moniae by acid and physiologic concentrations of nitrite. Mice received subcutaneous pantoprazole or saline every 12 hours for 3 days followed by orogastric inoculation of 100 colony-forming units of VRE or *K. pneumoniae* in combination with subcutaneous clindamycin. The presence and density of the pathogens in stool were monitored by plating samples onto selective media. Student's *t*-test was performed to compare the pH of gastric contents from pantoprazole versus saline-treated mice and chi-square test was performed to compare the frequency of acquisition of stool colonization with the pathogens. **Results:** Exposure to pH 2 buffer or fasting human gastric contents resulted in significant killing of the pathogens and physiological concentrations of nitrite-enhanced killing. In comparison to saline controls, pantoprazole-treated mice had elevated gastric pH (mean ± SD, 4.8 ± 0.87 versus 2.3 ± 0.3; $p < .001$) and increased frequency of establishment of intestinal colonization with the pathogens (75% versus 25% for VRE, $p = .01$; 100% versus 40% for *K. pneumoniae*, $p < .001$). For all mice that developed colonization, high densities of the pathogens were measured (range 6–9.8 $\log_{10}$CFU/g of stool). **Conclusions:** Proton pump inhibitor administration facilitates establishment of intestinal colonization by VRE and *K. pneumoniae* in clindamycin-treated mice. These data suggest that the widespread use of proton pump inhibitors in health care facilities could contribute to the dissemination of nosocomial pathogens.

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PHOSPHORYLATION OF β-CATENIN BY PROTEIN KINASE A IN VASCULAR SMOOTH MUSCLE CELLS. S. Taurin, N. Sandbo, Y. Qin, N.O. Dulin, Department of Medicine, The University of Chicago Chicago, IL.

Beta-catenin is a transcriptional coactivator that promotes cell proliferation by induction of genes such as cyclin D1, c-myc, and others. Enhanced β-catenin signaling was observed in many cancer cells and in vascular smooth muscle cells (VSMC) during restenosis. The canonical mechanism of regulation of β-catenin involves its phosphorylation by glycogen synthase kinase 3 (GSK3) that targets β-catenin to ubiquitination and degradation by the proteasome system. Mitogenic factors promote β-catenin signaling through inhibition of GSK3, resulting in reduced β-catenin phosphorylation, its stabilization, and subsequent accumulation in the nucleus, where it stimulates gene transcription. In the present study, we investigated the mechanism of VSMC proliferation induced by extracellular ATP (a VSMC mitogen relevant to the pathogenesis of atherosclerosis and restenosis). We found that (1) ATP potently induces the cyclin D1 transcription in VSMC; (2) stimulation of cyclin D1 transcription by ATP is dependent on β-catenin signaling and on protein kinase A (PKA) activity; (3) β-catenin is phosphorylated by PKA in vitro and in intact cells at two novel sites, Ser-552 and Ser-675; (4) phosphorylation by PKA promotes the transcriptional activity of β-catenin; (v) mutation of Ser-675 attenuates the promoting effect of PKA; (6) phosphorylation by PKA does not affect the GSK3-dependent phosphorylation of β-catenin, its stability or intracellular localization; but (7) phosphorylation at Ser-675 site promotes the binding of β-catenin to its transcriptional coactivator, CREB-binding protein (CBP). In conclusion, this study identifies a novel, noncanonical mechanism of modulation of β-catenin signaling through direct phosphorylation of β-catenin by PKA, promoting its interaction with CBP.

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SPHINGOSINE-1-PHOSPHATE-MEDIATED ENDOTHELIAL CELL MOTILITY IS REGULATED BY $S1P_1$ RECEPTOR, LIPID PHOSPHATE PHOSPHATASE-1, AND SPHINGOSINE KINASE 1. I. Gorshkova, E. Berdyshev, B. Saatian, Y. Zhao, S. Pendyala, J.G.N. Garcia, V. Natarajan, The University of Chicago, Chicago, IL.

The extracellular effects of S1P, a naturally occurring and platelet-derived bioactive lipid molecule, are mediated by $S1P_{1-5}$ family of G protein–coupled receptors. The exact role of different S1P receptors and signaling pathways regulating S1P-induced cell motility is not completely defined. Human pulmonary artery endothelial cells (HPAEC) expressed $S1P_1$, $S1P_3$, and $S1P_4$ receptors with a predominance of $S1P_1$, as determined by RT PCR. Exposure of HPAEC to S1P resulted in a potent induction of cell motility as determined by a scratch wound healing assay and by monitoring changes in transendothelial electrical resistance of wounded HPAEC by ECIS. Inverse agonist of $S1P_1$ receptor, SB649146, and $S1P_1$ siRNA attenuated S1P-induced EC motility. Furthermore, overexpression of sphingosine kinase 1 wild type and $S1P_1$ wild type increased intracellular content of S1P as well DHS1P (DHS1P > S1P) with a concomitant loss of cell motility in response to exogenous S1P. Overexpression of lipid phosphate phosphatase (LPP)1 wild type that enhanced hydrolysis of exogenous S1P blocked S1P-induced cell motility. These results suggest that a balance between $S1P_1$ expression and intracellular content of S1P and DHS1P seems to be critical for maintaining cellular sensitivity to external S1P stimulation.

