiovascular disease and type II diabetes. Patients diagnosed with some autoimmune diseases such as scleroderma, systemic lupus erythematosus, and mixed connective tissue disease, are more likely to develop pulmonary hypertension than individuals without autoimmunity. A common diagnostic marker for autoimmunity is the presence of autoantibodies – antibodies that bind to self-antigen. Autoantibodies and inflammatory immune responses can cause endothelial damage and lead to endothelial dysfunction. It's known that patients with metabolic syndrome and pulmonary hypertension have elevated levels of autoantibodies compared to healthy controls, but it is unclear whether animal models of these diseases share this characteristic. We therefore sought to determine whether autoantibodies are present in animal models of PH and MS. We isolated plasma from pulmonary hypertensive and metabolic syndrome rats, and separated the IgG present by affinity chromatography. We observed elevated levels of IgG in the disease models as compared to controls. We next asked whether the antibodies from the disease models inhibit cell proliferation. IgG fractions from PH, MS, and healthy control rats were used to treat pulmonary microvascular ECs over six days, and growth curves were generated. All three IgG fractions from MS rats inhibited growth at six days, while IgG fractions from PH rats exhibited varied effects on proliferation. These data suggest that autoantibodies may be found in the animal models of PH and MS, and contribute to the endothelial dysfunction of these diseases.

PREDICTING SECRETED BIOMARKERS FOR EARLY DETECTION CANCER USING LC-MS/MS ANALYSIS

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Identification of biomarkers from secretory proteins is highly desirable for early diagnosis of cancer progression. We have developed a method to identify and analyze biomarkers for cancer using a secretory fluid. The secretory fluid is separated by RP-HPLC and the fraction from 27-33 minutes representing the protein fraction is collected. The proteins are digested with 0.2 μ g trypsin in 50mM ABC/TCEP overnight at 37°C. Following overnight digestion with trypsin, the samples are injected in triplicate into a LTQ-Orbitrap MS with the injection volume based on the UV peak height from the chromatogram to normalize samples. The MS collected on one second scans (peptide mass data collected) and 5 per second MS/MS (sequence-related) scans of selected peptide masses. Resulting data was processed into a search file using the Xcalibur software package (ThermoElectron). The search files were then processed through an in-house copy of MASCOT™, a search engine used to identify proteins present in the sample. These protein profiles and abundances can then be compared. As a second approach for differential analysis between cancer and healthy controls, MS signals from LC-MS/MS data were chromatographically aligned to allow comparison of peptide quantities. Once aligned, protein masses, time, M/Z, and intensity were merged for common peptides using DifProWare, a software program developed in the USA MCI. The differential comparison of samples is both graphically and textually represented within the software and the potential biomarker peptides (and associated proteins) identified for further analysis. The graphical representation allows an easy selection of these possible biomarkers but final analyses are performed by statistical analyses of the spreadsheet output of DifProWare. Given the importance of finding biomarkers using secretory proteins for early detection and diagnosis of cancer, this method could decrease the time needed to discover possible protein candidates to be used as biomarkers.

FREEZING TEMPERATURES DO NOT DISASSEMBLE ENDOTHELIAL CELL MICROTUBULES
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Rationale: Microtubules are composed of α -tubulin and β -tubulin dimers. Microtubules yield tubulin dimers at freezing temperatures. These dimers reassemble spontaneously to form microtubule fibers at 37°C. In contrast, microtubules of poikilotherms that live in the polar seas do not disassemble at very low temperatures – a phenomenon known as microtubule cold-stability. Mammals have a population of cold-stable microtubules that have been identified in neurons, glial cells, and fibroblasts. This cold-stability is predominantly due to microtubule association with a family of proteins known as Stable Tubule Only Polypeptides (STOP). Whether other mammalian cells have cold-stable microtubules is currently unknown. Since one of the multiple functions of microtubules is to regulate cell shape, and endothelial cell shape is paramount for endothelial barrier function, we hypothesized that endothelial cells express STOP and therefore have cold-stable microtubules. **Materials and Methods:** Pulmonary artery endothelial cells (PAEC), pulmonary vein endothelial cells (PVEC), pulmonary microvascular endothelial cells (PMVEC), and HeLa cells (negative control) were grown on glass coverslips in complete DMEM until they reached 50-70% confluency. Cells on coverslips were exposed to ice-cold water (0°C) for 10 minutes. After cold-exposure, cells were fixed in methanol followed by a microtubule extraction protocol with triton X. Microtubule fibers were detected using immunofluorescence against β -tubulin. Slides were viewed with a PerkinElmer Ultraview RS-3 spinning disk confocal microscope. Immunoblots against STOP and immunoprecipitation were performed according to the established protocols. **Results:** After cold exposure, immunofluorescence revealed that HeLa cell microtubules disassembled while endothelial cell microtubules did not. Also, immunoblot showed that endothelial cells express STOP and immunoprecipitation revealed that STOP binds with endothelial microtubules only after cold exposure. **Conclusion and Future Directions:** Incubation of cultured endothelial cells at 0°C revealed a population of cold-stable microtubules. Lung endothelial cells also express STOP and STOP seems to play a role in cold-stability of endothelial cell microtubules. Since in mammals, this phenomenon has little to do with the need for cold-stability but indicates that some microtubules must be stabilized for specific functions, the implication of this newly identified population of microtubules for endothelial cell physiology is currently under investigation. Supported by HL-60024, HL-66299, and HL-076125

