

### ROLE OF RAC GUANOSINE TRIPHOSPHATASE IN SIMVASTATIN-MEDIATED ENDOTHELIAL CELL SIGNALING. J.R. Jacobson, W. Chen, J.G.N. Garcia, Section of Pulmonary and Critical Care Medicine, The University of Chicago, Chicago, IL.

The statins are a class of HMG CoA-reductase inhibitors used clinically for their ability to reduce serum cholesterol levels via inhibition of the prenylation pathway. However, not all of their clinical benefits, including both vascular barrier protection and reduced superoxide generation, can be attributed to their lipid-lowering properties. One potential mechanism of these effects is via inhibition of geranylgeranylation, a covalent modification that allows translocation to the cell membrane and activation of the small GTPases Rho and Rac, mediators of cytoskeletal rearrangement. While statins inhibit Rho, we previously reported the paradoxical activation of Rac (Rac-GTP) in endothelial cell (EC) after prolonged treatment with simvastatin (5  $\mu$ M, 16 hours). In this study, upon membrane fractionation and subsequent Western blotting, we report a 37% reduction in Rac translocation to the EC membrane by simvastatin (5  $\mu$ M, 16 hours) relative to untreated control cells, consistent with the inhibition of geranylgeranylation and evidence of a separate mechanism of simvastatin-mediated Rac activation. In addition, as Rac is required for activation of the NADPH oxidase complex and subsequent superoxide anion generation, we examined the effect of simvastatin on the peripheral translocation of P47<sup>phox</sup>, an NADPH oxidase component. Simvastatin affected a 47% reduction in P47<sup>phox</sup> at the EC membrane relative to control cells. Finally, we measured transendothelial electrical resistance and quantified the effects of simvastatin pretreatment on sphingosine 1-phosphate-induced barrier enhancement, an event dependent on Rac. SIP-induced (1  $\mu$ M) EC barrier function was increased by simvastatin (5  $\mu$ M, 16 hours) 70% at peak effect relative to SIP-treated control cells. These data are consistent with a functional role for Rac in simvastatin-mediated EC barrier protection and further define the mechanism by which simvastatin is able to directly modulate EC.

### HYPoxic VENTILATORY RESPONSE IN EARLY LUNG INJURY IS AUGMENTED BY OXYGEN-INDEPENDENT SENSITIZATION OF THE CAROTID BODY. E.J. Jaco, Y. Peng,<sup>2</sup> D. Nethery,<sup>1</sup> J.A. Faress,<sup>1</sup> J.A. Kern,<sup>1,2</sup> N.R. Prabhakar,<sup>2</sup> <sup>1</sup>Division of Pulmonary and Critical Care Medicine and <sup>2</sup>Department of Physiology, Case Western Reserve University, Cleveland, OH.

**Rationale:** Acute lung injury alters ventilatory control by impairing gas exchange. However, even before hypoxemia develops, lung inflammation itself may alter ventilatory control. The objective of the present study was to examine the impact of acute lung injury on ventilatory control by hypoxia and hypercapnia. **Methods:** Experiments were performed on adult male Sprague-Dawley rats challenged with intratracheal injections of either bleomycin (BM; 1 unit) or PBS. Five days after the injections, the extent of lung injury was evaluated, and ventilatory responses to hypoxia (12% O<sub>2</sub>) or hypercapnia (7% CO<sub>2</sub>) were measured by plethysmography in unanesthetized animals and by diaphragmatic EMG in anesthetized animals. Contribution of carotid body sensory afferents to ventilatory patterns was evaluated by comparing responses before and after glomectomy in anesthetized animals. **Results:** BM-treated animals had increased total cell count, percent neutrophils, and protein levels in lavage fluid with no alterations in lung collagen content suggesting acute lung injury but not fibrosis. Core body temperature, PaO<sub>2</sub> and PaCO<sub>2</sub> were comparable between both groups of animals. In unanesthetized animals ( $n = 16$ ), baseline ventilation and the hypoxic ventilatory responses were significantly higher in BM-injected animals compared to control animals (average increases in minute ventilation [V<sub>E</sub>]: BM +214  $\pm$  59 mL/kg/min vs Control +60  $\pm$  8 mL/kg/min;  $p = .003$ ), whereas respiratory stimulation by hypercapnia was not altered to the same degree ( $p = .672$ ). The selective enhancement of hypoxic ventilatory drive was also present in anesthetized, spontaneously breathing animals ( $n = 12$ ) where average increases in respiratory rate [RR] were greater in animals with lung injury ( $p = .036$ ). In contrast, this difference between control and BM-exposed animals was abolished following bilateral glomectomy ( $p = .786$ ). In these same animals, average decreases in RR in response to sudden administration of hyperoxia (FiO<sub>2</sub> change from 0.12  $\rightarrow$  1.0) was significantly greater in the BM-exposed group compared to control animals (BM -13.0  $\pm$  1.0 % vs control -9.7  $\pm$  1.0 %;  $p = .041$ ), and these differences were abolished following glomectomy ( $p = .128$ ). **Conclusions:** These data demonstrate that afferent sensory input from the carotid body contributes to a selective enhancement of hypoxic ventilatory drive in the absence of pulmonary fibrosis and arterial hypoxemia in early BM-induced lung injury.