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IDENTIFICATION OF ORGAN-SHARED AND STRAIN-INDEPENDENT CANDIDATE GENES DURING COMBINED VENTILATOR-ASSOCIATED LUNG AND KIDNEY INJURY. D.N. Grigoryev,[1] S. Sammani,[2] S.-F. Ma,[2] S.Q. Ye,[2] K.C. Barnes,[1] H. Rabb,[1] J.G.N. Garcia,[2] [1]Johns Hopkins University, Baltimore, MD; [2]The University of Chicago, Chicago, IL.

Combined ventilator-induced lung injury (VILI) and acute renal failure (ARF) have 80% mortality in the ICU. We hypothesized that analysis of global genetic changes in lung and kidney tissues during developing of ALI will identify similar genomic responses to injury that could underlie injurious effects in both organs. We performed gene expression profiling of lung and kidney tissues in three different mouse strains in a model of VILI, speculating that key transcriptional changes would be tissue and strain independent. Three inbred mouse strains C57BL/6J (B), C3H/HeJ (H), and DBA/2J (D) were selected and young 4-week-old mice were mechanically ventilated (V_t = 17 mL/kg, 110 breaths/min) for 2 hours (n = 6–8) and compared to spontaneous ventilated (n = 6) mice. Analysis of bronchoalveolar lavage proteins demonstrated a significant ($p < .05$) increase in B, H, and D strains by 133 ± 47SE μg/mL, 125 ± 28SE μg/mL, and 108 ± 53SE μg/mL, respectively, with no significant differences between groups at this age. Gene expression profiles of VILI-affected lung and kidney tissues were generated using MG-U74A (12,488 genes) GeneChips (n = 12) and simultaneously analyzed using *GC-Robust Multichip Average* and *Significance Analysis of Microarrays* softwares. Genes with the lowest false discovery rate (q = 0.092%) and ± 50% fold change (FC) were considered affected by VILI in both tissues. Gene Ontology (GO) analysis of identified genes was conducted by GenMAPP and MAPPFinder tools and biological processes with z score > 1.96 were considered significantly affected by VILI. This cross-tissue and cross-strain analysis identified 58 genes that behaved similarly in lung and kidney tissues during VILI. Gene ontology analysis of these genes revealed significant up-regulation of genes involved in response to stress (z = 2.51), including Mig-6 ($5.97FC_{Lung\ (L)}$, $3.02FC_{Kidney\ (K)}$) and Gadd-45{γ ($3.68FC_L$, $6.06FC_K$); and transcription activity (z = 4.04), including Ceb/pδ} ($3.97FC_L$, $3.74FC_K$) and Ceb/pβ ($2.87FC_L$, $4.58FC_K$). These data demonstrate that VILI activates specific transcription factors and stress responsive genes in both lung and kidney in all tested strains. Detailed studies of these pathways can reveal organ-shared VILI targets for the development of new therapeutic strategies for this devastating disorder.

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ZYXIN IS INVOLVED IN THROMBIN SIGNALING VIA INTERACTION WITH PROTEASE-ACTIVATED RECEPTOR 1. J. Han, J. Niu, G. Liu, T. Voyno-Yasenetskaya, University of Illinois at Chicago, Chicago, IL.

Introduction: Thrombin-induced increase in endothelial monolayer permeability depends on reorganization of cytoskeletal structures. Protease-activated receptor 1 (PAR-1) is the main receptor responsible for thrombin signaling in endothelial cells. Zyxin, a low-abundance phosphoprotein, serves as a molecular adaptor that mediates the productive juxtaposition of protein partners. Zyxin may participate in aspects of cytoskeletal assembly and dynamics by recruiting components of actin assembly machinery to specific sites in the cells. The subcellular distribution of zyxin is critical to its functions. In human umbilical vein endothelial cells (HUVECs), thrombin stimulation induces zyxin targeting to actin stress fibers. We proposed that zyxin participates in thrombin induced cytoskeletal reorganization by interaction with PAR-1. **Methods:** Coimmunoprecipitation and GST fusion protein pull-down assay were performed to identify the domains of zyxin to interact with carboxyl-terminal PAR-1 in COS-7 cells. Confocal immunofluorescence microscopy was utilized to observe the subcellular distribution of zyxin, PAR-1, actin stained with appropriate antibodies against these proteins. Zyxin-specific siRNA duplexes are generated to knock down the expression of endogenous zyxin. **Results:** We showed that PAR-1 interacted with zyxin both in vitro and in vivo. We have shown that LIM domains 1 and 2 of zyxin interacted with C-terminal domain of PAR-1. In human umbilical vein endothelial cells (HUVECs), thrombin promotes zyxin targeting to actin stress fibers. Down-regulation of zyxin using siRNA and disruption of the interaction between zyxin and PAR-1 resulted in reduced cellular responses to thrombin stimulation: actin stress fiber formation and serum response element (SRE)-dependent gene transcription. Zyxin-specific siRNA and C terminus of zyxin overexpression did not inhibit thrombin-induced RhoA activity, which plays a key role in thrombin-induced stress fiber formation and SRE activity and knockdown of endogenous zyxin could still attenuate the active RhoA-induced SRE activity, suggesting that zyxin may regulate thrombin-induced actin reorganization and SRE activity in an RhoA-independent manner. These results suggest that zyxin is involved in thrombin signaling by direct interaction with PAR-1.