ESSENTIAL ROLE OF LACTATE DEHYDROGENASE-A IN PULMONARY MICROVASCULAR ENDOTHELIAL CELL PROLIFERATION.

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Pulmonary microvascular endothelial cells (PMVECs) utilize aerobic glycolysis, characterized by glucose consumption and fermentation to lactate, to sustain their rapid proliferation. As a consequence, these cells generate lactic acidosis during growth. **Rationale:** Endothelial cells express both lactate dehydrogenase A (LDH-A) and B (LDH-B), although it is not presently clear whether both LDH-A and LDH-B are necessary to sustain high rates of glucose to lactate conversion. Thus, we tested the hypothesis that LDH-A is essential to sustain the rapid glucose to lactate conversion that underlies high PMVEC proliferation. **Methods:** To address this hypothesis, we developed a system for conditional expression of shRNA. PMVECs were first engineered to stably express the Tet-On reverse transactivator protein (rtTA) using a retroviral system. Cells expressing the construct were selected to homogeneity using blasticidin. Once established, cells were re-infected with lentivirus containing the shRNA sequence driven by doxycycline, enabling rtTA interaction with the Tet-operator sequences. Four separate shRNA constructs targeting LDH-A were screened; one was found to be particularly effective at reducing LDH-A protein. These cells were then selected to homogeneity using puromycin. **Results:** In cells stably selected to possess both constructs, doxycycline induced a concentration-dependent (from 0.01-10 $\mu\text{g}/\text{mL}$) decrease in LDH-A expression. A time course response was determined following doxycycline induction, and revealed that LDH-A levels decreased within six hours post-induction; downregulation was most apparent 72 hours after doxycycline treatment. To assess the functional consequences of LDH-A downregulation, standard growth curves were performed. In control experiments, PMVECs grew from 10^5 cells to 4×10^6 cells over a seven-day time course. During the growth curve, PMVECs consumed glucose and generated lactic acidosis. In contrast, glucose consumption, lactate production and PMVEC growth were all attenuated following suppression of LDH-A expression. **Conclusion:** LDH-A expression is a critical determinant of glucose consumption, lactate production and rapid proliferation in PMVECs.

UPREGULATION OF pHSP27 IN HYPOXIC PULMONARY HYPERTENSION

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Rationale: Pulmonary hypertension (PH) refers to a sustained elevation of pulmonary arterial pressure. Both vasoconstriction and pulmonary arterial wall remodeling play roles in the pathogenesis of PH. Exposing rats to chronic hypoxia (CH) is one model of PH that is used to investigate underlying pathophysiological mechanisms and therapeutic options. Hypoxia is also a stimulus of some forms of human PH. CH in rats causes activation of RhoA and Rho kinase in pulmonary arteries leading to sustained vasoconstriction and hypertension. The small heat shock protein (Hsp), Hsp27, is a member of a family of highly conserved, constitutively expressed proteins. It regulates vascular smooth muscle tone in conjunction with intracellular calcium signaling. Phosphorylated Hsp27 (pHsp27) promotes contraction. Hypoxia induces expression of several heat shock proteins in rat lungs, including Hsp27. Thus, interactions among pHsp27, calcium signaling, and activity of RhoA/Rho kinase might play a role in the development of PH through regulation of vascular smooth muscle tone.

