studies were focused on the function of the 5-HT4 receptor in the CNS, none of the studies showed its expression and function in the endothelial cells. In the present study, we provide evidence for the first time that 5-HT4 receptor is expressed in the human umbilical vein endothelial cells (HUVECs). We demonstrate the transcription of 5-HT4 mRNA in the HUVECs using reverse transcription polimerase chain reaction. Additionally, we show 5-HT4 receptor expression in HUVECs by immunoblotting and immunofluorescent analysis with 5-HT4 specific antibody. Importantly, we determine that overexpression of 5-HT4 receptor leads to a pronounced cell rounding and intercellular gap formation in HUVECs. We are currently investigating the mechanism underlying 5-HT4 receptor-induced actin cytoskeleton changes in the endothelial cells. These data suggest that by activating 5-HT4 receptor, serotonin could be involved in regulation of actin cytoskeleton dynamics in the endothelial cells that may affect the endothelial barrier integrity.

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OVEREXPRESSION OF ANTIOXIDANT ENZYMES IN LUNG EPITHELIAL CELLS PREVENTS DAMAGE FROM AIRBORNE PARTICULATE MATTER–INDUCED OXIDANT INJURY. S. Soberanes,[1] V. Panduri,[1] H. Wang,[1] G. Mutlu,[1] G.R. Budinger,[1] D.W. Kamp,[1,2]
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Elevated levels of air pollution particles are associated with increased morbidity and mortality from acute and chronic cardiopulmonary injury. One mechanism underlying these effects involves oxidative damage to lung epithelial cells. We previously showed that Düsseldorf particulate matter (DPM) causes alveolar epithelial cell DNA damage and apoptosis by a mitochondria-regulated death pathway. In this work, we used several different types of well-characterized particulates to determine whether mitochondria-derived reactive oxygen species (ROS) are the primary cause of apoptosis. Washington particulate matter (WPM), residual oil fly ash (ROFA), and DPM each increased ROS production (ASSAY HERE) and apoptosis (DNA fragmentation) as compared to inert particulates such as desert dust (DD) and Mount St Helen volcanic dust (MSH) (Table 1). Notably, WPM, ROFA, and DPM did not induce ROS production or apoptosis in ;gr0-A549 cells, which are incapable of mitochondrial ROS production. We also found that overexpression of MnSOD or CuZnSOD using adenoviral expression vectors blocks DPM-induced A549 cell ROS production and apoptosis as compared to null/sham adenoviral controls. We conclude that a diverse group of toxic airborne particulates, unlike inert particulates, induce mitochondria-derived ROS production and lung epithelial cell apoptosis. We propose that strategies aimed at reducing mitochondrial-derived ROS levels will protect the lung epithelium exposed to airborne particulate matter.

TABLE 1 Elevated Levels of ROS in Airborne Particulate Matter Increases A549 Cell Apoptosis

	DPM	*WPM*	*ROFA*	*DD*	*MSH*
ROS (% over control)	301.3 ± 20.0*	319.3 ± 8.0*	146.1 ± 15*	8.38 ± 2.1	9.77 ± 1.7
DNA frag. (% over control)	70.4 ± 5.9*	37.4 ± 5.3*	58.8 ± 9.1*	0.98 ± 1.1	0.98 ± 0.25

Mean ± SEM ($n = 3$). *$p < .05$ vs untreated cells.

