model of IBD. To understand the phenotype, we utilized macrophages to investigate the mechanism behind autophagy and proinflammatory cytokine secretion. In addition, we analyzed the development of colonoids in a co-culture system with macrophages with or without a functional autophagy pathway. Lastly, pharmacological modulation of autophagy to control inflammation was assessed. RESULTS/ANTICIPATED RESULTS: Mice with autophagy-deficient macrophages were highly susceptible to intestinal barrier disruption. Susceptibility was due to enhanced proinflammatory cytokine secretion and intestinal permeability. Furthermore, proinflammatory macrophages (due to an autophagy defect) co-cultured with colonoids, significantly decreased the number of mucus producing goblet cells. Finally, pharmacologically modulation of autophagy reduced the secretion of proinflammatory cytokine by macrophages and reduced intestinal permeability. DISCUSSION/SIGNIFICANCE OF IMPACT: Our results strongly suggest autophagy modulation can dampen inflammation and enhance the intestinal epithelial barrier.

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MicroRNA-451: A potential key player in the development of diabetic nephropathy in an insulin resistant mouse model

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OBJECTIVES/SPECIFIC AIMS: MicroRNAs (miRNA) affect transcription of a number of genes involved in the development and progression of diabetic nephropathy (DN), and have become attractive therapeutic targets and biomarkers. Elevated renal gluconeogenesis, fibrosis, and albuminuria are early markers of incipient DN. Recent studies report that renal miRNA-451 may protect against DN and reduce renal gluconeogenesis in rodent models. MiRNA-451 is thought to act by targeting select factors resulting from disrupted insulin and growth factor signaling and the mechanistic-target of rapamycin (mTOR) in early DN. This study aimed to elucidate the role of miRNA-451 in the development and progression of DN. METHODS/STUDY POPULATION: To further elucidate the role of miRNA-451 in DN, we placed male insulin-resistant, TALLYHO/Jng mice on a high-fat diet (60% kCal). The mice were divided into 2 treatment groups and received 8 consecutive weekly intraperitoneal injections of locked nucleic acid (LNA) miR-451-inhibitor or LNA-scrambled compound (2 mg/kg·bw; n = 8/treatment). Mice were euthanized after 12 weeks (4 weeks sans injections) and kidneys, liver, pancreas and abdominal adipose tissue were harvested for analysis. RESULTS/ANTICIPATED RESULTS: Renal homogenate expression of miRNA-451 was drastically reduced in inhibitor-treated mice (~6-fold) in comparison with scramble-treated mice. Western blotting of cortex homogenates for indicators of fibrosis and targets of miRNA-451 revealed a significant reduction in collagen IV (marker of epithelial integrity) in inhibitor-treated mice. In addition, metalloproteinase type 9 (MMP9, a known type IV collagenase), YWHAZ (a scaffolding protein and known target of miR-451), mTOR, and fructose bisphosphatase (FBP1, a rate-limiting gene in gluconeogenesis) were significantly increased in this group in comparison to scramble-treated mice. However, no differences were found in protein levels for glucose-6-phosphatase (G-6-Pase) or phosphoenolpyruvate (PEPCK), 2 additional gluconeogenic rate-limiting genes. MiRNA-451 antagonist did not significantly affect final body weight or blood glucose; however, mean blood sodium concentrations were slightly, but significantly higher (2%) in the LNA-inhibitor treated group (when compared with the scramble-treated group). No differences in blood potassium or chloride were found. Anion gap was 90% higher in the LNA-inhibitor treated group when compared with scramble-treated mice. No differences in urinary albumin to creatinine ratio were found between the two treatment groups. However, Masson Trichrome scoring revealed a 59% increase in fibrosis in inhibitor-treated mice. DISCUSSION/SIGNIFICANCE OF IMPACT: Collectively, these findings support a potentially protective role of miRNA-451 in attenuating signaling via mTOR that may alter both renal gluconeogenic potential (contributing to the diabetic phenotype) and activation and progression of renal fibrosis. Therapies to enhance miRNA-451 signaling may be beneficial to reduce renal pathology associated with DN.