Objectives: To determine if pHsp27 plays a role in the sustained pulmonary arterial constriction that accounts for pulmonary hypertension in CH rats, and, if so, whether pHsp27 signaling occurs upstream or downstream of RhoA/Rho kinase activation.

Methods: Hypertensive pulmonary arteries were isolated from rats exposed to CH (10% O₂) for 3 weeks. Normotensive pulmonary arteries were collected from control normoxic rats. The left and right main branches were then cut into four 3-millimeter rings, which were mounted in wire myographs to measure isometric tension responses. The normotensive and hypertensive rings were allowed to equilibrate for 1 h to optimal resting tensions. After equilibration, saline (vehicle control), nitro-L-arginine (nitric oxide synthase inhibitor), U46619 (thromboxane A₂ mimetic), or serotonin was added each to one ring and tension was measured for 20 min. The tissues were then collected and processed for western blot analysis using anti-Hsp27 and anti-pHsp27 antibodies.

Preliminary Results: Compared to normotensive pulmonary arteries, hypertensive arteries showed increased expression of pHsp27 both under resting conditions and after eliciting contractions with inhibition of nitric oxide synthesis or the G protein coupled agonists.

Conclusions: The resting and stimulated expression of pHsp27 is increased in hypoxia-induced hypertensive rat pulmonary arteries and may play a role in the sustained vasoconstriction of these vessels. Additional experiments are planned to determine the relationship between pHsp27 and activation of RhoA/Rho kinase in the hypertensive pulmonary arteries.

ACTIVATION OF GLUCOSE-6-PHOSPHATE DEHYDROGENASE PROMOTES ACUTE HYPOXIC PULMONARY ARTERY CONTRACTION

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Hypoxic pulmonary vasoconstriction (HPV) is a physiological response to a decrease in airway O_2 tension, but the underlying mechanism is incompletely understood. We studied the contribution of glucose-6-phosphate dehydrogenase (G6PD), an important regulator of NADPH redox and production of reactive oxygen species, to the development of HPV. We found that hypoxia (95% N_2 -5% CO_2) increased contraction of bovine pulmonary artery (PA) precontracted with KCl or serotonin. Depletion of extracellular glucose reduced NADPH, NADH and HPV, substantiating the idea that glucose metabolism and G6PD play roles in the response of PA to hypoxia. Our data also show that inhibition of glycolysis and mitochondrial respiration (indicated by an increase in NAD^+ and decrease in the ATP-to-ADP ratio) by hypoxia, or by inhibitors of pyruvate dehydrogenase or electron transport chain complexes I or III, increased generation of reactive oxygen species, which in turn activated G6PD. Silencing G6PD expression in PA using a targeted siRNA abolished HPV and diminished Ca^{2+} -independent and Ca^{2+} -dependent myosin light chain phosphorylation otherwise increased by hypoxia. Similarly, G6PD expression and activity were significantly reduced in lungs from G6PDMut(-/-) mice, and there was a corresponding reduction in HPV. Finally, regression analysis relating G6PD activity and the NADPH-to- $NADP^+$ ratio to the HPV response clearly indicated a positive linear relationship between G6PD activity and HPV. Based on these findings, we propose that G6PD and NADPH redox are crucially involved in the mechanism of HPV and, in turn, may play a key role in increasing pulmonary arterial pressure, which is involved in the development of pulmonary hypertension.

NUCLEAR OXIDANT STRESS INDUCTION BY HYPOXIA AND ESTROGEN

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Increases in oxidative DNA damage have recently been linked to gene expression, with the emerging paradigm being that oxidative DNA damage ultimately results in recruitment of transcription factors, enhancing gene expression. Hypoxia and estrogen (E2) both target the pulmonary endothelium and induce oxidative DNA damage and gene expression. Given that both hypoxia and estrogen induce oxidative DNA stress, we hypothesized that the physiological stimuli, hypoxia and E2, induce an oxidative nuclear state in pulmonary artery endothelial cells (PAECs). To test this hypothesis we utilized a novel ratiometric, redox sensitive green fluorescent protein with a nuclear localizing sequence (roGFP-NLS) to probe for alterations in the nuclear redox state. PAECs were grown to confluence and allowed to transfect for 3 hr with the roGFP-NLS plasmid construct. Twenty-four hours post transfection, cells were subjected to experimental conditions (control, 1% O₂ or E2[100nM]) for 60 min. Nuclear localization was verified in the 480 excitation channel and probe functionality was determined by subjecting cells to a reducing and an oxidizing agent. Photomicrographs were taken at 400 nm and 480 nm excitation with emission detected at 510 nm for both channels. Fluorescent intensities for nuclei were quantified with a ratio based on excitations wavelength (Ratio = 400/480). Images were divided utilizing the same ratio (image @ 400/ image @ 480) with MetaMorph software and pseudo-colored to allow for visualization of changes in nuclear redox state. Initial calculated ratios and images demonstrated baseline nuclear redox heterogeneity. Addition of physiological stimuli (hypoxia and E2) showed marked increase, visually and quantitatively, in nuclear oxidation after 60 min compared with the initial ratio and controls. Therefore, we conclude that both hypoxia and E2 induce a shift in the nuclear redox state toward oxidation, supporting a role for reactive oxygen species in nuclear signaling.

