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

Maurice B Fluitt, Lijun Li, Narayan Shivapurkar and Carolyn Ecelbarger

Georgetown University

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

William Thomas Harris and Farruk Kabir

University of Alabama at Birmingham

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.

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

Sankalp Malhotra, Daniel J. Wozniak[1] and Don Hayes Jr.[2]

[1] Department of Microbial Infection and Immunity, Department of Microbiology, The Ohio State University College of Medicine;

[2] Pulmonary Medicine, Transplant Program Nationwide Children's Hospital

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