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EFFECT OF CONCENTRATED BRONCHOALVEOLAR LAVAGE FROM HIV-POSITIVE SUBJECTS TO PRIME ALVEOLAR MACROPHAGES AGAINST *MYCOBACTERIUM TUBERCULOSIS*. C.J. Huffer, R.B. Day, S. Lodhi, Y. Wang, P.A. Smith, H.L. Twigg III, Indiana University School of Medicine, Indianapolis, IN.

Background and Objective: *Mycobacterium tuberculosis* (MTB) is a common cause of pulmonary disease in HIV-infected patients. Normal alveolar macrophages (AM) phagocytize MTB and are then activated by cytokines to kill ingested MTB. We have previously shown that HIV alveolar AM are intrinsically resistant to MTB infection compared to normal AM, likely due to an AM-activating factor secreted by alveolar T cells. In this work, we examined the ability of concentrated BAL from HIV-infected subjects to activate AM to kill MTB. **Methods:** BAL from HIV-infected subjects before and after starting antiretroviral therapy were concentrated 5 times using 10 kD spin columns and then cultured with normal AM for 24 hours. Virulent and avirulent MTB at an MOI of 1:1 was then added and subsequent infection determined using a BACTEC methodology. BAL cytokine concentrations were measured using a cytometric bead array assay system. Time to initial and maximal infection of the AM by MTB was assessed. **Results:** Virulent and avirulent MTB grew equally well in AM not treated with HIV BAL. However, pretreatment with HIV BAL significantly delayed AM infection (both initial and maximal) by avirulent MTB compared to virulent MTB (initial infection: 12.2 ± 0.3 days vs 9.8 ± 0.3 days, $p < .001$; maximal infection measured at day 16: 0/39 avirulent vs 34/39 virulent, $p < .001$). There was not a significant correlation between levels of IFN-γ in BAL and time to initial infection by MTB. While treatment with antiretroviral agents lowered IFN-γ concentrations in BAL, there was no difference in BAL from untreated compared to treated subjects in the ability to prime AM against MTB. **Conclusions:** BAL from HIV-infected subjects primes AM against infection against avirulent MTB but not virulent MTB. This is consistent with the known sensitivity of avirulent MTB to macrophage activating cytokines. The lack of correlation between BAL IFN-γ concentrations and the ability to prime AM against MTB suggests that other soluble factors are involved and are currently under investigation.

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CARDIOMYOPATHY RESULTS IN DYSREGULATION OF ENDOCARDIAL THROMBOMODULIN AND AN ALTERED PROTEIN C ACTIVATION PATHWAY. A.Y. Kim, H.M. Russell, J. Raman, M. Gupta, X. Tian, R.M. Lang, J.L. Miller, The University of Chicago, Chicago, IL.

Cardiomyopathy (CM) is associated with a significant risk for thrombosis and an increased risk for thromboembolic events. Early studies invoked blood stasis or flow disturbance as the principal underlying mechanisms. More recent evidence has linked CM with a hypercoagulable state, as demonstrated by elevated levels of thrombin-antithrombin complexes, prothrombin fragment F1+2, and fibrinopeptide A, all indices of thrombin activation. Thrombomodulin (TM), an integral membrane protein found on the endocardial endothelium (EE), is a potent thrombin inhibitor via activation of the protein C pathway. We hypothesized that the increased ventricular wall tension characteristic of CM would result in reduced TM protein expression on the surface of EE, which would, in turn, result in an impairment of local activated protein C (APC) activity, thereby increasing the risk for thrombosis. Using a rabbit pacing-induced CM model, we quantified TM protein expression on EE over time by digital immunohistochemical analysis (n = 4/time point), expressed as arbitrary units (AU)/mm of left ventricular (LV) perimeter. Baseline TM staining of LV endocardial segments was 1,669 ± 128 AU/mm. After 8 weeks of rapid pacing, TM protein expression on EE was markedly decreased (175 ± 41 AU/mm, $p < .0001$ vs control LV). TM protein expression remained significantly low after 15 weeks of pacing compared to control LV (142 ± 32 AU/mm, $p < .0001$). Western blot analysis confirmed the immunohistochem-