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miRNA manipulation to improve CFTR correction in cystic fibrosis

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OBJECTIVES/SPECIFIC AIMS: CFTR is the mutant protein that causes cystic fibrosis (CF), a fatal respiratory diseases affecting 1 in 3500 children. CFTR modulators are small molecules that directly address mutant CFTR function. Improving correction of the F508del CFTR mutation (affecting 90% of CF patients) is one of the most pressing unmet needs in CF. Currently available F508del therapeutics only marginally improve CF, In vitro, we have identified a miRNA that impairs utility of CFTR directed therapies. miR-145 is upregulated by TGF-β (a genetic modifier of CF lung disease) with a direct binding site on the 3'-untranslated region of CFTR mRNA. Binding of miR-145 to CFTR destabilizes mRNA transcript and impedes protein translation. Overexpression of miR-145 abolishes benefit of F508del CFTR correction. Antagonists to miR-145 block TGF-β suppression of CFTR function and augment response to CFTR correction. This project evaluate in vivo impact of TGF-beta and miRNA manipulation on CFTR functional readouts including nasal potential difference (NPD) and short circuit current (Isc) across tracheal explants in addition to standard biochemical measures. METHODS/STUDY POPULATION: Wild-type Sprague-Dawley rats were inoculated with an adenoviral vector containing bioactive TGF-beta or sham at 1×10^9 pfu/animal placed in the left nares. Seven days post-inoculation, functional, and biochemical measures were conducted. NPD was measured with a microelectrode placed in the left nare and grounded the tail. The nare was sequentially perfused with standard Ringer's solution, amiloride (to block the ENaC sodium channel), low chloride Ringer's (to stimulate chloride efflux), forskolin (to open the CFTR channel) and CFTRinh-172 (to block the CFTR channel. Tracheal explants were harvested, microdissected, and placed on modified Ussing chambers. RESULTS/ANTICIPATED RESULTS: We have inoculated WT rats with bioactive TGF-β Versus sham delivered by intranasal inoculation of an adenoviral vector. Functional readout of CFTR function is by Isc across tracheal epithelia and NPD. Lung homogenates are analyzed for TGF-β signaling, miRNA expression, and CFTR transcripts. Both tracheal explants and NPD indicate TGF-β stimulation diminishes CFTR function in vivo. In tracheal explants, TGF-β exposure diminishes CFTR response to forskolin-stimulation by 75%. Loss of current after CFTR inhibition (CFTRinh-172) is halved. By nasal PD, TGF-β inoculation similarly halves the bioelectric response to low chloride and forskolin stimulation. Evaluation by qPCR reveals a strong increase in TGF-β signaling demarcated by PAI-1, prompting a reduction in CFTR mRNA. miR-145 is expressed highly in rat pulmonary tissue, but no change in overall miR-145 levels was detected between TGF-β and sham exposed rats. This finding reflects what we have observed in human lungs, with a localized increased miR-145 expression in CF epithelia, but similarly high levels of miR-145 in both CF and non-CF whole lung homogenates. Although expressed at lower levels than miR-145, we did find increased expression in TGF-β relevant miR-101, miR-494, and miR-144 that have a predicted binding site on rat 3'-UTR in TGF-β exposed Versus sham lungs. DISCUSSION/SIGNIFICANCE OF IMPACT: Our data indicate the relevance of TGF-β stimulation to suppress CFTR synthesis and function in vivo. Future work will evaluate whether these additional miRNA with CFTR binding sites may mediate TGF-β suppression of CFTR in the rat model, and the utility of miRNA manipulation to augment F508del CFTR correction.

2301

Mucoidal pseudomonas aeruginosa infection is associated with regional inflammation in the cystic fibrosis lung

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OBJECTIVES/SPECIFIC AIMS: Cystic fibrosis (CF) is a life-shortening genetic disease that affects approximately 30,000 patients in the United States. CF patients suffer from chronic pulmonary infections that are associated with hyperinflammation and irreversible damage to the lower airways. As CF patients age, Pseudomonas aeruginosa (P.a.) is the predominant pathogen that infects the respiratory tract. The P.a. strains initially infecting the CF lung have a nonmucoid colony morphology, whereas, once chronic infection is established, these bacteria mutate leading to the emergence of mucoid P.a. variants with heightened resistance to both antibiotics and host immunity. Both nonmucoid and mucoid P.a. variants are often co-isolated on microbiological cultures of sputum collected from CF patients. However, the CF lung is known to exhibit heterogeneity in inflammation and infecting microbes across different lung regions that cannot be studied using routine sputum collection alone. Here, using a standardized bronchoscopic protocol, bronchoalveolar lavage (BAL) fluid was prospectively collected from each lobe of a CF cohort undergoing clinically indicated surgical procedures. We sought