COMPARISON OF THE INTRA-HOST QUASISPECIES OF TAURA SYNDROME VIRUS IN ACUTE AND CHRONIC INFECTIONS IN *LITOPENAEUS VANNAMEI*

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Taura Syndrome (TS), a severe viral disease affecting penaeid shrimp aquaculture, is caused by Taura Syndrome Virus (TSV). TSV is an icosahedral, non-enveloped viral particle approximately 30 nm in diameter which contains a single-stranded, positive-sense RNA genome of 10,205 bases in length. RNA viruses have been shown to exist within the host as a diverse population of mutants known as a quasispecies. The quasispecies nature of TSV was investigated and the quasispecies characteristics of acute and chronic TSV infections in experimentally infected Kona stock *Litopenaeus vannamei* were compared to determine if the chronic TSV infection exhibits increased intra-host genetic heterogeneity relative to the acute infection. A total of 50 animals were injected intramuscularly with 0.2 ml of TSV infected homogenate per gm of body weight and maintained at 27°C in a maturation tank of aerated artificial seawater at salinity 15 ppt. for 28 d. By 7 d post injection, 8 shrimp were moribund with signs of acute TS. Hemolymph was collected from these animals and RNA extracted. At 28 d post-injection, 7 survivors remained which showed signs of chronic TS. Hemolymph was collected from the 7 survivors and RNA extracted. All extracted RNA samples tested positive for TSV by RT-PCR. High Fidelity RT-PCR and cloning of the viral CP2/VP1 gene was performed. Sequencing was performed on 20 to 25 clones per sample to allow for analysis of individual intra-host viral genomes. The chronic TS infected group had a significantly greater overall quasispecies complexity and diversity than the acute TS infected group. Conclusion: These results support the hypotheses that TSV exists within the host as a quasispecies and that chronic TSV infections show greater intra-host genetic heterogeneity than acute TSV infections in *Litopenaeus vannamei*.

ENHANCEMENT OF BETA CELL VIABILITY THROUGH THE USE OF A FUSION PROTEIN

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The functional loss and death of beta cells through oxidative stress plays a principal role in the development of insulin-dependent diabetes mellitus. A promising treatment option for type one diabetes is intrahepatic islet cell transplantation. Unfortunately, islets are lost during isolation and transplantation due to hypoxic conditions and oxidative stress. Previous work from our lab has shown that reactive oxygen species (ROS), such as superoxide (O_2^-) and hydroxyl radical (OH^-) are particularly damaging to mitochondrial DNA. mtDNA receive some protection through a base excision repair pathway. One component of this repair pathway is a DNA glycosylase, which removes adducted bases and cleaves the DNA backbone on the 3' side of the lesion. We hypothesize that oxidative stress is a central component of the pathogenesis of type one diabetes and a key factor affecting islet cell transplantation to treat type one diabetes. To test this hypothesis, we stably transfected a radiation induced rat insulinoma cell line (INS-1) with a plasmid containing the DNA repair glycosylase, 8-oxoguanine DNA glycosylase (hOGG) fused to a mitochondrial targeting sequence (MTS). In addition, we used the protein transduction domain (PTD) of the HIV-1 transcriptional activator (TAT protein) fused to the DNA repair glycosylase, Endonuclease III (EndoIII), as a delivery method into β -cells isolated from Sprague-Dawley neonates. The INS-1 cells stably transfected with MTS-hOGG, and the β -cells transduced with MTS-OGG-TAT were both treated with alloxan, a reactive oxygen species generator, and mtDNA repair and cellular viability were analyzed. The hOGG1 transfected INS-1 cells showed enhanced viability and mtDNA repair. The cultured beta cells also showed enhanced viability. We believe that this fusion protein has the potential to increase the viability of islets used in transplantation to treat type one diabetes.

