Diabetic Complications Consortium

Application Title: Modeling the Diabetic Gut: how microbes fuel metabolic disorders
Principal Investigator: Soumita Das

1. Project Accomplishments:

The gut barrier separates trillions of microbes from the largest immune system in the body; when compromised, a 'leaky' gut barrier fuels systemic inflammation, which hastens the progression of chronic diseases including diabetes. Strategies to detect and repair the leaky gut barrier remain urgent and unmet needs. Recently, a stress-polarity signaling (SPS)-pathway has been described in which the metabolic sensor, AMP-Kinase augments epithelial polarity exclusively under energetic stress and suppresses tumor formation. Using murine and human colon-derived organoids, and enteroid-derived monolayers (EDMs) that are exposed to stressors, we reveal that the SPS-pathway is active in the intestinal epithelium and requires a catalytically active AMP-kinase. Its pharmacologic augmentation resists stress-induced collapse of the epithelium when challenged with microbes or microbial products.

Specific Aims:

Aim 1: Determine the role of DM-associated microbes and microbial products in gut barrier and in the activation of the SPS- pathway in colonic enteroid-derived monolayers (EDMs).

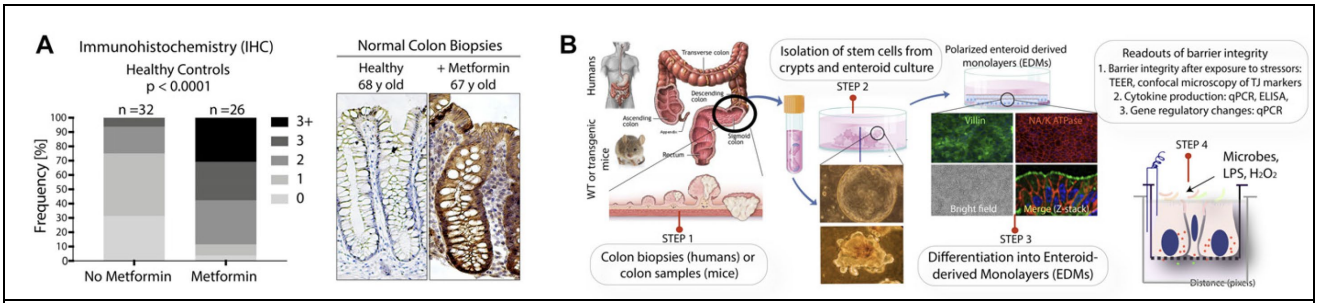

Figure 1. The stress polarity signaling (SPS) pathway is active in the gut lining, and its pharmacologic augmentation requires the catalytic activity of AMP-kinase.
Left: The SPS-pathway was evaluated in normal adult colon by immunohistochemistry (IHC) on FFPE colonic biopsies using anti-pS245-GIV, and the staining intensity in the epithelium was scored (see **Figure S1**). Bar graph displays the proportion of patients in each group with varying intensities of staining. Two-sided Fisher's exact test was used to calculate significance. *Right*: Representative images are presented from healthy adult without metformin (lowest '0' staining) and with metformin (highest '> 3+' staining) within the cohort.

Schematic showing the key steps involved during the development of the stem cell-based organoid model, "gut-in-a-dish". Fresh biopsies obtained from the colons of mice and humans (STEP 1) are used as source of stem cells to grow organoids (STEP 2). Organoids are differentiated into polarized enteroid-derived monolayers (EDMs; STEP 3) for co-culture studies with microbes and microbial products (exposed apical surface) to mimic the gut lumen in physiology and enable the assessment of barrier integrity (STEP 4).

Result: We found that the Stress-Polarity Signaling (SPS)-pathway is active in the colon epithelium. The intensity of staining varies among patients, a larger proportion of patients on Metformin displayed positive staining and at a stronger intensity compared to the cohort of patients, not on metformin (Fig 1). These findings indicate that the SPS-pathway, as determined by pS245-GIV as a surrogate marker (Fig 2), is active in the human colon epithelium and that although its degree of

activation varies considerably in the normal colon, it appears to be consistently enhanced in patients exposed to the widely-prescribed AMPK-agonist Metformin.