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to investigate if there is an association between infecting P.a. variants (nonmucoid, mucoid, or mixed populations), the lung lobes in which these variants are found, and regional proinflammatory cytokine production. METHODS/STUDY POPULATION: We performed BAL on 16 CF patients with clinically stable disease. For each patient, we obtained BAL fluid from the right upper lobe, right middle lobe, right lower lobe, left upper lobe, lingula, and left lower lobe. We plated BAL fluid on nonselective and P.a.-selective medium to quantitate bacteria and to identify P.a. colony subtypes (nonmucoid, mucoid, or mixed). We further used a V-PLEX human cytokine array to quantitate inflammatory cytokine concentrations (IL-1β, TNF-α, IL-6, IL-8, and IL-10) within BAL fluid specimens. Our specimen collection was approved by the local IRB with informed consent and assent obtained from patient volunteers. RESULTS/ANTICIPATED RESULTS: Based on microbiological analysis, each lobar BAL specimen was classified as uninfected with P. a. or infected with nonmucoid, mucoid, or mixed (both nonmucoid and mucoid) P.a. variants. There was no observed propensity of mucoid or nonmucoid variants to be confined to certain lung lobes in our cohort. However, infection with mucoid P.a. variants was associated with higher concentrations of IL-1β ($p < 0.001$), TNF-α ($p < 0.001$), IL-8 ($p < 0.001$), and IL-10 ($p < 0.001$) within lobar BAL fluid compared with P.a.-free specimens. Specimens with mucoid variants also had greater concentrations of TNF-α ($p < 0.01$), IL-8 ($p < 0.001$), and IL-10 ($p < 0.05$) compared with specimens with only nonmucoid P.a. variants. Patients infected with mixed mucoid and nonmucoid variants showed higher concentrations of TNF-α and IL-10 ($p < 0.05$) as well as nonsignificant trends for higher concentrations of IL-1β and IL-6 compared to P.a.-free samples. Interestingly, the presence of nonmucoid P.a. variants was inversely correlated with IL-6 ($p < 0.05$). Total bacterial burden (both P.a. and non-P. a. species) within BAL fluids was positively correlated with higher proinflammatory cytokine concentrations. Additionally, independent of bacterial colonization, the upper lobes (right upper lobe and left upper lobe) of the lungs showed trends towards higher proinflammatory cytokine concentrations compared with the lower lobes (right lower lobe and left lower lobe). DISCUSSION/SIGNIFICANCE OF IMPACT: Our results demonstrate that P.a. variants (mucoid or nonmucoid) appear not to be geographically restricted in ability to colonize any lobe of the CF lung. Moreover, infection with mucoid P.a. (either alone or in mixed populations with nonmucoid variants) is associated with higher inflammatory cytokine concentrations in the CF lung. Given that infection with mucoid P.a. predicts deterioration in pulmonary function, this study provides a rationale for further investigation of cytokines as diagnostic/prognostic correlates of infection and lung disease in CF.

2012

Multivariate air pollutant exposure prediction in South Carolina

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OBJECTIVES/SPECIFIC AIMS: The objective of this project is the application of complex fusion models, which combine observed and modeled data, to areas with sparse monitoring networks with multiple chemical components is under-developed. Such models could provide improved accuracy and coverage for air quality measurement predictions, an area greatly limited by the amount of missing data. METHODS/STUDY POPULATION: This project focuses on the development of methods for improved estimation of pollutant concentrations when only sparse monitor networks are found. Sparse monitoring networks are defined as areas where fewer than three criteria air pollutants (based on EPA standards) are monitored. Particularly, a multivariate air pollutant statistical model to predict spatio-temporally resolved concentration fields for multiple pollutants simultaneously is developed and evaluated. The multivariate predictions allow monitored pollutants to inform the prediction of nonmonitored pollutants in sparse networks. RESULTS/ANTICIPATED RESULTS: Daily, ZIP code level pollutant concentration estimates will be provided for 8 pollutants across South Carolina, and goodness of fit metrics for model variants and previously established methods will be compared. DISCUSSION/SIGNIFICANCE OF IMPACT: These methods utilize only widely available data resources, meaning that the improved predictive accuracy of sparsely monitored pollutant concentrations can benefit future studies in any US area by improving estimation of health effects and saving resources needed for supplemental air pollutant monitoring campaigns. Our method for estimation attempts to improve predictive accuracy and data availability for sparsely monitored pollutants and areas.