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EXPANSION OF SUBPOPULATIONS OF *BACTEROIDES* THAT HYPERPRODUCE BETA-LACTAMASE PREVENTS CEFTRIAXONE-INDUCED DISRUPTION OF THE HUMAN INTESTINAL MICROFLORA IN A CONTINUOUS-FLOW CULTURE MODEL. U. Stiefel,[1,2] N.J. Pultz,[2] C.J. Donskey,[1,2] [1]Case Western Reserve University, [2]VA Medical Center, Cleveland, OH.
Background: The indigenous intestinal microflora may generate beta-lactamase activity in response to beta-lactam antibiotic selective pressure. We hypothesized that expansion of subpopulations of *Bacteroides fragilis* group organisms that hyperproduce cephalosporinases could prevent disruption of the indigenous microflora in the face of ceftriaxone selective pressure. **Methods:** In vitro mixing studies were performed to determine the ability of *Bacteroides fragilis* TAL3636 (BF3636), a hyperproducer of a broad-spectrum beta-lactamase, to raise the minimum bactericidal concentration (MBC) of susceptible organisms to ceftriaxone. Continuous-flow (CF) cultures of human colonic microflora were established with or without the addition of BF3636 and increasing concentrations of ceftriaxone were added to the inflowing media. Beta-lactamase activity, ceftriaxone concentrations, and densities of bacterial populations were monitored. A mouse model was also utilized to evaluate the ability of BF 3636 to preserve colonization resistance in vivo. Concomitant with injections of ceftriaxone, mice received orogastric inoculation of CF culture containing BF3636, BF3636 culture alone, or normal saline (control group). Mice subsequently received a culture of vancomycin-resistant enterococcus (VRE) by orogastric gavage. Stool samples were collected on succeeding days and plated for the presence of VRE and resistant *Bacteroides* species. **Results:** In mixing studies, BF3636 raised the MBC of a ceftriaxone-susceptible *Escherichia coli* strain from < 0.25 to 64, whereas organisms that did not produce beta-lactamase did not ($p < .001$). The CF culture containing intestinal microflora was not affected when 8 µg/mL of ceftriaxone was added to the inflowing media, but 100 µg/mL resulted in disruption of the microflora and detection of ceftriaxone in the culture. In the CF culture containing BF3636, the population of *Bacteroides* species with high-level resistance to cephalosporins expanded over time and beta-lactamase activity increased; infusion of 100 µg/mL of ceftriaxone did not disrupt the indigenous microflora and ceftriaxone levels remained undetectable. In vivo, ceftriaxone-treated mice that had been orally inoculated with CF culture containing BF3636 did not develop intestinal overgrowth with VRE despite exposure; this was in contrast to ceftriaxone-treated mice that had received an oral inoculation with saline alone. **Conclusion:** Expansion of subpopulations of *Bacteroides* species that hyperproduce cephalosporinases can inactivate high concentrations of ceftriaxone and preserve the indigenous intestinal microflora. CF culture containing beta-lactamase-producing *Bacteroides* species was able to preserve colonization resistance of ceftriaxone-treated mice against VRE.

