LIGHTNING TALK:

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In situ, O-glycomic, and functional characterization of the gut mucus sialic acid O-acetylome of complex O-glycans derived from human and murine Mucin-2

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Background and Rationale: The mucus system provides innate immune defense to protect our gastrointestinal tract from pathogens, and promote homeostasis with our resident microbiota. This system is constituted by the gel-forming mucin MUC2 (Muc2 in mouse), which is ~80% complex O-linked glycans by weight. Sialic acid (Sia) is a key capping monosaccharide on complex O-glycans which interacts with and is metabolized by the microbiota. Sia can undergo enzymatic modifications including the addition of O-acetyl groups to carbons 4, 7, 8, & 9. The 9-O-acetyltransferase CasD1 is responsible for the 9-OAc Sia variants, and OAc groups are known to migrate between carbons via a poorly understood mechanism. Functionally, OAc-modification in known to inhibit microbial sialidase activities which is a key step in complex O-glycan catabolism. However, the extent of these O-acetylation modifications in human and murine Mucin-2, and how they influence the function of the mucus system, is unclear.

Methodology: We used a novel method to visualize and purify human MUC2 from human fecal material, as well as viral-derived probes that target specific OAc-Sia analogues to visualize their spatial arrangement and microbial interaction in situ. We also performed biochemical analysis via in-gel Alcian blue staining on composite agarose-polyacrylamide gels following electrophoresis (AgPAGE). For glycomic analysis, OAc-Sia analogues were quantitated by HPLC-MS after derivatization with 4,5-dimethyl-1,2-diaminobenzamine (DMBA). For functional data in mice, we generated intestinal epithelial cell-specific Casd1 KO mice (*Casd1*$^{flox/flox}$;VillinCre or IEC *Casd1*$^{-/-}$ mice) and analyzed their mucins. Sialidase activities were quantified in the supernatants of colon fecal materials from WT and IEC *Casd1*$^{-/-}$ mutants mice using a fluorogenic substrate 4-MU-NeuNAc (2-(4-Methylumbelliferyl)-α-D-N-acetylneuraminic acid.

Results: We found Sias on both human MUC2 and murine Muc2 were heavily O-acetylated, with ~75% of Sias on human MUC2 having 9-OAc-based modification; of these, ~42% of analogues were represented by 7,9 di-O-acetyl variants, ~20% with single 9-OAc modification, and 5% each with either single 7- or 8 -OAc modification. 4 OAc-Sia analogous were rarely observed. In mice, 9-OAc modification also dominated, although they were less abundant (~45%) than on human. IEC *Casd1*$^{-/-}$ mice were viable and healthy with knockdown confirmed by 9-OAc Sia-specific probes on mucus and goblet cells in situ. Consistent with the known role of OAc Sia in inhibiting sialidases, loss of Casd1-depdent O-acetylation was associated with increased sialidase activities as assessed by heightened 4 MU signal on fecal supernatants in WT vs littermate IEC *Casd1*$^{-/-}$ mice. However, mucins migrated at similar rate by AgPAGE and appeared intact overall in situ. Interestingly, glycomic analysis of mucins purified from WT and IEC *Casd1*$^{-/-}$ mice indicated that loss of Casd1 activity ablated 7,8, & 9 O-acetylation status on murine Muc2, suggesting Casd1 activity is the source of the majority of Sia O-acetylation.

Conclusion: These studies are the first to chemically and functionally characterize Sia O-acetylation on human and murine Mucin-2. Studies are ongoing to determine the impact on microbiome and inflammation susceptibility.