### THE SOY ISOFLAVONE GENISTEIN BLOCKS TRANSFORMING GROWTH FACTOR $\beta_1$ -STIMULATED LUNG FIBROBLAST TO MYOFIBROBLAST TRANSFORMATION.

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**Rationale:** Genistein is a dietary isoflavone and a broad-spectrum tyrosine kinase inhibitor contained in soy products. An epidemiologic study of asthma revealed that subjects with high consumption of dietary soy isoflavones had better lung function than those with lower intake. In a guinea pig model of allergic asthma, genistein reduced methacholine-induced bronchoconstriction. To explore the mechanisms underlying these observations, we tested genistein's ability to block lung myofibroblast differentiation, a key phenotypic change in asthmatic airway remodeling. **Methods:** Human fetal lung fibroblasts (IMR-90) were grown to subconfluence in DMEM containing 10% FBS, serum-deprived for 24 hours, and treated with genistein (10  $\mu$ M) for an additional 24 hrs. Cells were then stimulated with TGF- $\beta_1$  (2 ng/mL) for 24 hours, and  $\alpha$ -smooth muscle actin ( $\alpha$ SMA) expression, a marker of the myofibroblast phenotype, was assessed by both immunoblot and immunofluorescence microscopy. To determine the intracellular mechanism of inhibitory actions by genistein, we also assessed phosphorylation of Smad2 by immunoblot in the presence and absence of genistein after 30-minute stimulation with TGF- $\beta_1$ . **Results:** Treatment with genistein resulted in 54.9  $\pm$  17.1% reduction in TGF- $\beta_1$  induced expression of  $\alpha$ SMA. Immunofluorescence microscopy revealed a decrease in  $\alpha$ SMA staining intensity and stress fiber formation in genistein-treated cells. In addition, Smad2 phosphorylation was inhibited by 71.1  $\pm$  16.7% in genistein-treated cells. **Conclusions:** These results demonstrate that genistein interferes with TGF- $\beta_1$ -stimulated myofibroblast differentiation and suggest that tyrosine kinase inhibition may have a role in modulation of asthmatic airway remodeling.

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### A DIRECT HAPLOTYPE TYPING METHOD OF PUTATIVE FUNCTIONAL VARIANTS IN PROMOTER REGIONS OF THREE CANDIDATE GENES IN ACUTE LUNG INJURY.

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**Rationale:** Due to their functional relevance, single nucleotide polymorphisms (SNPs) and short tandem repeats (STRs) have been used in association studies of complex diseases. The combination of both types of polymorphisms in short genomic regions (< 500 bp), termed SNPSTRs, constitutes a powerful tool as it allows for the empiric determination of gametic phase, or haplotype, thus adding a new level of complexity over single variants. Our aim is to select a set of SNPSTR pairs in candidate genes in acute lung injury (ALI) and to develop a multiplex method to directly determine the haplotypes of each of these SNPSTR pairs. **Methods:** Candidate genes identified by microarray analysis of animal models of ALI were selected. Functional relevance of SNPs and STRs in the promoter region was established for the selected candidate genes using *PubMed*. *Primer3* software (<http://frodo.wi.mit.edu/cgi-bin/primer3/primer3\_www.cgi>) was used to design SNPSTR primers as well as the complementary fluorescently labeled primers for SNP alleles. A three-step direct haplotyping method was performed as follows: (1) coamplification of the genomic regions of interest by multiplex PCR, (2) linear amplification of the amplicons with FAM- and HEX-labeled fluorescent primers, and (3) identification of haplotypes by *GeneMapper* software v3.7. **Results:** Three candidate genes (*IL-10*, *HMOX-1*, and *CXCL2*) were identified with reported functionally relevant SNPSTRs and were arranged into a multiplex protocol. Genotyped variants were -1087 G/A SNP and the -1121 C<sub>A</sub> STR for *IL-10*, -413 A/T SNP and the -199 G<sub>T</sub><sub>n</sub> for *HMOX-1*, and -437 A/G SNP and the -665 C<sub>A</sub><sub>n</sub> STR for *CXCL2*. A Coriell CEPH DNA panel, consisting of individuals of European and African American descent (23 and 24 individuals, respectively), was then used to develop our haplotyping method. SNP alleles and STR repeats were confirmed with direct DNA sequencing. **Conclusion:** This SNPSTR method represents an efficient and robust approach to obtain direct haplotypes in three candidate genes, which will advantage association studies of genetic variants in ALI.