Aim 2. Assess the impact of microbes, microbial products and nutritional components on the secretion of peptide hormones (PYY, GLP-1/2) that are secreted from the gut lining using intestinal EDMs

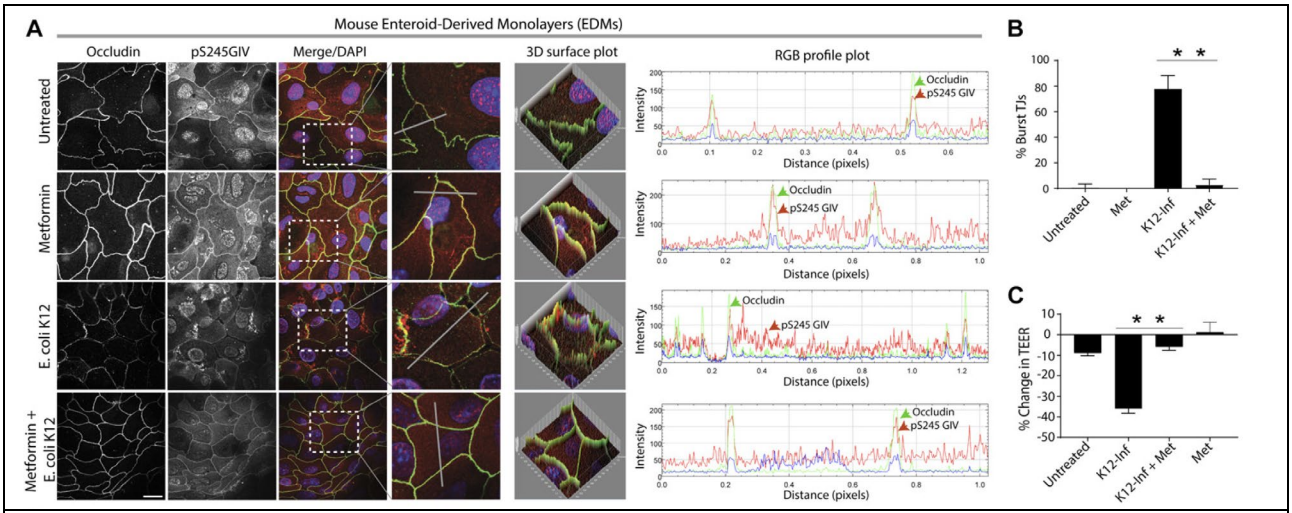

Figure 2. Pharmacologic augmentation of the SPS-pathway protects the gut barrier against diverse stressors such as microbes and microbial products.

A. Mouse EDMs were infected with E. coli K12 with or without prior exposure to metformin (1 mM for 16 h), fixed, stained with anti-pS245-GIV (red; a surrogate measure of the SPS-pathway), occludin (green; a bona-fide TJ marker) and nucleus (DAPI) and analyzed by confocal microscopy. A-left: Images displayed are representative of three independent experiments. Co-localization of pS245-GIV and occludin was assessed using ImageJ plug-ins: RGB profile plot (A-middle) and 3D surface plots (A-right). Scale bars = 10 µm.

B. Bar graphs display the % of tight junctions (TJs) that appeared broken and/or splitting ('burst'-appearing) on Y axis encountered in 8-10 randomly chosen fields from three independent experiments in A. Findings were analyzed by one-way ANOVA followed by multiple comparison test. Error bars represent mean +/- S.E.M, n = 3; **, $p < 0.01$.

C. Bar graphs display the change in TEER across the EDMs in A. Findings were analyzed by one-way ANOVA followed by multiple comparison test. Error bars represent mean +/- S.E.M; n = 3; **, $p < 0.01$.

Result: The SPS-pathway protects the gut barrier against diverse stressors such as microbes and microbial products. We used the 'gut-in-a-dish' model to study the role of the SPS-pathway in EDMs under stress. Our choice of stressors included those that are physiologically encountered within the gut lumen, e.g., a) live commensal microbes (E. coli; Figure 2A-C). We found that the microbe induced barrier disruption in untreated EDMs, as determined by occludin staining (Figure 2A-B) and by the observed drops in Trans-epithelial electrical resistance (TEER).

2. Publications:

Ghosh P*, Swanson L, Sayed IM, Mittal Y, Lim BB, Ibeawuchi SR, Foretz M, Viollet B, Sahoo D and **Das S***. The Stress Polarity Signaling (SPS)-Pathway Serves as a Marker and a Target in the Leaky Gut barrier: Implications in Aging and Cancer. Life Science Alliance. Feb 10, 2020 0;3(3). pii: e201900481. PMID: 32041849 (* equally contributed)