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Neural correlates of externally Versus internally guided dance-based therapies for people with Parkinson's disease

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OBJECTIVES/SPECIFIC AIMS: Parkinson's disease (PD) is a condition that affects over a million Americans, and despite current medical therapies, the progression of the disease results in impaired generation of internally timed or guided (IG) movements. To address this loss of motor function, previous rehabilitation therapies have focused on remediating the affected striatal-thalamic-cortical circuits (STC), primarily thought to be responsible in generating timed motor patterns. However, given the disease leads to the cell death of dopaminergic cells that are essential for proper STC function, we propose a motor therapy aimed at utilizing a compensatory parallel cerebellar-thalamic-cortical (CTC) pathway, recruited to perform externally guided (EG) movements, in which gait initiation is driven from sensory input. Our previous study has shown efficacy in our novel argentine tango therapy and improves behavioral measures above the relevant MCID threshold, but it has not been established that the CTC are in the causal pathway that are responsible for these changes. Using neural measures from task fMRI, we have begun to characterize networks that have changed and quantify any associations with behavioral metrics. METHODS/STUDY POPULATION: Patients were randomly assigned to an IG (n = 18), EG (n = 18), or education contact control (n = 14). Participants were assessed preintervention and postintervention for behavioral motor and cognitive measures and neurophysiologically with task based fMRI. In the task, participants performed a foot tapping task under both IG (tap their foot in previously learned rhythm) or EG (tap immediately after receiving a tactile cue on their hand) conditions. The fMRI data were preprocessed using AFNI and registered to MNI standard space. The brainnetome atlas was applied and the average time series of each region of interest (ROI) was used to increase the signal to noise ratio. The activation of these ROI with respect to the stimulus was modeled using GLM, and we estimated the area under the curve during the task blocks. A 1-way ANOVA analysis on these betas were performed between the pre and the post intervention time points and the ROIs that were above a significance of 0.95 were identified and corrected for multiple comparisons. The change in beta in all ROIs for each individual were calculated and then correlated with the changes in the behavioral data, to see which changes in ROI areas matched the best with the behavioral changes. RESULTS/ANTICIPATED RESULTS: The EG group showed significant changes only in the EG task in 2 areas—inferior frontal gyrus and inferior temporal sulcus. Correlating to the cognitive behavioral measures show reduced error from the Inferior frontal gyrus (corr > 0.5) best reflect changes in observed. There were no changes to either the STC or the CTC pathways. The IG group showed no changes behaviorally and showed no changes neurally as well. The control group showed no changes behaviorally, but neuronally certain DMN nodes, such as the precuneus and inferior temporal regions showed a significant change for both tasks. DISCUSSION/SIGNIFICANCE OF IMPACT: Addressing the damaged STC pathway directly through IG therapy may not be effective. The EG therapy may not be able to enhance the STC pathway. However, the therapy appears to utilize new areas in the frontal regions and correlates with positively with changes in spatial memory and balance tasks. Contrary to our hypothesis the CTC circuit was not upregulated for performance of the IG or EG task, but therapy may have enhanced recruitment of other cognitively engaged areas. The educational control group interestingly showed changes in the DMN network, which has been shown to be linked to attention during tasks blocks.

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Neural correlates of face processing in autism spectrum disorder: A quantitative meta-analysis of current literature and future directions

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OBJECTIVES/SPECIFIC AIMS: Autism spectrum disorder (ASD) affects 1 in 68 people and includes restricted, repetitive behavior, and social communication deficits. Aspects of face processing (i.e., identity, emotion perception) are impaired in some with ASD. Neuroimaging studies have shown aberrant

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