PROGRESS IN THE SEARCH FOR CF-CAUSING CFTR MUTATIONS IN DOGS

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Naturally-occurring, cystic fibrosis-causing mutations in the CFTR gene have not been identified in any non-human animal species. Since domestic dogs are known to develop medical conditions associated with atypical CF in humans, (e.g. idiopathic bronchiectasis, pancreatitis, and aplasia of the vas deferens), we hypothesized that some dogs also carry CFTR mutations. Temporal temperature-gradient gel electrophoresis (TTGE) was used to screen dogs for CFTR mutations. Canine whole blood was obtained from veterinary clinics and diagnostic laboratories. We screened DNA from 182 dogs diagnosed with pancreatitis, 14 dogs diagnosed with bronchiectasis, and 167 dogs admitted to clinics for any illness (at-large dogs). Thus far, 27 dogs have been identified with one of four missense mutations: R812W, P1281T, R1456W and P1464H. P1281T and P1464H mutations occur in relatively unconserved residues. R1456W is analogous to the human R1453W mutation, which has about 20% of normal CFTR function and is associated with pancreatitis and panbronchiolitis. R812W disrupts a highly conserved protein kinase A (PKA) recognition site within the regulatory domain. Mutations that disrupt this PKA recognition site in human CFTR have been associated with male infertility and mild CF. We conclude that naturally occurring CFTR mutations are relatively common in domestic dogs and can be detected with TTGE.

NS1 AND NS2 OF RESPIRATORY SYNCYTIAL VIRUS TARGET MULTIPLE COMPONENTS OF THE INTERFERON INDUCTION AND RESPONSE PATHWAYS OF THE INNATE IMMUNE SYSTEM

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Respiratory Syncytial Virus (RSV) belongs to genus *Pneumovirus* and family *Paramyxoviridae*. RSV infects the epithelia of respiratory airways and is the most common cause of bronchiolitis in children under one year of age in the US. RSV leads to the hospitalization of more than 100,000 infants each year and has very limited and expensive therapeutic options that do not significantly alter the course of the infection. Moreover, RSV has no licensed vaccine. Type I Interferons (IFNs) are produced by host cells upon virus infection, and are key mediators for initiation of antiviral responses by the innate immune system and the later recruitment of the adaptive immune system. RSV is able to inhibit this crucial induction of type I IFNs, circumventing the host's antiviral defenses thus leading to disease. This mechanism of inhibition is confounded in infants who have less developed immune systems leading to a possibility of more severe disease. RSV's genome encodes two NS, or Non-Structural proteins; NS1 and NS2. These NS proteins were found to be the key players in inhibiting production of IFNs and IFNs' antiviral effects. Our research focuses on characterizing the functions and functional domains of NS proteins and has identified key host targets. NS1 was found to decrease steady state levels of TRAF3 and IKK ϵ which are key signaling components of the IFN induction pathway. NS2 was described by us and others to degrade STAT2, thus inhibiting the signaling pathway required for the induction of the antiviral effects of type I IFNs. The C-terminal 20 amino acids (AA) of NS1 were not required for its effect on TRAF3, whereas the C-terminal 10 AAs were required for the reduction of IKK ϵ . The C-terminal 4 AAs of NS2 were required for its ability to degrade STAT2. Finally, we discovered that NS1 and NS2 can interact *in vitro* and can individually interact with the host MAP1B protein. Characterizing the functions and functional domains of NS proteins should eventually allow the production of an attenuated RSV strain for vaccine purposes and allow for the design of novel antiviral therapies.