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### ABNORMAL CYCLIC ADENOSINE MONOPHOSPHATE PRODUCTION IN HUMAN CORTICAL FRAGILE X NEURAL TISSUE: A PROOF OF PRINCIPLE STUDY. D.J. Kelley,

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Fragile X syndrome, the most common inheritable cause of mental retardation, is due to a CGG trinucleotide amplification on the X chromosome (Xq27.3) in the 5' untranslated region of the fragile X mental retardation 1 gene (FMR1) that suppresses production of the fragile X mental retardation protein (FMRP). Based on a decade of studies by Berry-Kravis who established the cyclic AMP (cAMP) cascade as dysfunctional in fragile X using a variety of non-neuronal cell types and who identified a direct relationship between levels of FMRP expression and cAMP levels in a mouse neural cell line overexpressing FMRP (Berry-Kravis and Ciurlionis, 1998), we hypothesized that human fragile X neural tissue would produce less cyclic AMP upon stimulation. Using an assay in which reductions in fluorescence intensity are associated with increasing cAMP levels (Mediomics, LLC), we quantified cAMP levels in neurospheres (NS) and differentiated cells (DC) from the human cortical fragile X (M049) and control (M037, M045, M046) fetal stem cell lines incubated with the phosphodiesterase inhibitor IBMX (3-isobutyl-1-methylxanthine) in the presence or absence of forskolin, an adenylate cyclase agonist. As hypothesized, the fragile X differentiated cell line (fractional decrease in raw fluorescence [FDRF] = 0.32) shows a stimulated cAMP production that is reduced relative to the three differentiated control stem cell lines (mean FDRF  $\pm$  SD = 0.70  $\pm$  0.09). When differentiated cells are compared to neurospheres, all three control cell lines showed a marked increase in the levels of cAMP production in differentiated cells compared to undifferentiated neurospheres (mean FDRF difference [DC-NS] = 0.52); however, stimulated cAMP levels in differentiated cells and neurospheres are comparable in the fragile X line (FDRF difference [DC-NS] = 0.05). To our knowledge, these results are the first demonstration of an altered cAMP cascade in human fragile X neural tissue and suggest a developmental role for FMRP in the cAMP cascade. With only one human fragile X fetal stem cell line available, these results require replication with more fragile X neural tissue samples as they become available. Nevertheless, this proof of principle study identifies the human fragile X cAMP cascade as a potentially useful pharmacotherapeutic target that deserves further investigation.

### THE EFFECT OF ACIDOSIS AND HYPERKALEMIA ON THE IKr BLOCKING ACTION OF ANTIARRHYTHMIC DRUGS. C. Lin, X. Ke, I. Cvetanovic, V. Ranade, J.C. Somborg,

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Regional myocardial acidosis resulting from the impaired coronary blood flow has been observed in both animal models and in man. The ischemic myocardium also releases potassium into the extracellular space, which can cause regional hyperkalemia. Antiarrhythmic agents are frequently prescribed for patients with ischemic heart disease and regional changes in pH and potassium may alter the effect of these agents. In this study, we evaluated the effect of extracellular acidosis and hyperkalemia on the action of IKr blocking antiarrhythmic drug-quinidine (Q). The IKr channel was studied at room temperature by employing human-ether-a-go-go-related gene (HERG) expressed in *Xenopus oocytes* and two-electrode voltage clamp technique was employed for recording. The pH of the recording bath solution was adjusted with NaOH to 6.8 or 7.4 and the recording bath solutions contained either 5 or 7.5 mmol/L KCl (5 or 7.5 K). The recording solution with 5 K, pH 7.4 represented the normal condition and 7.5 K, pH 6.8 represented acidic and hyperkalemic conditions. Q 3, 10, and 30  $\mu$ M when applied at 5 K, pH 7.4 inhibited current by 17  $\pm$  3, 39  $\pm$  3, and 63  $\pm$  4%. The percentage current block by Q at 7.5 K, pH 7.4 was similar to current block at 5 K, pH 7.4. Q at 7.5 K, pH 7.4 decreased HERG current by 18  $\pm$  1, 42  $\pm$  3, and 65  $\pm$  3%. But if Q was applied at 5 K, pH 6.8, the HERG inhibitory effect of Q was decreased, and 3, 10, and 30  $\mu$ M Q produced 8  $\pm$  2, 24  $\pm$  3, and 50  $\pm$  4% current block. Q 3, 10, 30  $\mu$ M administered in acidic and hyperkalemic condition (7.5 K, pH 6.8) caused 13  $\pm$  2, 25  $\pm$  2, and 47  $\pm$  2% current inhibition, which was similar to the inhibitory effect of Q at 5 K, pH 6.8. There was a significant difference in current block by Q between 5 K, pH 7.4 and 5 K, pH 6.8, and there was also a significant difference in current inhibition by Q between 5 K, pH 7.4 and 7.5 K, pH 6.8 ( $p < .05$ ). Our data suggest that extracellular acidosis (pH 6.8)