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THE N-TERMINUS OF FOG-2 INTERACTS WITH METASTASIS-ASSOCIATED PROTEINS 1 AND 2 TO MEDIATE TRANSCRIPTIONAL REPRESSION DURING CARDIAC DEVELOPMENT. S.A. Samant, A.E. Roche, E.C. Svensson, Department of Medicine, The University of Chicago, Chicago, IL.
Mutations in several transcription factors that regulate cardiac development have recently been described to cause congenital heart disease in humans. A greater understanding of the molecular mechanisms that regulate heart formation may help identify other genes that when mutated will result in human congenital heart disease. FOG-2 is one such gene that encodes a transcriptional corepressor expressed in the developing heart. It is critical for proper cardiac morphogenesis as mice deficient in this factor die during midgestation of cardiac malformations. We have previously shown that FOG-2 physically interacts with GATA4 and attenuates GATA4's ability to activate cardiac specific gene expression. This repression is mediated by a domain of FOG-2 localized to its N-terminus, termed the FOG repression motif. To gain further insights into the molecular mechanism responsible for this repression, we took a biochemical approach to identify factors that interact with FOG repression motif. Using MALDI-TOF mass spectrometry, we identified seven proteins from rat neonatal cardiac nuclear extracts that copurified with a FOG-2-GST fusion protein. All of these proteins have been previously described to be subunits of a nucleosome-remodeling complex called the NuRD complex. To determine which of the NuRD subunits directly interact with the FOG repression motif, we used a series of in vitro binding assays. We found that MTA-1 and MTA-2 specifically bound to the N-terminus of FOG-2 but not to a mutant form of the N-terminus that is unable to mediate repression. In situ hybridization revealed that both MTA-1 and MTA-2 are expressed in the developing heart during mouse embryogenesis. Taken together, these results suggest that FOG-2 mediates transcriptional repression during cardiac development by the recruitment of the NuRD complex to GATA-dependent promoters leading to the remodeling of the local chromatin structure and the attenuation of gene expression.
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THE ROLE OF PROTEIN PHOSPHATASE 2A IN THE REGULATION OF ENDOTHELIAL CELL CYTOSKELETON STRUCTURE. K. Tar,[1,2,#] C. Csortos,[1,2,#] I. Czikora,[2] G. Olah,[1] S-F. Ma,[1] R. Wadgaonkar,[1] P. Gergely,[2] J.G.N. Garcia,[1] A.D. Verin,[1*] [1]Department of Medicine, Division of the Biological Sciences, The University of Chicago, Chicago, IL; [2]Department of Medical Chemistry, Research Center for Molecular Medicine, University of Debrecen, Medical and Health Science Center, Debrecen, Hungary.
Our recently published data suggested the involvement of protein phosphatase 2A (PP2A) in endothelial cell (EC) barrier regulation (Tar et al, 2004). In order to further elucidate the role of PP2A in the regulation of EC cytoskeleton and permeability, PP2A catalytic (PP2Ac) and A regulatory (PP2Aa) subunits were cloned and human pulmonary arterial EC (HPAEC) were transfected with PP2A mammalian expression constructs or infected with PP2A recombinant adenoviruses. Immunostaining of PP2Ac or of PP2Aa+c overexpressing HPAEC indicated actin cytoskeleton rearrangement. PP2A overexpression hindered or at least dramatically reduced thrombin- or nocodazole-induced F-actin stress fiber formation and microtubule (MT) dissolution. Accordingly, it also attenuated thrombin- or nocodazole-induced decrease in transendothelial electrical resistance indicative of barrier protection. Inhibition of PP2A by okadaic acid abolished its effect on agonist-induced changes in EC cytoskeleton; this indicates a critical role of PP2A activity in EC cytoskeletal maintenance. The overexpression of PP2A significantly attenuated thrombin- or nocodazole-induced phosphorylation of HSP27 and tau, two cytoskeletal proteins, which potentially could be involved in agonist-induced cytoskeletal rearrangement and in the increase of permeability. PP2A-mediated dephosphorylation of HSP27 and tau correlated with PP2A-induced preservation of EC cytoskeleton and barrier maintenance. Collectively, our observations clearly demonstrate the crucial role of PP2A in EC barrier protection.
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AMBIENT PARTICULATE MATTER INDUCES MYOFIBROBLAST DIFFERENTIATION VIA MACROPHAGE-DEPENDENT TRANSFORMING GROWTH FACTOR β TYPE I (ALK5) SIGNALING. K. Thavarajah, R. Kalhan, A. Nair, M.C. Nlend, N. Wang, P.H.S. Sporn, Northwestern University, Chicago, IL.
Rationale: Increased levels of ambient particulate matter have been associated with increased pulmonary morbidity and mortality. To investigate if particulate matter induces airway remodeling, we studied the effects of particulate matter (< 10 µm in diameter) collected from Dusseldorf, Germany (DPM), on fibroblast to myofibroblast differentiation. **Methods:** Human fetal lung fibroblasts (IMR-90) were grown to subconfluence, serum-starved for 48 hours, and exposed to either TGF-β1 (2 ng/mL), DPM, or conditioned medium from human monocytic (THP-1) cells exposed to DPM. The role of TGF-β1 signaling was assessed by the addition of SB431542 (10 µM), a TGF-β type I (ALK5) receptor inhibitor. After 48 hours, cells were lysed and analyzed by immunoblot for α-smooth muscle actin (αSMA), a marker of myofibroblast differentiation. **Results:** Direct exposure of IMR-90 cells to DPM did not increase αSMA expression. However, conditioned medium of THP-1 cells exposed to DPM induced increased αSMA expression in fibroblasts. This increase was blocked by the ALK5 inhibitor SB431542. **Conclusions:** Ambient particulate matter triggers macrophage-dependent induction of myofibroblast differentiation via ALK5 receptor signaling. We speculate that particulate matter induces airway remodeling.

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REGULATION OF 4-HYDROXY-2-NONENAL–MEDIATED ENDOTHELIAL BARRIER FUNCTION BY FOCAL ADHESION AND ADHERENS JUNCTION PROTEINS. P.V. Usatyuk, V. Natarajan, School of Medicine, The University of Chicago, Chicago, IL.
4-Hydroxy-2-nonenal (4-HNE), a highly reactive aldehyde generated by peroxidation of membrane lipids, altered endothelial cell (EC) barrier function, resulting in vascular leakiness. Here we report that 4-HNE mediates EC barrier dysfunction by modulating focal adhesion and adherens junction proteins that affect cell-cell and cell-matrix interactions via integrins. Treatment of bovine lung microvascular endothelial cells (BLMVECs) with 4-HNE, in a dose-dependent manner, modulated cell-cell adhesion contacts, enhanced intracellular ROS formation, and increased permeability. Interestingly, 4-HNE did not alter