THE ROLE OF SLINGSHOT PHOSPHATASE AND COFILIN AS MEDIATORS OF VSMC MIGRATION AND NEOINTIMA FORMATION

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Vascular smooth muscle cell (VSMC) migration is a critical component of neointima formation following vascular injury. Ultimately, rearrangement of the actin cytoskeleton is essential for cell migration and our recent findings demonstrated that cofilin, a protein which depolymerizes F-actin is critical for VSMC migration. Cofilin activation (dephosphorylation) at Ser3 is regulated by the Slingshot (SSH) phosphatase family. Our studies confirm that all three SSH isoforms (SSH1, -2, -3) are expressed in both cultured VSMCs and carotid artery tissue. In vitro studies examining the role of the SSH isoforms on cofilin activation and VSMC migration demonstrated that only SSH1 was involved in the regulation of PDGF-induced VSMC migration. Thus, we hypothesized that cofilin activation by SSH1 is a key mechanism regulating VSMC migration during neointima formation following vascular injury. To test this hypothesis, carotid arteries were injured with a Fogarty 2F arterial embolectomy catheter. The degree of vascular injury was quantified 14 days post injury by comparing wall thickness, cross-sectional wall area and media/intima ratio in H&E stained cross sections. Immunoblot analysis revealed that both cofilin and SSH1 expression were elevated at 4, 7 and 14 days post injury (vs. non-injured). Furthermore, IHC staining of cross sections from injured arteries confirmed that localization of both cofilin and SSH1 expression was primarily in the neointima. Taken together, our studies suggest that SSH1 dependent-cofilin activation in VSMC migration may potentially contribute to neointima formation following vascular injury in vivo.

DIFFERENTIAL PROTEOMIC ANALYSIS OF RICKETTSIA PROWAZEKII PROPAGATED IN DIVERSE HOST BACKGROUNDS

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Rickettsia prowazekii is an obligate, intracellular, parasitic bacterium that is vectored by the human body louse. This pathogen, the causative agent of epidemic typhus, has a small genome predicted to code for 835 proteins. The obligate nature of *Rickettsia prowazekii* intracellular growth places severe restrictions on the analysis of rickettsial gene expression. Identifying proteins that are differentially expressed between strains that vary in virulence, or when the rickettsiae transition between different hosts, is critical to an understanding of rickettsial pathogenicity. We employed an LC-LTQ-Orbitrap mass spectrometer that allows the simultaneous acquisition of high precision MS-only data and tandem mass spectrometry (MS/MS) sequence data. We have developed a novel combination of commercially available algorithms and in-house software that utilizes both the quantitative MS-only data and comprehensive peptide coverage generated from MS/MS. This method results in the assignment of peptide identities with intensity values, allowing for the differential comparison of complex protein samples. Additionally, quantitative MS-only based analyses allow for statistical comparisons within data sets to provide confidence in peptide assignment and differential protein abundance data. Using these protocols we are able to identify proteins within a sample and to differentially compare samples, yielding quantitative intensity ratios to analyze changes in the total proteome profile of *R. prowazekii* grown in different host backgrounds. Total protein extracted from rickettsiae grown in embryonated chicken egg yolk sacs, a murine fibroblast L929 cell line, the *Ixodes scapularis* ISE6 tick cell line, or a *Spodoptera frugiperda* (SF21) cell line was analyzed. The ability to directly compare, in a global manner, differential rickettsial protein expression provides the means to identify important proteins involved in intracellular survival and virulence. Here we report global changes found during these analyses, including the upregulation of many shock-related protein in the samples cultivated in tissue culture.

SECRETOTRANIN II IS COSTORED WITH CHORIONIC GONADOTROPIN IN SQUIRREL MONKEY PITUITARY GONADOTROPHS

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Secretogranin II (SgII) is a member of the granin family of tyrosine-sulfated, acidic proteins found in neuroendocrine and endocrine cells. SgII, along with other members of the granin family, chromogranins A and B (CgA and CgB), aggregate at low pH and high calcium concentrations and are thought to function as mediators of sorting into secretory vesicles of the regulated secretory pathway. Unlike the constitutive secretory pathway, where secretory products are released in an unstimulated manner, regulated secretion is complex, involving secretory products from vesicles which have undergone processing and maturation and then are released in response to a specific stimulus. One such product is luteinizing hormone (LH), which is stored and secreted with SgII in secretory vesicles of the anterior pituitary gland in response to gonadotropin releasing hormone (GnRH) stimulation. Squirrel monkeys, members of the New World primates, do not express LH in the pituitary, but rather CG is expressed. Thus, we hypothesized that in order for squirrel monkey CG secreted from the pituitary to maintain a regulated secretion, it follows that this protein is stored with a granin protein, most likely SgII. Here, we show that SgII and CG colocalize in squirrel monkey pituitary tissue.

AORTIC ENDOTHELIAL CELLS TRANSPORT MORE GLUCOSE THAN PULMONARY ENDOTHELIAL CELLS *IN VITRO*.

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Diabetes is characterized by hyperglycemia resulting from insulin resistance or deficiency. Increases in intracellular glucose can lead to endothelial dysfunction. While systemic vascular disease is a major cause of morbidity and mortality in diabetics, pulmonary vascular disease is less clinically apparent. Since both circulations are exposed to similar circulating glucose and insulin concentrations, we hypothesize that increased glucose loading of systemic endothelial cells might explain this difference. Using bovine aortic and pulmonary artery endothelial cells from the same animal (AECs or PAECs, respectively), we found that AECs transported 5-fold more glucose than PAECs. Because shear stress is different between the two vascular beds, we examined the effect of shear stress on glucose transport. Flow adapted cells decreased radiolabelled glucose uptake but remained higher in AECs. Glucose enters endothelial cells primarily through the glucose transporter, Glut-1. To determine if an increase in Glut-1 expression might explain the difference in glucose uptake, we compared cells using Western Blot Analysis. AECs expressed more Glut-1 than the PAECs. The increased glucose loading of AECs compared to PAECs may be important in determining the different effects of diabetes in the systemic and pulmonary circulations. This research is supported by the NIH grants R01HL070273 and T32HL076125.

IMPACT OF CYCLIC STRAIN ON INTEGRIN EXPRESSION IN RAT PULMONARY MICROVASCULAR ENDOTHELIAL CELLS

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Detachment of pulmonary microvascular endothelial cells (PMVEC) is observed in mechanical stress-induced, TRPV4-mediated (vanilloid transient receptor potential 4 channel) acute lung injury. Integrin dimers, comprised of α and β subunits, that mediate PMVEC tethering and detachment have not been identified. The goals of this study were to characterize integrin expression and to identify the effect of cyclic strain on integrin expression. Rat PMVEC were cultured on collagen I or pronectin flexwell membranes to 90% confluence and then subjected to 0% or 10% uniaxial strain at 30 cycles/min for 24 h. Using PCR and primers for rat integrins, expression of integrin subunits $\alpha 1$, $\alpha 2$, $\alpha 3$, $\alpha 5$, $\alpha 6$, $\alpha 7$, αv , $\beta 1$, and $\beta 6$, but not $\alpha 4$, $\alpha 8$ or $\beta 3$, was identified in PMVEC, irrespective of strain or matrix composition. However, integrin $\beta 4$ expression decreased with cyclic strain in PMVEC cultured on either matrix. Based on these results, extracellular ligands for integrin dimers, and matrix composition of the alveolar basement membrane, we predict that $\alpha 1\beta 1$, $\alpha 2\beta 1$, $\alpha 3\beta 1$, $\alpha 6\beta 1$, $\alpha 7\beta 1$, and/or $\alpha 6\beta 4$ could tether PMVEC to matrix in vivo and be a target for detachment in TRPV4-mediated acute lung injury. Supported by HL066299 and HL076125.

HYPEROXIA-INDUCED MITOCHONDRIAL DNA DAMAGE IN ALVEOLAR TYPE II EPITHELIAL CELLS.

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Premature infants born with under developed lungs are ill equipped to handle exposure to elevated oxygen tensions. Normal fetal lung development occurs at 3% oxygen (fetal normoxia), which promotes lung branching and maturation. In the preterm infant, the abrupt increase in inspired oxygen disrupts lung development. Hyperoxic exposure impairs type II epithelial cell function and inhibits alveolarization. Hyperoxia increases reactive oxygen species (ROS); these ROS target the cellular structures including mitochondrial DNA (mtDNA). MtDNA is a target of oxidative stress and, when left unrepaired, mtDNA lesions can lead to mitochondrial dysfunction and ultimately cell death. Preliminary data suggests that hyperoxia-induced mtDNA damage inhibits fetal lung morphogenesis. We hypothesize that neonatal exposure to hyperoxia induces mtDNA damage in alveolar type II epithelial cells leading to impaired alveolarization. A fetal lung explant model was used to test this idea. Hyperoxia impaired distal lung branching and was accompanied by mtDNA lesions. A mtDNA repair enzyme targeted to the mitochondria restored fetal lung branching in hyperoxia-exposed fetal lung explants. MtDNA damage was also measured in type II epithelial cells cultured in hyperoxia. These data suggest that mtDNA in alveolar type II epithelial cells is a target of hyperoxia-induced ROS. Future studies will determine if mtDNA damage is a proximate cause of mitochondrial dysfunction and cell death in vivo in a neonatal model of hyperoxia exposure.